11-29-2011

EGFR Regulation of Epidermal Barrier Function

Nhu Quynh T. Tran

EGFR REGULATION OF EPIDERMAL BARRIER FUNCTION

by

Nhu Quynh T. Tran

A Dissertation

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

Major: Biology

The University of Memphis

December 2011

Dedication

I dedicate this dissertation to my husband, my son, and my parents. They have offered me unconditional love and supported me through the course of this dissertation; especially my dear husband, without him, I could not have come this far. Thank you.

Acknowledgments

I would like to acknowledge and extend my heartfelt gratitude to the following people, who have made the completion of this Dissertation possible. First, I would like to thank my advisor Dr. Thomas R. Sutter for advising and encouraging me throughout my research. I thank him for believing in my competence and teaching me how to become an independent thinker and a good scientist. I thank Dr. Judith A. Cole for being a great professor, a good listener and supporter. I thank Dr. Carrie H. Sutter for always being available for technical help and support, and also for giving my son many of her kids' toys. I would like to thank other committee members Drs. Andrew Liu and Ramin Homayouni for their guidance over the years. My special thanks to all the past and present members of the Sutter research group for their help and support. Their friendship over the years has made me a stronger and better person. I would also like to thank Dr. Warren Haggard and Jonathan McCanless for the use of the fluorometer; Dr. Christopher Waters (The University of Tennessee Health Science Center- UTHSC) for lending the Endohm system; Meifen Lu, Linda White, Dr. Anand Kulkarni, and Crystal Stanton (all from UTHSC) for their generous help in histology and slide scanning; Dr. Shuhong Qiao, Dr. Marina Kedrov and Yunming Hu (UTHSC) for the confocal microscopy training; Dr. Omar Skalli and Renada Scott for helpful advise regarding histology and the immunofluorescence assay.

Finally, and most importantly, I would like to thank my husband, who has played a significant role throughout my graduate work. He is not only a great mentor, but also a great companion in life. I thank him for being so patient, but yet so definite and firm. His faith and confidence in my abilities and in me have helped me to succeed.

Abstract

Tran, Nhu Quynh T. Ph.D. The University of Memphis. December 2011. EGFR Regulation of Epidermal Barrier Function. Major Professor: Thomas R. Sutter, Ph.D.

Keratinocyte terminal differentiation is the process that ultimately forms the epidermal barrier that is essential for mammals to survive in the *ex utero* environment. This process is tightly controlled by the expression of many well-characterized genes. Although a few of these genes are known to be regulated by the epidermal growth factor receptor (EGFR), an important regulator of multiple epidermal functions, neither the genome-wide scale of EGFR-mediated regulation nor the mechanisms by which EGFR signaling controls keratinocyte differentiation are well understood. Using microarray analysis we identified 2,676 genes that are regulated by EGF, a ligand of the EGFR. We further discovered, and separately confirmed by functional assays, that EGFR activation abrogates all essential metabolic processes of keratinocyte differentiation by (1) decreasing the expression of lipid matrix biosynthetic enzymes, (2) regulating numerous genes forming the cornified envelope, and (3) suppressing the expression of tight junction proteins. In organotypic cultures of skin, the collective effect of EGF impaired epidermal barrier integrity, evidenced by increased transepidermal water loss. As defective epidermal differentiation and disruption of the epidermal barrier are primary features of many human skin diseases, we used bioinformatics analysis to identify genes that are known to be associated with human skin diseases. In comparison to non-EGF-regulated genes, the EGF-regulated gene list was significantly enriched for disease genes. Further validation of the expression profiles of many of the 114 identified skin disease genes included the transcription factors GATA binding protein 3 (GATA3) and Kruppel-like factor 4 (KLF4), both required for establishing the barrier function of the skin in

developing mice. These results provide a new systems level understanding of the actions of EGFR signaling to inhibit keratinocyte differentiation. As the overall effect of this inhibition is to impair epidermal barrier integrity, this study clarifies how dysregulation of the EGFR and its ligands may contribute to diseases of the skin.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ACTB	β-actin
acylGC	Acylglucosylceramide
AD	atopic dermatitis
calcium	Ca^{2+}
Cat #	catalog number
cdc42	cell division cycle 42
CDSN	Corneodesmosin
CE	cornified envelope
Cer	Ceramide
CLDN	Claudin
cont.	Continue
crk	v-crk sarcoma virus CT10 oncogene homolog
CTSD	cathepsin D
DMSO	dimethyl sulfoxide
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ELOVL	elongation of VLCFA
ERK	extracellular signal-regulated kinase
FFA	free fatty acid
FLG	Filaggrin
GDP	guanosine diphosphate
Grb2	growth factor receptor-bound protein 2
GTP	guanosine triphosphate
h	Hour
IVL	Involucrin
JNK	Jun N-terminal kinase
KEGG	Kyoto Encyclopedia of Genes and Genomes
KHG	keratohyalin granules
KLK	Kallikrein
KRT	Keratin
KSFM	keratinocyte serum-free medium
LOR	Loricrin
MAPK	mitogen-activated protein kinase
MEK	MAPK/ERK kinase
min	Minute
NHEK	normal human epidermal keratinocyte
PBS	phosphate buffered saline
PI3K	phophoinositide 3-kinase
PKC	protein kinase C
PLC	phopholipase C
pY	Phosphotyrosine
qRT-PCR	quantitative reverse transcriptase-polymerase chain reaction
Rac1	ras-related C3 botulinum toxin substrate 1
Ras	rat sarcoma

SB	stratum basale
sec	Second
SC	stratum corneum
SG	stratum granulosum
SH3	src homolog 3 domain
Sos	son of sevenless
SPRR	small proline-rich protein
Src	v-src sarcoma viral oncogene homolog
TER	transepithelial electrical resistance
TEWL	transepidermal water loss
TF	transcription factor
TGF-α	transforming growth factor α
TGM	transglutaminase
TJ	tight junction
TJP1 (ZO-1)	tight junction protein 1 (zona occluden 1)
TUBA1C	tubulin, alpha 1c
VLCFA	very long chain fatty acid

INTRODUCTION AND BACKGROUND

Origins of the Skin Epithelium

In mammals, the skin is the largest organ in the integumentary system, consisting of 3 different layers, the epidermis, dermis, and hypodermis (subcutaneous tissue) (Figure 1). The epidermis originates from the outermost layer, or ectoderm, of a 3-layered embryo. When cells in this layer respond to Wnt signaling, they are fated to develop into the epidermis. Wnt signaling blocks the ability of ectoderm to respond to FGF signaling, causing expression of bone morphogenetic proteins (BMPs) which signal the epidermis, instead of the nervous systems to develop (Stern, 2005). The ectodermal cells produce predominantly mucoproteins (in fish) or keratinaceous proteins in land dwelling animals (Flaxman and Maderson, 1976). In higher vertebrates, there are two distinct categories of keratinaceous proteins, the hair or alpha-type and the feather or beta-type (Baden and Maderson, 1970). In the vertebrate epidermis, the alpha-keratin is mostly synthesized (Flaxman, 1972).

The dermis is mesenchymal tissue that originates from the middle layer, or mesoderm of the embryo (Flaxman and Maderson, 1976). The mesenchymal cells produce dermal fibroblasts which secrete the precursors of self-assembling, extracellular collagen, elastin, glycoproteins, and glucosaminoglycans (Flaxman and Maderson, 1976). These cells also give rise to dermal blood vessels and fat cells. Mesenchymal cell fate is specified by Wnt signaling (Atit et al., 2006). Interaction between the mesenchyme and ectoderm is essential throughout the entire life of the organism. This interaction induces the formation of hair placodes which respond to signals produced by FGFs and BMP-inhibitory factors to determine their position and density (Fuchs, 2007). In parallel,

epidermal cells are also directed by the ectodermal Wnt signals to grow downward to form the hair bud or placode (Fuchs, 2007). The formation of the hair follicle may also be controlled by epidermal growth factor receptor (EGFR) signaling. Studies in chicks indicate that elevated levels of EGF increases the proliferation of interbud epidermal cells, whereas inhibition of EGFR signaling increases the acquisition of a feather bud fate (Atit et al., 2003). In mammalian skin, EGFR signaling is associated with enhanced epidermal proliferation and hair loss (Blanpain and Fuchs, 2006).

At the end of embryonic development, the interfollicular epidermis reaches maturity and is composed of multiple layers that form the outermost structure of the skin. The epidermis undergoes a process called homeostasis in which dividing cells in the innermost layer continually move outwards to replace terminally differentiated cells being sloughed off from the skin surface. Structure and function of the epidermis are discussed in the next sections.

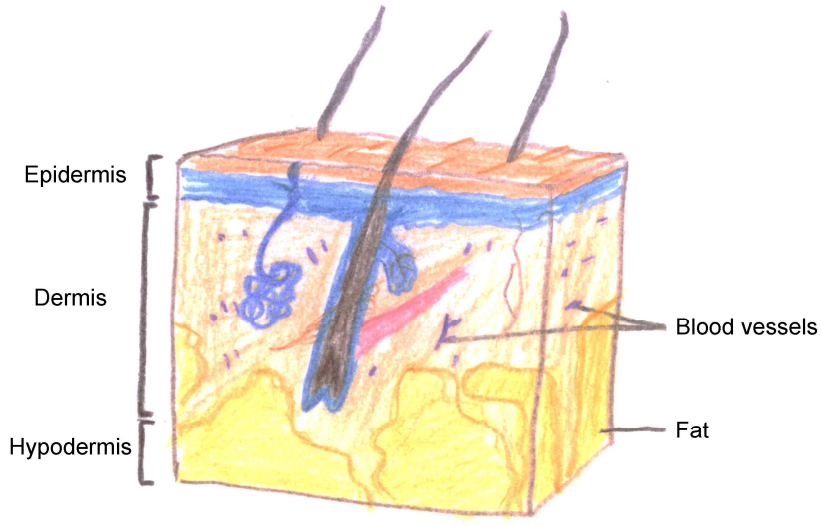

Figure 1. **Normal skin**
Skin consists of 3 major layers: epidermis, dermis, and hypodermis. The epidermis is a stratified epithelium (Figure 2). The dermis is connective tissue with hair follicle, blood vessels, and sebaceous glands embedded in it. The hypodermis consists mostly of adipocytes. Adapted from http://blog.celluliterxworks.com/blog/page/5/.

Structure and Function of the Epidermis

The epidermis is the outer layer of the skin. It consists of 4 principal layers, the stratum corneum (SC), the stratum granulosum (SG), the stratum spinosum (SP), and the stratum basale (SB) (Figure 2). Together, these layers form a barrier which is essential for life. The barrier prevents the body from dehydration in a dry environment and reduces hydration in a humid environment. It blocks the penetration of microorganisms and destructive chemicals. It protects the body from harmful ultraviolet radiation. The epidermis is capable of self-repair and has the mechanical strength to withstand damage. These functions of the skin barrier are accomplished by epidermal cells, the keratinocytes. The keratinocytes are the major cell population in the epidermis. They play a critical role in providing the properties of the epidermal barrier.

Keratinocytes

The structure of the keratinocyte depends on its position within the epidermis and its state of differentiation. In all keratinocytes the primary elements of the cytoskeleton are the intermediate filaments. These intermediate filaments are made of keratin proteins, which are bundled and span throughout the cytoplasm from the nuclear envelope to the desmosomes (Leigh et al., 1994). Keratins are alpha-helical molecules and can be divided into two subfamilies: Type I (acidic keratins; pI < 5.5) and Type II (basic keratins; pI > 6) (Eichner et al., 1984). An acidic and a basic keratin are paired to form heteropolymers, which are assembled into filaments (Coulombe and Fuchs, 1990). The expression of keratins changes during epidermal differentiation. For example, early differentiation markers such as *KRT1* and *KRT10* start to replace *KRT5* and *KRT14* as the cells migrate to the stratum spinosum from the stratum basale.

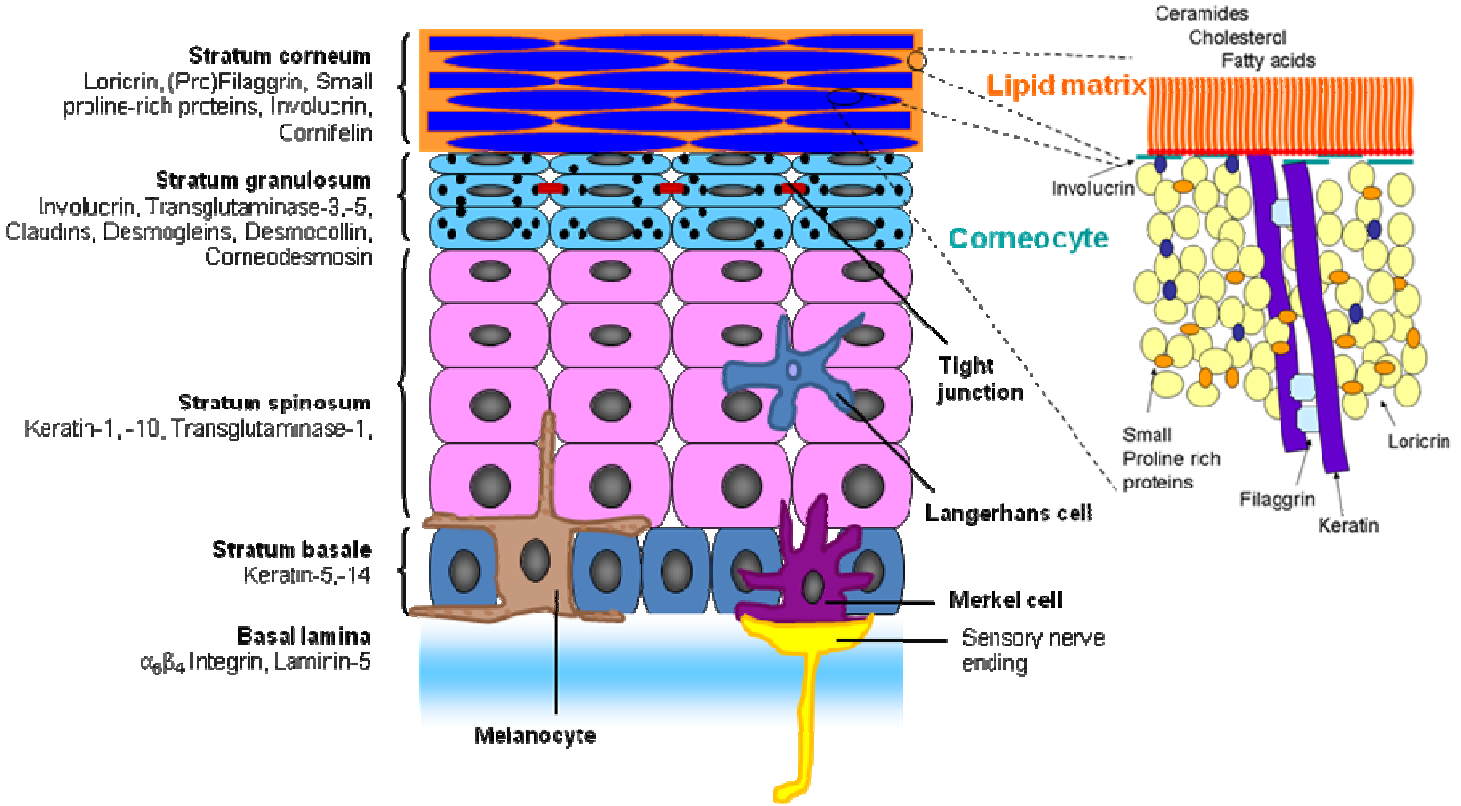

Figure 2. The Four Principal Layers of the Epidermis

The epidermis is a stratified keratinized squamous epithelium consisting of four different layers: stratum basale, stratum spinosum, stratum granulosum, and stratum corneum. The major cell type in the epidermis is the keratinocyte, which undergoes terminal differentiation to form the corneocyte. Other cell types include melanocytes (UV protection), Langerhans cells (immunity), and Merkel cells (touch). The locations of certain markers of keratinocytes differentiation such as KRT5, KRT14, KRT1, KRT10, IVL, FLG, LOR, and TGM1 are shown. Adapted from (Candi et al., 2005; Garrett et al., 2002).

Other cytoskeleton elements include microfilaments and microtubules, which contribute to the movement of cells in culture and in tissue. These structures enhance the communication within the cell and between the cell and its environment (Leigh et al., 1994). It has been shown that there is a physical association between intermediate filaments and microfilaments in mouse epidermal keratinocytes before and after raising the Ca^{2+} level in culture media (Green et al., 1987). During mitosis and keratinocyte differentiation, the pattern of organization of the intermediate filaments, microfilaments, and microtubules cytoskeleton coordinately change (Lewis et al., 1987; Zamansky et al., 1991).

A second feature common to keratinocytes of all layers, except the stratum corneum (the outermost layer), are desmosomes and focal junctions between adjacent keratinocytes. These structures attach keratin filaments within the cell and include intracellular and extracellular components that function in cell-to-cell adhesion (Steinberg et al., 1987). Keratinocytes adhere at desmosomes through interaction of transmembrane glycoproteins tha belong to the cadherin families known as desmogleins and desmocollins (Figure 3A). Desmosomes have mirror image plaques that sandwich a membrane core region. The plaques are present beneath the plasma membrane of each keratinocytes and associate with keratin filaments via plaque proteins such as plakoglobin and desmoplakin (Figure 3A) (Green and Jones, 1996). The intermediate filament anchorage of the plaques creates a transcellular network that is thought to resist forces of mechanical stress (Green and Jones, 1996). Keratinocytes in cultures form focal contacts with their substrate and form adherens and gap junctions with each other in cultures and in tissue (Burridge et al., 1988).

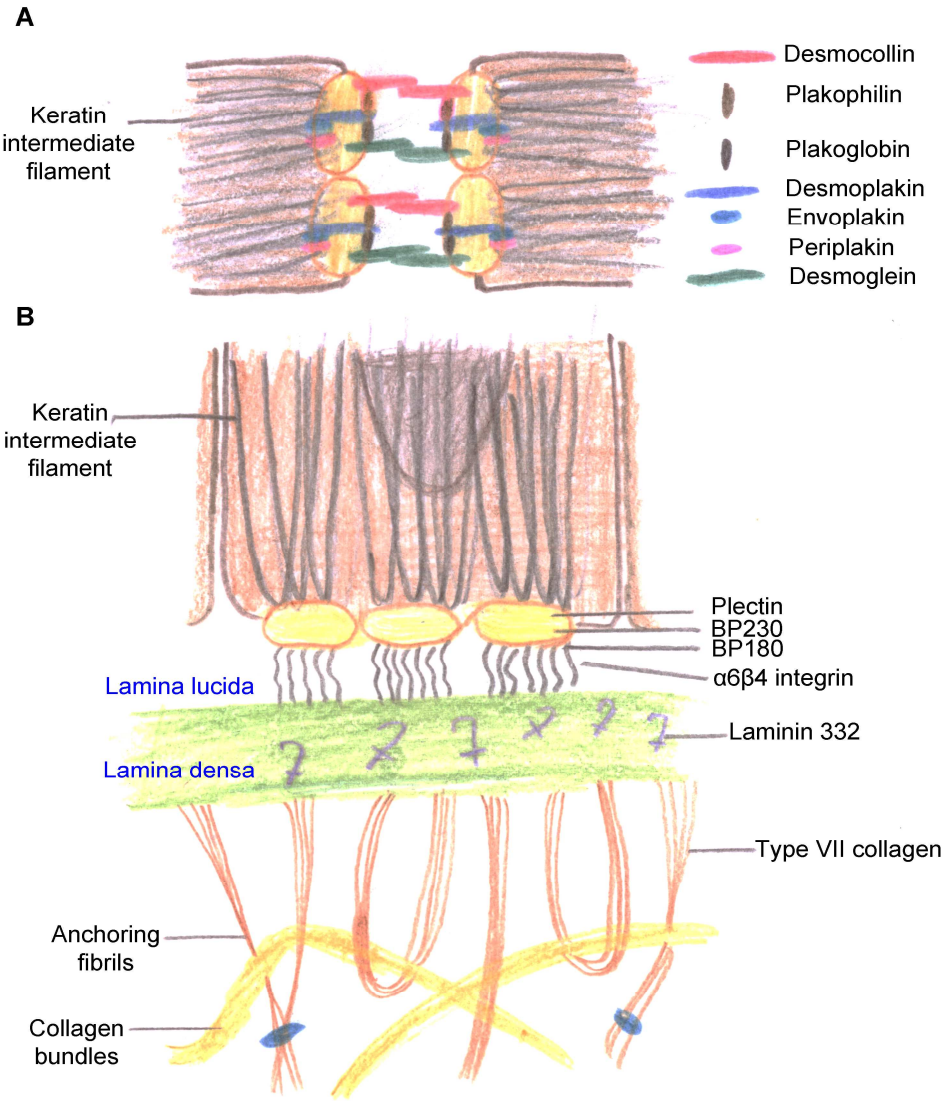

Figure 3. Desmosomes and Hemidesomsomes Structures of a Basal Keratinocyte

(A) Desmosomes. Desmosomes are primary components that maintain the integrity of epidermal cell cohesion. The intracellular desmosomal plaque proteins include desmoplakin, plakoglobin, plakophilin, envoplakin, and periplakin, which interact with intercellular plaques to connect transmembrane adhesion molecules such as the desmogleins and desmocollins with keratins of the cytoskeleton.

(B) Hemidesmosomes are components of the basement membrane. Hemidesmosomes connect the basal keratinocytes to the basement membrane. The intracellular hemidesmosomal proteins include BP230 and plectin that are linked to keratins and interact with the cytoplasmic domains of BP180 and α6β4 integrin, which in turn interact with laminin 332 via their ectodomains (Hertl et al., 2006).

Layers of the Epidermis

Stratum Basale

Cells in the stratum basale have a columnar shape and attach to the basement membrane (Figure 2). The association between the basal cells and the basement membrane is very important for the physical and mechanical integration of the epidermis as well as the regulatory signal to restrain or differentiate (Watt et al., 1988). The proliferation of keratinocytes is only found in the stratum basale. This basal layer contains epidermal stem cells that continuously provide new cells to repopulate the epidermis (Lavker et al., 1993). Besides stem cells, there are transit amplifying cells and postmitotic cells that are ready to move into the suprabasal layers. Basal keratinocytes also contain melanosomes, the pigment granules that provide tissues with color and photoprotection. These melanosomes produce, store, and transport melanin pigments. They are synthesized in melanocytes (Wasmeier et al., 2008) (Figure 2) and phagocytized into vacuoles either in clusters (caucasian skin) or individually (black skin). Most of these vacuoles or melanosome complexes are degraded within the spinous layer (Leigh et al., 1994).

The mechanical strength of basal cells is provided by the structure, composition, organization and stability of the keratin filament cytoskeleton even though keratin comprises only 30% of the protein present in the basal cells (Leigh et al., 1994). The cytoskeleton of the basal layer contains KRT5, KRT14, and small amounts of KRT15, which anchor the epidermis firmly to its substratum (Porter and Lane, 2003). The keratin intermediate filaments are grouped in bundles surrounding the nucleus, but do not attach to it (Figure 3). Mutations in basal cells keratin polypeptides and abnormaltities in

keratin filament assembly are seen in disorders such as epidermolysis bullosa simplex (EBS) (Bonifas et al., 1991; Coulombe et al., 1991).

The interface between the basal keratinocyte and the dermis is a complex structure. The basement membrane is joined with the plasma membrane of the basal cells by hemidesmosomes (Figure 3B). The hemidesmosome and the desmosome exhibit similar structural characteristics, but each also has unique morphologic and compositional features (Green and Jones, 1996). Both junctions are composed of a tripartite electron-dense plaque with cytoplasmic and membrane-related domains. The proteins of the hemidesmosomal plaque are well characterized. The primary protein of the hemidesmosomal plaque is the bullous pemphigoid (BP) antigen (BP230) (Tanaka et al., 1991). BP230 proteins are found in the regions of hemidesmosome plaque to which keratin intermediate filaments attach (Jones et al., 1994). BP230 has some structural and amino acid homology with one of the desmosomal plaque proteins (Tanaka et al., 1991). The membrane-bound molecules of hemidesmosomes include integrins, which are heterodimeric matrix receptors. Besides linking the extracellular matrix and the cytoskeleton of the cells, these receptors also act to transduce signals (Giancotti et al., 1992). There are α and β subunits in each receptor. The α6 subunit can bind either the β1 or β4, but binds preferentially with the β4 integrin subunit (Giancotti et al., 1992). This β4 integrin is unique among the β integrins with its extended carboxy-terminal cytoplasmic tail of 1000 amino acids (Hogervorst et al., 1990). This tail is physically associated with one or more protein kinases. When the extracellular ligands interact with the α6β4 receptors, β4 becomes phosphorylated on tyrosine (Mainiero et al., 1995). It has been shown that a tyrosine phosphorylation site in the cytoplasmic domain of β4

triggers binding of the signaling adaptor Shc (Src homologous collagen protein), which upon phosphorylation recruits the adaptor Grb2 (growth factor receptor binding protein 2), linking the integrin to the Ras signaling pathway (Mainiero et al., 1995). Another membrane molecule is the BP180 protein, also known as type XVII collagen (Giudice et al., 1991; Hopkinson et al., 1992) (Figure 3B). This protein has been shown to be missing from the skin of individuals carrying generalized atrophic benign epidermolysis bullosa (Jonkman et al., 1995; McGrath et al., 1995). The lack of BP180 proteins in the skin weakens the attachment of the epidermal cells to the basement membrane, leading to blistering. In autoimmune diseases such as bullous pemphigoid and gestationis, BP180 is a target for pathogenic antibodies (Giudice et al., 1993). The matrix molecules include laminin 332 (previously known as laminin 5), which is composed of three subunits termed α3, β3, and γ2 (Tryggvason, 1993). They are concentrated in the basement membrane zone immediately underlying the hemidesmosome (Jones et al., 1994). Laminin 332 can interact with several cell-surface receptors including α6β4, α3β1, EGFR and syndecan 1 (Marinkovich, 2007). This protein has been shown to be deficient in the skin of patients with a genetic disease called junctional epidermolysis bullosa (Kivirikko et al., 1995; Pulkkinen et al., 1994).

In addition to structural proteins, there are other proteins with regulatory function such as basonuclin (BNC1), a zinc-finger protein mainly expressed in basal keratinocytes and the outer root sheath of hair follicles. Immunocytological experiments using Ki-67, a common marker of proliferating cells, and bromodeoxyuridine (BrdU) incorporation into DNA, have demonstrated that BNC1 is present in cells that are able to enter the growth cycle, but disappears in terminally differentiated cells that have irreversibly withdrawn

from the cell cycle (Tseng and Green, 1994). The ability of BNC1 to associate with ribosomal RNA genes on human keratinocyte mitotic chromosomes, as well as its own gene promoter, has identified BNC1 as a transcription factor with the unsual ability to interact with promoters of both RNA polymerases I and II (Tian et al., 2001; Tseng et al., 1999). This notion is supported by a study in which mouse oocytes with *Bnc1* knock-down exhibits a large perturbed number of RNA polymerase II transcripts (Ma et al., 2006).

Stratum Spinosum

As cells leave the basal layer and move to the spinosum layer, their shape changes from columnar to polygonal (Figure 2). Spinous cells are joined by 'spines' that extend from the cell surface (Leigh et al., 1994). The spines are formed by bundles of keratin filaments that insert into the desmosomal plaques of opposing cells. As keratinocytes migrate to the stratum spinosum from the stratum basale, newly synthesized keratin polypeptides are added, thus increasing the quantity and diversity of keratin species. During this journey, many genes have been sequentially turned on and off to support the differentiation of keratinocytes. For example, early differentiation markers such as *KRT1* and *KRT10* start to replace *KRT5* and *KRT14*. Other important genes are also activated in the stratum spinosum such as involucrin (*IVL*), an important component of the cornified envelope, and transglutaminase-1 (*TGM1*), the enzyme that cross-links IVL and other substrates during the synthesis of the cornified envelope (Holbrook and Wolff, 1987).

The first appearance of lamellar granules, also known as lamellar bodies, is seen in the uppermost spinous cells. These structures have been observed by electron microscopy as round and oblong membrane-bound organelles containing disk-like lipid

bilayers (Odland and Holbrook, 1981). They contain glucosylceramides, and other lipids, various hydrolytic enzymes (Madison et al., 1998), and other proteins including corneodesmosin (CDSN) (Serre et al., 1991). Previously, lamellar granules were thought of as discrete granules produced from the Golgi apparatus, which then migrated to the cell surface, and fused with the plasma membrane. However, this view was recently challenged by Norlen (Norlen, 2001). Norlen proposed the "membrane folding" model in which the *trans*-Golgi network and lamellar bodies of the uppermost SG cells, as well as the multilamellar lipid matrix of the intercellular space at the border zone between the SG and SC, are part of one continuous membrane structure (Norlen, 2001). In 2003, Norlen and his colleagues used a cryotransmission electron microscopy technique and provided evidence to support this hypothesis (Norlen et al., 2003). The activities of the lamellar granules are very important for the permeability barrier formation and will be discussed in more detail in the section describing the granular cell layer.

Stratum Granulosum

The stratum granulosum is typically comprised of two to three layers of granular keratinocytes (Figure 2). In the palm and sole, there are more granular layers. Hallmarks of this layer are keratohyalin granules (KHG), which are composed of the intermediate filament-associated protein, profilaggin (proFLG). ProFLG is synthesized in the granular cells and deposited to the intersections of keratin filament bundles (Leigh et al., 1994). KHG become larger as the cells move upward. In some of the uppermost granular cells, the filaggrin subunits of proFLG may start to assist the aggregation and alignment of the keratin filaments (Leigh et al., 1994). The quantity and composition of the keratins in the granular cells determine the structure of the KHG. Reduction in *KRT1* and *KRT10*

expression is observed in disorders of keratinization where KHG are often globular in shape and may have different substructures (Holbrook and Wolff, 1987). In a group of ichthyosis vulgaris patients, KHG and proFLG are reduced or absent (Sybert et al., 1985).

Another characteristic feature of the SG is the presence of the lamellar granules (LG). LG are branched tubular structures continuous with the *trans*-Golgi network (Norlen et al., 2003). LG contain many cargoes including glucosylceramides (GlcCer), sphingomyelin (SM) and other lipids (Madison et al., 1998), various hydrolytic enzymes, such as proteases, acid phosphatases, glucocidase, and lipases, and other proteins including cathepsin D (CTSD), CDSN, kallikerin (KLK)7, and KLK8 (Ishida-Yamamoto et al., 2004). These cargoes are synthesized at different times during keratinocyte differentiation and are transported independently through the trans-Golgi network and LG (Ishida-Yamamoto et al., 2004). KLK7, KLK8, CDSN, and GlcCer are individually transported as aggregates and directly form bulbous protrusions of the trans-Golgi network. CTSD proteins are packed into small vesicles, some of which may join with LG from the trans-Golgi network. As the LG move toward the apical surface, they fuse with the plasma membrane of the uppermost granular cells and extrude their contents into the intercellular space (Ishida-Yamamoto et al., 2004). The major lipid components of the intercellular lipid matrix are ceramides (Cer), cholesterol (CHOL), and free fatty acid (FFA). The lower pH at the SG/SC interface activates hydrolytic enzymes such as β-glucocerebrosidase (GBA) and acid sphingomyelinase to cleave glucose and choline phosphate from GlcCer and SM in order to form Cers. Sterol sulfatase and phospholipase(s) are activated to help form CHOL and FFA from

cholesterol sulfate and glycerophospholipids, respectively. GlcCer and SM are modified forms of ceramide products synthesized from *de novo* and salvage pathways, which occur as a series of steps catalyzed by enzymes located in the endoplasmic reticulum (ER) (Uchida and Holleran, 2008). The initial condensation step, catalyzed by serine palmitoyltransferase (SPTLC), is the rate limiting step of the *de novo* synthesis, forming 3-ketodihydro-sphingosine (Hornemann et al., 2009). This product is reduced to sphinganine, which is then acylated by ceramide synthases (LASS) to form dihydroceramide. In the epidermis, LASS3 is the most relevant isozyme, due to its fatty acyl-CoA chain length specificity (Stiban et al., 2010). Dihydroceramide is then desaturated by DEGS1/2 or hydroxylated by DEGS2 to form Cer and phytoceramide, respectively. In addition to *de novo* synthesis, salvage synthesis also occurs to form Cer5 (AS) and Cer2 (NS) via acylation of sphingosine by LASS. Ceramides can also be synthesized from FFA that are acylated to sphingoid bases. FFA synthesis is first catalyzed by fatty acid synthase (FASN) in the cytosol. Fatty acid chain elongation occurs in the ER using metabolically-linked enzymes ELOVL 1-7 (condensation), hydroxysteroid (17-beta) dehydrogenase (HSD17B, reduction), protein tyrosine phosphatase-like, member B (PTPLB, dehydration), trans-2,3-enoyl-CoA reductase (TECR, reduction) (Jakobsson et al., 2006). The initial step of elongation is the rate-limiting step (Jump, 2009). Among the ELOVL family of enzymes, ELOVL3 and ELOVL6 catalyze the majority of the fatty acids present in the epidermal barrier. ELOVL1 and ELOVL 4 catalyze the elongation of the VLCFA present in the ceramides of the epidermal barrier (Ohno et al., 2010). In addition, FFA are hydroxylated to form 2-OH FFA by FA2H. Ceramides containing 2-OH FFA include Cer7 (AH), Cer6 (AP)

and Cer5 (AS), and are critical for proper formation of the epidermal barrier (Uchida et al., 2007).

Cells in the granular layer are interconnected by a junctional complex including tight junctions (TJ), desmosomes, adheren, and gap junctions. TJ are the most apical components in the junctional complex. They seal neighbouring cells together to prevent diffusion of solutes through the intercellular spaces. Components of TJ, *e.g.* claudin (CLDN)1, CLDN4, occludin (OCLN), and tight junction protein 1 (TJP1) are expressed throughout the epidermis. However, the formation of TJ is only found in the apical-most layer of the SG (Furuse et al., 2002). Defects in TJ are observed in patients with AD (De Benedetto et al.). Mice lacking *Clnd1* die within 1 day of birth due to tremendous TEWL (Furuse et al., 2002), marking CLDN1 a key TJ protein. A mutation and reduction in human CLDN1 has been reported in patients suffered from neonatal ichthyosis-sclerosing cholangitis and from AD, respectively (De Benedetto et al.; Hadj-Rabia et al., 2004). These studies indicate that tight junctions play a critical role in the permeability barrier function of mammalian skin.

Stratum Corneum

The stratum corneum is the outermost layer of the epidermis (Figure 2), which has been described as the series of bricks (corneocytes) glued by mortar (lipids). Corneocytes are terminally differentiated, cornified, flattened, hexagonal-shaped cells that undergo a specialized type of cell death leading to loss of the cell nucleus. They are filled with water-retaining keratin proteins surrounded by cornified envelope (CE) and lipids. The insoluble CE structures are formed beneath the plasma membrane of the corneocytes and covalently bound to ceramide lipids in order to provide an effective physical and water

barrier function in the skin. CE formation requires deposition of filaggrin (FLG), which aggregates the keratin filaments into tight bundles, causing the flattened shape of corneocytes. This formation creates a template or scaffold for the subsequent maturation steps of CE assembly. Besides FLG, a series of other structural proteins including IVL, LOR, trichohyalin (THH), and small proline-rich proteins (SPRR) are synthesized and then catalytically cross-linked by several TGMs (Candi et al., 2005). Corneocytes are linked one to another by corneodesmosomes, modified desmosomal structures that are composed mainly of desmoglein-1 (DSG1), desmocolin-1 (DSC1), and CDSN (Candi et al., 2005). In normal epidermis, the corneocytes are continually shed by the physiological process called desquamation to balance the proliferation rate in the basal layer, thereby maintaining epidermal homeostasis. Desquamation requires corneodesmosome degradation by several proteolytic enzymes such as SC chymotryptic enzyme (SCCE) and the SC tryptic enzyme (SCTE) (Brattsand and Egelrud, 1999; Ekholm et al., 2000).

Corneocytes have the biggest dimensions of all the keratinocytes. One corneocyte is equivalent in area to approximately 25 basal cells (Leigh et al., 1994). Cell size and the number of corneocyte layers vary, depending on the region of the skin, sex, and age of the individual (Plewig and Marples, 1970). For example, there are hundreds more of cell layers in the palms and soles than in other skin parts of the body (Leigh et al., 1994). The SC functions as a barrier that protects our body from excessive water loss. The water impermeability of this layer is 1000 times higher than that of other membranes of living organisms (Potts and Francoeur, 1991).

Nonkeratinocyte Cells of the Epidermis

In addition to keratinocytes, there are melanocytes, Langerhans, and Merkel cells (Figure 2). Melanocytes migrate into the epidermis from the neural crest early in the embryonic development (Holbrook, 1989; Rawles, 1947). Langerhans cells originate from the bone marrow (Stingl et al., 1980). The origin of the Merkel cells has been controversial. It has been thought that these cells arise from either the skin or neural crest lineages, but no definite proof was given. Recently, Morrisson and colleagues have shown that Merkel cells originate from an epidermal lineage, based on mice with a conditional knockout of *Atoh1* (atonal homolog 1), a transcription factor essential for the production of Merkel cells (Maricich et al., 2009; Morrison et al., 2009). Knocking out *Atoh1* in the neural crest did not affect the Merkel cell population, whereas loss of *Atoh1* expression in the skin deleted all Merkel cells (Morrison et al., 2009).

Melanocytes and Merkel cells are found in the stratum basale, while Langerhans cells often appear in the suprabasal layer (Figure 2). Melanocytes are uniformly distributed within the basal layer in a ratio of 1 melanocyte to approximately 10 keratinocytes. However, each melanocyte, via its dendrites, supplies melanin to about 36 nearby keratinocytes (Jimbow et al., 1976). Human skin and hair color is determined by the amount of melanin (eumelanin and pheomelanin, respectively) produced by the melanosome, a unique intracytoplasmic organelles of melanocytes (Boissy and Nordlund, 1996).

Langerhans cells are dendritic cells and are typically found in the spinous layer of the epidermis. They have dark nuclei and pale or clear cytoplasm. They contain large granules called Birbeck granules, which make them distinguishable from other cell types

(Birbeck, 1962). Langerhans cells are considered to be the primary antigen-presenting cell of the skin. They contain antigen markers, human leukocyte antigen (HLA)-D, T4 antigen, and some of the CD1 antigens that are not expressed by other epidermal cells (Ray and Schmitt, 1988). Compared to keratinocytes, Langerhans cells have been shown to be sensitive to the effect of ultraviolet A and B radiation. A single doses of 60 mJ/cm^2 of UVB spectrum almost completely eliminated all Langerhans cell membrane markers (Aberer et al., 1981). Langerhan cells play an important role in immunogenic and tolerogenic aspects of epidermal cells.

Merkel cells are neuroendocrine cells that are intimately associated with a nerve terminal. They contain small membrane-bound dense-core granules that resemble neuroendocrine cells. The number of Merkel cells in the skin is lower than melanocytes and Langerhans cells. Merkel cells are present at higher density in regions of high touch sensitivity such as the palm, buccal mucosa and lips, face and foot (Lacour et al., 1991; Moll et al., 1990).

Factors that Regulate Keratinocyte Growth and Differentiation

Calcium Ions (Ca^{2+})

In vitro, the addition of extracellular Ca^{2+} mimics the gradient existing *in vivo* where calcium is low in basal and significantly increased in the mid to upper granular toward cornified layers (Forslind et al., 1997; Menon et al., 1992). With concentrations of extracellular Ca^{2+} between 1.2 mM to 2 mM, primary human keratinocytes can establish close intercellular contacts, stratify, cornify, and express biochemical markers of differentiation such as *KRT1, KRT10*, *IVL, LO*R, *FLG*, and *TGM1*, resembling those that occur *in vivo* (Dotto, 1999). After 24 hours in high calcium, more than 95% of

keratinocytes are found to be arrested in G1 phase of the cell cycle (Missero et al., 1996), whereas more than 50% of the attached cells are reversibly arrested when switched back to low calcium containing medium (Topley et al., 1999). Thus, increased extracellular calcium may serve as a primary trigger for keratinocyte differentiation both *in vitro* and *in vivo*.

Increasing extracellular calcium to the same level that leads to increased differentiation has been shown to increase intracellular calcium (Hennings et al., 1989; Yuspa et al., 1989). This increase in intracellular Ca^{2+} is thought to happen in two ways. Firstly, exogeneous calcium binds the calcium receptor and stimulates non-receptor tyrosine kinases, fyn and src, which activates phosphatidylinositol-3-kinase (PI3K) via phosphorylation of the PI3K regulatory subunit, p85α, leading to phosphotidylinositol 3,4,5-triphosphate (PIP3) formation. PIP3 then binds to and activate phospholipases C (PLC)γ1 (Xie et al., 2005), which increases diacylglycerol (DAG), an endogenous protein kinase C (PKC) activator (Jaken and Yuspa, 1988), and inositol triphosphate (IP3) production. IP3 then binds to IP3 receptors in the Golgi and endoplasmic reticulum (ER), releasing intracellular Ca^{2+} and hence triggering keratinocyte differentiation (Bikle et al., 1996; Xie et al., 2005). Secondly, the increase in extracellular calcium results in calcium influx through calcium-gated chloride channels, causing Cl^- to exit the cells, which results in a depolarization of the plasma membrane. This depolarization leads to the opening of voltage gated calcium channels, allowing influx of calcium from the external environment (Reiss et al., 1991). A rising level of intracellular calcium causes genomic and nongenomic effects, such as a redistribution of desmoplakin to the membrane (Watt

et al., 1984), increases in IVL, LOR, and TGM1 protein within hours (Rice and Green, 1979), and increased cornification at 1-2 days (Pillai et al., 1990).

Confluent Cell Density

Confluent cell density of keratinocytes in culture has been shown to strongly induce commitment to terminal differentiation (Lee et al., 1998; Poumay and Pittelkow, 1995). In normal human epidermis, keratinocytes undergo constant renewal, during which stem cells in the basal layer of the epidermis replace keratinocytes that are constantly lost or shred off during terminal differentiation. The differentiation process is initiated when stem cells are replaced by transit amplifying cells (Watt et al., 2006), which are in equilibrium with cells that have withdrawn reversibly and irreversibly from the cell cycle to commit to terminal differentiation (Okuyama et al., 2004). At cell confluence, a large majority of proliferative keratinocytes rapidly undergo irreversible growth arrest, as demonstrated by a dramatic loss of keratinocyte clonogenicity (Poumay and Pittelkow, 1995). Cell density induces expression of multiple keratinocyte differentiation markers such as *SPRR1*, *KRT1*, *IVL*, *LOR*, *FLG*, and *TGM1*, independent of extracellular Ca^{2+} concentration of the medium (Lee et al., 1998; Poumay and Pittelkow, 1995). In NHEK cultures, density-mediated keratinocyte differentiation is associated with activation of PKCα, as demonstrated by its translocation to the particulate fraction, and the blockage of keratinocyte differentiation markers by the inhibition of this PKC isoform activation (Lee et al., 1998).

Kolly and colleagues have demonstrated that confluency plays an key role in driving proliferating keratinocytes into terminal differentiation by studying the effect of confluency at low (up to 0.9 mM) and high (1.2 and 1.8 mM) calcium (Kolly et al., 2005).

When keratinocytes reach confluency, they are contact inhibited. As cells are contact inhibited, Notch1 is activated by the binding of Delta1 or Jagged, Notch1 ligands expressed on neighboring cells (Kolly et al., 2005; Mumm and Kopan, 2000). Notch1 is a key determinant of keratinocyte growth arrest and differentiation. Activation of Notch1 causes growth suppression by inducing $p21^{WAF1/Cip1}$ expression (Rangarajan et al., 2001), one of the earliest cell cycle regulatory events essential for keratinocyte terminal differentiation (Missero et al., 1996). At confluence, c-Myc is also inhibited (Kolly et al., 2005). The protooncogene c-Myc plays a key role in promoting the exit of stem cells into the transit amplifying compartment (Arnold and Watt, 2001). Low levels of c-Myc expression are required for transit amplifying cells to commit to terminal differentiation (Waikel et al., 1999). These results indicate that confluency, independent of calcium concentration, is a key regulator of keratinocyte proliferation and terminal differentiation.

Cell-cell Adhesions

Control of cell-cell contact has been shown to play an inherent role in the regulation of skin cell differentiation (Charest et al., 2009; Hines et al., 1999; Owens et al., 2000). With the formation of cell-cell contacts, the cells begin to stratify to generate multiple epidermal layers, with the basal-like cells remaining attached to the dermis while the more differentiated cells forming the suprabasal layers (Hennings et al., 1989). Differentiating keratinocytes develop cell-cell junctions through various transmembrane proteins, such as E-cadherin and P-cadherin. *In vivo*, loss of E-cadherin in keratinocytes leads to down-regulation of markers of differentiation (Young et al., 2003b). *In vitro* human keratinocytes, blocking E-cadherin activity with antibodies leads to abnormal stratification and delayed localization of junction proteins (Wheelock and Jensen, 1992).

Blocking the function of both E-cadherin and P-cadherin with antibodies prevents the induction of differentiation markers such as TGM1, LOR, and proFLG proteins in vitro; however inhibition of only E-cadherin increases protein levels of LOR and proFLG (Hines et al., 1999), indicating that cell-cell contact affects differentiation through multiple junction proteins. Charest and colleagues demonstrated that cadherin-mediated cell-cell contact regulates keratinocyte differentiation by using a micropatterned surfaces *in vitro* cell model, which provides a controlled and simple strategy to inhibit or permit cell-cell contact between isolated pairs of cells while controlling cell spreading, shape, and density (Charest et al., 2009). Micropatterned surfaces are microcontact printing of self-assembled monolayers of alkanethiolates on gold. These surfaces are printed with bowtie patterns using a polydimethylsiloxane stamp. Each bowtie has 15 distinct fields, each with several hundred replicates and is coated with the extracellular matrix protein fibronectin (Charest et al., 2009).

Biology and Structure of EGF

EGF was first described by Cohen in the 1960s. The protein was isolated from murine submaxillary glands and caused tooth eruption and premature eyelid opening in new-born mice (Cohen, 1962). It was named epidermal growth factor after the observation that its direct addition to organ cultures of chick embryonic skin led to a significant increase in epidermal cell number and size (Cohen, 1965). It is a small peptide (6045 Da) that produces a variety of biological responses, including promotion of proliferation and differentiation of skin tissue (Cohen and Carpenter, 1975), corneal epithelial tissue (Savage and Cohen, 1973), lung and tracheal epithelia (Catterton et al., 1979); potentiation of 3-methylcholanthrene carcinogenesis (Reynolds et al., 1965);

phosphorylation of nuclear proteins (Huff and Guroff, 1978); formation of fatty liver (Heimberg et al., 1965); and inhibition of gastric acid secretion (Bower et al., 1975). In humans, EGF has been found in many body fluids such as urine, saliva, breast milk, amniotic fluids, and plasma (Carpenter, 1980b). It is a major growth-promoting agent in human milk (Carpenter, 1980a). The human equivalent of mouse EGF is urogastrone, a hormone that inhibits gastric acid secretion (Gregory, 1975). In cultured cells and tissues, exogenous EGF has multiple functions. Some of the functions include activation of glycolysis (Diamond et al., 1978; Schneider et al., 1978), phosphorylation of nuclear protein (Huff and Guroff, 1978), stimulation of macromolecular (hyaluronic acid, RNA, protein, DNA) synthesis (Cohen and Stastny, 1968; Hoober and Cohen, 1967; Lembach, 1976), enhanced cell proliferation (Gospodarowicz et al., 1977; Hollenberg, 1975), and alteration of viral growth (Knox et al., 1978).

EGF consists of 53 amino acid residues. In humans, EGF is translated from a gene that is located on chromosome 4. This gene contains 24 exons separated by large non-coding regions (Bell et al., 1986; Savage et al., 1972). The precursor of EGF has 1207 amino acids which are translated from a 4.8 kb mRNA. This mRNA is spliced from an initial transcript of approximately 110 kb (Bell et al., 1986). The EGF precursor contains a hydrophobic domain, assumed to be required for anchoring the protein to the membrane (Rall et al., 1985), and 7 EGF-like domains (Gray et al., 1983) with unknown functions. The tertiary structure of EGF is non-glycosylated and stabilized by three intramolecular disulfide bonds that are the characteristic for EGF and EGF-like growth factors and also required for its biological activity (Savage et al., 1973; Taylor et al., 1972).

Structure and Function of the Human EGF Receptor

EGF exerts its function via EGF receptors (EGFRs). The EGFR (ErbB1 or Her1) is a 170-kDa transmembrane glycoprotein. It belongs to the ErbB family, which contains four related receptor tyrosine kinases: EGFR (ErbB1, Her1), ErbB2 (Her2), ErbB3 (Her3), and ErbB4 (Her4). The EGFR and ErbB2 are the most closely related with 49% identity (64% similarity) and EGFR/ErbB3 being the furthest from one another with 37% identity (53% similarity) (Jorissen et al., 2003).

The extracellular domain (or ectodomain) of the EGFR contains the amino terminus with 62 amino acid residues, two ligand binding (L1 and L2) and two cysteine-rich domains (CR1 and CR2) (Figure 4) (Carpenter and Zendegui, 1986). EGF binds to the L1 and L2 regions (Garrett et al., 2002; Ogiso et al., 2002). The hydrophobic domain of the EGFR was originally identified by hydrophobicity analysis of the EGFR sequence. This region contains 23 amino acid residues that span the membrane (Ullrich et al.,

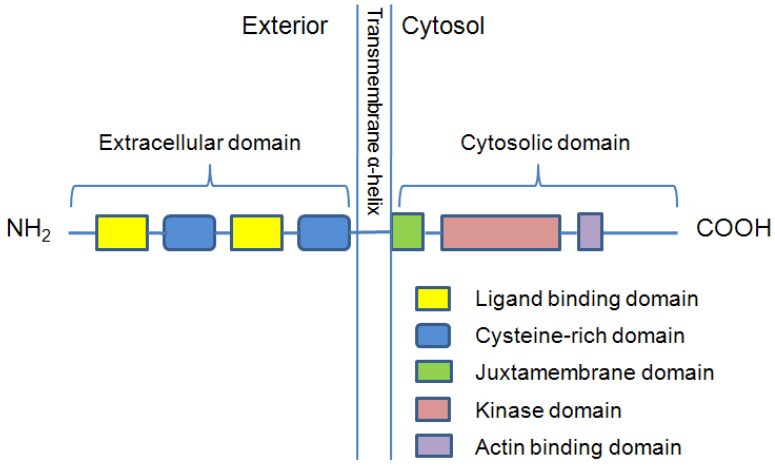

Figure 4. Architecture of the Epidermal Growth Factor Receptor
The extracellular domain contains the two ligand binding (L1 and L2) and two cysteine-rich domains (CR1 and CR2). The cytosolic domain contains juxtamembrane, kinase, and actin binding domains. Adapted from (Carpenter and Zendegui, 1986; Garrett et al., 2002).

1984). However, nuclear magnetic resonance analysis of the EGFR transmembrane peptide and the beginning of the cytoplasmic domain indicated that the transmembrane domain is α- helical and expands to the juxtamembrane domain (Rigby et al., 1998). The juxtamembrane region has multiple regulatory functions including downregulation and internalization of ligand-receptor complexes (Kil and Carlin, 2000), basolateral sorting of the EGFR in polarized cells (He et al., 2002), and direct binding with proteins such as EGFR kinase substrate (EPS8) (Castagnino et al., 1995) and calmodulin (Li and Villalobo, 2002; Martin-Nieto and Villalobo, 1998). Continuing into the cytosol is the highly conserved tyrosine kinase domain of the EGFR. The three-dimensional structure of the EGF kinase domain is similar to other tyrosine kinases. The ATP binding region is located between the N-terminal region and the larger C-terminal region. The carboxy terminus of the EGFR is composed of 542 amino acid residues. The C-terminal domain contains tyrosine residues that can be phosphorylated by the receptor itself (Downward et al., 1984). This autophosphorylation modulates EGFR-mediated signal transduction. Phosphorylation at serine/threonine residues on the kinase domain has been suggested to be important for the downregulation and endocytosis processes of the receptor (Zwang and Yarden, 2006). The C-terminal domain also contains a binding site for actin (den Hartigh et al., 1992) which is believed to play a role in the formation of higher order receptor oligomers and/or receptor clustering after phosphorylation.

Signal Transduction

In the absence of ligand binding, EGFRs exist in monomeric and dimeric forms, but both are inactive (Yu et al., 2002). When ligands such as EGF bind, there is a change in the conformation of the receptor that reorients the intracellular domains to form a

structure having an active kinase (Moriki et al., 2001). This configuration allows the tyrosine kinase domains of the two ligand-bound monomers to transphosphorylate each other. Tyrosine phosphorylation serves as the first and crucial step in the EGFR-mediated signal transduction.

Different ligands and combinations of homo- and heterodimerizations within the EGFR family lead to a diverse set of signaling events. Some basic features of EGFR signal transduction can be summarized as follows. Upon ligand binding, receptor dimerization occurs, leading to autophosphorylation of distinct tyrosine residues. This autophosphorylation creates docking sites for various cytoplasmic adaptor proteins and enzymes with SH2 (Src homology 2) and PTB (phosphotyrosine binding) domains. The SH2 domain binds specifically to the residues located downstream of the EGFR phosphotyrosine (pY) while PTB binds to the residues located upstream of the pY. Docking proteins are important to receptor tyrosine kinases as they recruit signaling molecules to the receptors, regulating variety of cellular responses (Figure 5).

Activation of the Mitogen-activated Protein Kinase (MAPK) Cascade

The MAPK cascade includes four major groups of MAP kinases including extracellular regulated kinases (ERKs) 1 and 2, jun N-terminal kinases (JNKs), p38, and the extracellular signal regulated kinase-5 (ERK5) (Kyriakis and Avruch, 2001). These serine/threonine kinases are evolutionarily conserved in all eukaryotes (Garrington and Johnson, 1999). Signaling through ERK1/2 is the best characterized pathway among other MAPK pathways. The first biochemical event in this cascade is the activation of the proto-oncogene Ras. Ras is activated by a guanine nucleotide exchange factor, Sos. In this cascading event, the adaptor protein Grb2 is a key component of EGFR signaling

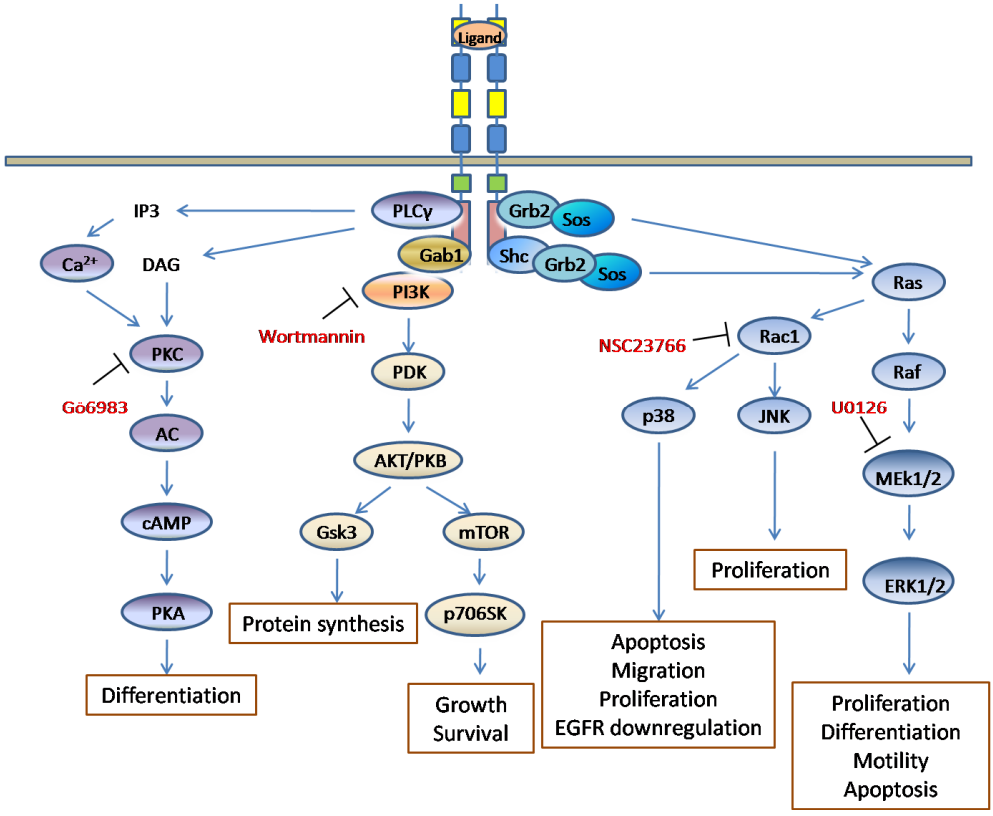

Figure 5. EGFR Signal Transduction
Activation of the EGFR leads to a number of serine/threonine cascades. This includes the MAPK, PI3K, and PLCγ signaling pathways, producing diverse cellular responses such as proliferation, differentiation, motility, and apoptosis. Adapted from (Davis, 2000; Garrington and Johnson, 1999; Johnson and Vaillancourt, 1994; Kyriakis and Avruch, 2001; Prenzel et al., 2001). Arrow indicates activation. Flat head line indicates inhibition. Chemicals known to inhibit PKC, PI3K, P38, and MEK1/2 are Gö6983, wortmannin, SB203580, and U0126, respectively.

to Ras (Lowenstein et al., 1992). Grb2 forms a complex with Sos through its SH3 domain. The Grb2/Sos complex binds to the tyrosine phosphorylated EGFR via the SH2 domain in Grb2, thus recruiting Sos to the plasma membrane where it stimulates the exchange of GDP for GTP for Ras. Ras-GTP binds to and activate the serine/threonine kinase Raf-1 and subsequently the activation of the dual specificity kinase MEK1/2 (Hallberg et al., 1994). This in turn leads to the phosphorylation and activation of ERK1 and ERK2, allowing them to translocate to the nucleus where they catalyze the

phosphorylation of nuclear transcription factors such as Elk1, cFos (Prenzel et al., 2001), cJun, cMyc, and NFκB. Sos can also be recruited to the plasma membrane by binding of Grb2/Sos to Shc, another adaptor protein with SH2 and PTB domains (Margolis, 1999). These signaling cascades regulate diverse cellular responses such as proliferation, differentiation, motility, and apoptosis (Davis, 2000; Johnson and Vaillancourt, 1994).

Signaling through the JNK and p38 stress-activated kinases is less well understood than for ERK1/2. EGF activates JNK via the small GTP-binding proteins Rac1 and Cdc42 (Coso et al., 1995; Minden et al., 1995). The adaptor Crk protein has been shown to be critical for JNK activation following EGF treatment of Crk-transfected COS7 cells (Dolfi et al., 1998). JNK activity is not detected in healthy human epidermis, but is elevated in psoriasis (Takahashi et al., 2002). In cultures of keratinocytes, JNK activation has been shown to be associated with epidermal proliferation (Zhang et al., 2004), while inhibition of JNK promotes differentiation of epidermal keratinocytes (Gazel et al., 2006). The activation of the p38 pathway is required for the migration of cultured human keratinocytes on dermal collagen (Li et al., 2001), and for the downregulation of EGFR, explaining its role in the regulation of EGF-stimulated epithelial wound healing and proliferation (Frey et al., 2006).

Activation of Phosphotidylinositol-3-kinase (PI3K)

EGF activates the lipid kinase PI3K, which consists of a p85 regulatory domain and a p110 catalytic domain. The activation of PI3K by EGF is relatively weak compared to other receptor tyrosine kinases since EGFR does not have a binding sites for the SH2 domain of PI3K (Soltoff et al., 1994). The EGFR activates PI3K via the adaptor protein Gab1. PI3K activation leads to membrane recruitment and activation of the

serine/threonine kinase PDK1. Subsequently, protein kinase B (PKB/Akt) is phosphorylated (Prenzel et al., 2001). These cascading events stimulate a variety of cellular responses. It has been shown that activation of PKB leads to phosphorylation and inactivation of BAD, preventing apoptotic cell death by inhibiting its formation with the apoptotic proteins Bcl-2 and Bcl-xl (Datta et al., 1999). PKB can also inhibit apoptosis by phosphorylating the transcription factor FKHR1, suppressing proapoptotic gene expression (Brunet et al., 1999). PDK1 and PKB have been suggested to play a role in the control of protein synthesis, gluconeogenesis and glycolysis in response to insulin stimulation through phosphorylation of the S6 kinase, glycogen synthase kinase-3 (GSK3) and phosphofructokinase (Toker and Newton, 2000). Activation of PI3K also results in activation of mammalian target of rapamycin (mTOR) and the p70 S6 kinase (S6K). This signaling cascade controls the expression of several genes that involved in cell growth and survival (Heinonen et al., 2008).

Activation of Phospholipase C (PLC)

EGF activates the autophosphorylation of the EGFR, providing the docking site for the SH2 domain of PLCγ (Jorissen et al., 2003). PLCγ is recruited to the plasma membrane and is tyrosine phosphorylated and activated by the EGFR. Activated PLCγ hydrolizes the phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)IP_2) substrate to form two second messengers, 1,2-diacylglycerol (DAG) and inositol 1,3,5-trisphosphate (IP_3). IP3 mediates the release of Ca^{2+} from intracellular stores. Ca^{2+} binds to calmodulin, activating a family of Ca^{2+}/calmodulin-dependent protein kinases. Both Ca^{2+} and DAG activate members of the serine/threonine kinase PKC. Activation of certain PKC isoforms alters the growth and differentiation of human epidermal keratinocytes (Szegedi

et al., 2009). Specifically, PKCα activation is required and sufficient to trigger irreversible growth arrest during human keratinocyte differentiation (Jerome-Morais et al., 2009).

EGFR and Keratinocyte Differentiation

Epidermal development is tightly regulated by cytokines and growth factors (Fuchs and Raghavan, 2002). These molecules provide spatial and temporal signaling that controls keratinocyte proliferation, differentiation, migration, and finally, terminal differentiation and cornification. TGF-α and EGF are related growth factors that activate EGFR to regulate growth and differentiation in epidermal keratinocytes (Schneider et al., 2008). Dysregulation of EGFR signaling results in abnormal "wavy hair" and "curly whiskers" phenotypes of spontaneous loss-of-function mutations in the mouse TGF-α gene (named *waved-1* or *wa1*) or in the EGFR (named *wave-2* or *wa2*), repectively (Mann et al., 1993; Threadgill et al., 1995). Depending on the genetic background of the mice, complete loss of the EGFR can cause death at embryonic or mid-gestation stage, or after a few weeks of postnatal life (Sibilia and Wagner, 1995; Threadgill et al., 1995). Mice lacking ERBB2, ERBB3, or ERBB4 die during embryonic development (Gassmann et al., 1995; Lee et al., 1995; Riethmacher et al., 1997). Abnormally high levels of EGFR activities cause a hairless phenotype and skin cancers (Ferby et al., 2006).

In keratinocyte cultures, EGFR signaling is known to have an inhibitory effect on multiple pro-differentiation signals and down-regulates the expression of both early and late differentiation markers, as well as inhibits the formation of cornified envelope (CE) (Sun and Green, 1976). In keratinocyte cultures containing growing colonies, the percentage of cornified cells is reduced in the presence of EGF compared to that of

untreated cells (Sun and Green, 1976). Keratinocytes undergoing differentiation first enter reversible cell cycle withdrawal and then irreversible cell cycle withdrawal. During this process, many differentiation-specific proteins are expressed (Poumay and Pittelkow, 1995). Early differentiation markers such as KRT1 and KRT10 are induced in cells that undergo differentiation. The induction of these genes is suppressed by the addition of EGF to the culture medium (Poumay and Pittelkow, 1995). A study has shown that the suppression of KRT1 by EGF is due to the repression of Notch1 expression, an important regulator of cell fate decisions in the epidermis (Kolev et al., 2008). EGF also moderately reduces activity of TGM1 and inhibits late markers of differentiation such as FLG (Marchese et al., 1990; Monzon et al., 1996). Organotypic cultures grown at 33ºC in the presence of EGF exhibit no LOR expression and abnormal expression of SPRR2 and SPRR3 (Gibbs et al., 1998). Inhibition of the EGFR with neutralizing antibodies or EGFR tyrosine kinase inhibitors diminishes the effects of EGF and promotes terminal differentiation (Hashimoto, 2000; Jost et al., 2000; Peus et al., 1997; Sutter et al., 2009). These reports convincingly demonstrate an important function of EGFR in the regulation of keratinocyte proliferation and differentiation. However, only two reports have shed new light into the molecular mechanisms by which EGFR signaling determines epidermal keratinocyte cell fate. In the first study, EGFR/ERK signaling was identified as a negative regulator of the expression of Notch 1, whose signaling promotes keratinocyte differentiation by down-regulating the expression of p53. The transcriptional suppression of p53 was shown to be involved by the EGFR effector c-Jun (Kolev et al., 2008). The second study, using an organotypic raft model, identified DSG1 as a suppressor of EGFR signaling. DSG1 is required to suppress the sustained

activation of EGFR/ERK signaling in the granular layer and acts together with other desmosomal proteins such as DSG2 and DSC3 to support a signaling network that balances keratinocyte proliferation and differentiation to maintain epidermal tissue homeostasis (Getsios et al., 2009). These studies have demonstrated that epidermal homeostasis is regulated spatiotemporally, in part, by EGFR signaling.

The EGFR in Dermatological Diseases

Defective epidermal differentiation and disrupted skin barrier are primary features of many human skin diseases such as psoriasis, allergic contact dermatitis, and atopic dermatitis. Common characteristics among these diseases are epidermal hyperplasia and abnormalities in expression of the EGFR and its ligands. In normal skin, the EGFR is expressed throughout the entire epidermis, but is more concentrated at the basal cell layer (Nanney et al., 1984). In psoriasis vulgaris, the level of EGFR expression is increased two- to four-fold in active lesions, and the EGFR are persistently expressed in the stratum spinosum and stratum corneum (Sporn et al., 1987). In addition, EGFR ligands such as TGF-α (Pastore et al., 2005) and amphiregulin (Chokki et al., 2006) are released in the psoriatic lesions, upregulating the expression of interleukin (IL)-8, a cytokine whose induction serves as a secondary amplification mechanism leading to epidermal hyperplasia (Reich et al., 2001). IL-8 can contribute to the activation of the metalloprotease-dependent release of EGFR ligands by acting on its specific G-protein-couple receptor (Tanida et al., 2004). In lesional skin of atopic dermatitis (AD) and allergic contact dermatitis, the expression of the EGFR prominently extends to the suprabasal layers. TGF-α is stained quite faintly in basal keratinocytes of normal skin. On the other hand, TGF-α is detected clearly throughout the basal and suprabasal

keratinocyte layers in patients with psoriasis, AD, and allergic contact dermatitis (Mascia et al., 2003). High levels of EGFR are also present in seborrheic keratoses and acrochodons of patients with dysplastic nevus syndrome who are pregnant or taking sex steroid hormones (Ellis et al., 1990). Seborrheic keratoses and acrochodons (skin tags) are characterized histologically by hyperkeratosis, epidermal acanthosis, and papillomatosis (Lever and Schaumburg-Lever, 1990). This increase is thought to be due to the increased EGF and estrogen levels, as seen in pregnancy (Ellis et al., 1990). EGFR distribution is also affected in the viral diseases of skin. The level of EGFR is decreased and abnormally distributed in verruca vulgaris as well as in molluscum contagiosum, a pox virus (Nanney et al., 1988). Further, elevated mRNA and protein levels of the EGFR are observed in cancers derived from epidermal cells, such as melanomas (de Wit et al., 1992; Derynck et al., 1987). In keratinocyte cancer cell lines and tumors, NOTCH1 gene expression and activity are significantly reduced. EGFR signaling has been identified to play an essential role in the negative regulation of NOTCH1 gene transcription in normal human keratinocytes and cancer cells (Kolev et al., 2008). In cancer cells, inhibition of EGFR signaling induces NOTCH1 gene expression through p53. Suppression of Notch signaling by EGF may lead to apoptosis (Kolev et al., 2008). These findings have emphasized the importance of the EGFR as targets of cancer therapy and skin diseases.

Because the EGFR plays a critical role in cancer progression, wound healing, and skin inflammatory diseases, anti-EGFR therapies have been introduced as treatment options. Currently, anti-EGFR monoclonal antibody (cetuximab) and EGFR tyrosine kinase inhibitors (gefitinib and erlotinib) have been approved for patients with colorectal and non-small-cell lung cancer. However, a common adverse effect of these agents is

pustular or aceneiform eruption which can be severe enough to lead to treatment modification or cessation (Agero et al., 2006; Perez-Soler et al., 2005). It has been suggested by many clinical trials that these skin lesions or rashes are an indicator of effective target inhibition and activity of EGFR-targeted agents (Perez-Soler and Saltz, 2005). The lesions may also be derived from the impairment of many EGFR-dependent homeostatic functions of the skin (Lacouture, 2006). These observations strongly suggest that chronically reduced activity of EGFR in skin may not be an appropriate treatment for inflammatory disorders associated with epithelial hyperproliferation.

Specific Research Objectives

Aim 1: Determine the Role of the EGFR in Epidermal Barrier Function

EGFR signaling is an essential regulator of fundamental functions in mammalian cells including proliferation, migration and survival (Jost et al., 2000; Pastore et al., 2008). Molecular events driven by EGFRs to mediate both proliferation and migration in human epidermal keratinocyte have been extensively studied using both experimental and computational approaches (Citri and Yarden, 2006; Pastore et al., 2008). However, little is known about EGFR-dependent mechanism on keratinocyte differentiation. Using NHEK cultured in serum-free medium to investigate the effect of different pharmacologic and physiologic factors on keratinocyte proliferation and differentiation reveals that proliferation and differentiation are controlled in an integrated manner (Wilke et al., 1988; Wille et al., 1984). Subsequent studies aiming to establish the molecular mechanisms underlying this effect have identified confluent cell density as a primary biological mechanism that regulates keratinocyte commitment to terminal differentiation and differentiated gene expression (Poumay and Pittelkow, 1995). Later

studies using gene expression profiles by microarray have identified large sets of differentiation-related genes in keratinocytes subjected to confluence-induced differentiation (Paragh et al., 2010). Although EGFR activation is known to regulate the expression of a few of these differentiation-related genes (Gibbs et al., 1998; Marchese et al., 1990; Monzon et al., 1996; Poumay and Pittelkow, 1995), its role in regulating gene expression at a genome-wide level has not yet been studied. Furthermore, using the confluence-induced differentiation model, we showed recently that in addition to blocking the expression of cornified envelope precursor genes, EGF also suppressed the expression of critical genes in the sphingolipid and ceramide biosynthetic pathway (Sutter et al., 2009). Specifically, EGF inhibits the expression of genes such as FLG, UDP-glucose ceramide glucosyltransferase (UGCG), and sphingolipid delta(4)-desaturase (DEGS2), that are involved in the cornification and epidermal barrier function of the skin (Sutter et al., 2009). Because lipid biosynthesis is essential for maintenance of the epidermal barrier (Holleran et al., 2006), we performed further studies to identify differentiation associated metabolic processes that are regulated by EGFR signaling.

We combined genome-wide microarray and functional analyses to explore the effects of EGF, a ligand of the EGFR, on normal human epidermal keratinocytes undergoing density-induced differentiation. We identified 2,676 density-dependent EGF-regulated genes. Contrary to the effects of density, EGF downregulated the expression of 91% of the density-upregulated genes and upregulated 96% of the density-downregulated genes, suggesting that EGF plays a critical role in inhibiting keratinocyte differentiation. Specifically, EGF significantly reduced free fatty acid synthesis by half, suppressing the expression of ELOVL4, PTPLB and TECR, enzymes that form stearic acid and oleic acid,

two major fatty acid components in the SC. EGF also inhibited mRNA levels of genes encoding enzymes in the *de novo* and salvage ceramide pathways, causing a decrease in ceramides 1, 3, 6, 7, 8, as well as the acyl-glucosylceramides. Our expression and protein results strongly suggest that activation of EGFR signaling inhibits cornified envelope formation by altering levels of enzymes and structural proteins essential for the synthesis of this differentiated structure. Further, we showed that EGF caused a significant reduction in levels of tight junction proteins such as CLDN1 and TJP1, leading to an increase in paracellular permeability and disruption of tight junction barrier function. EGF impaired the epidermal barrier integrity as a whole by increasing the transepidermal water loss in organotypic culture. Finally, bioinformatics and statistical analyses revealed that genes associated with skin diseases were enriched in the set of EGF-regulated genes. Our work advances the current understanding of EGFR signaling in regulating epidermal barrier function. We identified many new EGFR-regulated genes and, more importantly, connected the function of these genes to major processes involved in epidermal differentiation. These findings provide a reference for subsequent studies of EGFR in the regulation of epidermal cell fate and homeostasis and may lead to novel therapeutic approaches for the treatment of dermatological diseases.

Aim 2: Identify the EGFR Signaling Pathway(s) that Mediate(s) Its Identified Effects on Epidermal Barrier Function

In normal human epidermis, keratinocytes undergo ongoing renewal by a process called homeostasis, during which stem cells in the basal layer of the epidermis replace the keratinocytes that are constantly lost or shed during terminal differentiation. A model of epidermal homeostasis has been proposed and modified with rate constants to

demonstrate that the number of stem cells is determined by the surrounding transit amplifying cells. The transit amplifying cells are in turn in equilibrium with cells that have withdrawn reversibly from the cell cycle and can commit to terminal differentiation by withdrawing irreversibly from the cell cycle (Figure 6) (Okuyama et al., 2004; Wilke et al., 1988). This dynamic model, together with our previous observations that EGFR signaling affects keratinocyte cell fate by regulating the expression levels of genes responsible for the basal and suprabasal phenotypes, has led us to hypothesize that EGF not only affects the essential processes required for epidermal barrier performance, but

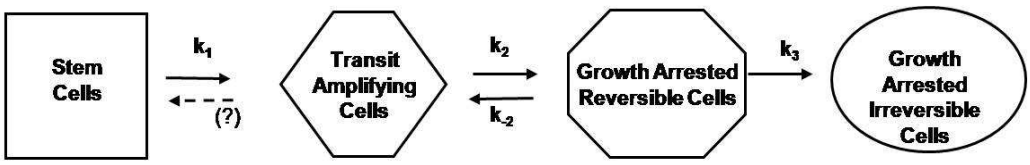

Figure 6. Dynamic Model of Keratinocyte Stem Cell Renewal and Differentiation (Okuyama et al., 2004)
This model shows a dynamic equilibrium between proliferative and terminal differentiated keratinocytes. The number of stem cells is determined by the surrounding transit amplifying cells which are in turn in equilibrium with cells that have withdrawn reversibly from the cell cycle and can commit to terminal differentiation by withdrawing irreversibly from the cell cycle.

also the transition between growth arrested reversible cells and transit amplifying cells (k_{-2}), by inhibiting and inducing the transcription factors (TFs) that regulate differentiation and proliferation, respectively. Our next aim is to understand the gene regulatory networks that control the homeostatic effects of EGF. Identifying regulatory networks and mapping TFs with their targets is a central problem and an ultimate goal in understanding the underlying mechanisms of cellular responses such as growth control and cell-cycle progression. Transcription factors are mediators that determine when and which genes are to be expressed. Understanding the factors that regulate the expression

of downstream target genes provides insight into the mis-regulated gene expression that is found in many human diseases.

To accomplish this task, we performed a time course microarray experiment. In this experiment, we had time-matched controls for each time point in order to identify the effects of EGF on gene expression over time. The data analysis revealed that EGF determined keratinocyte cell fate through a complex transcriptional regulatory network. After controlling for false discovery rate at 1%, EGF temporally changed the mRNA levels of 3,033 genes. Among these genes, we identified 245 TFs that were significantly changed by EGF. Specifically, we found that EGF controlled keratinocyte homeostasis by inhibiting the expression of pro-differentiation TFs and keratinocyte differentiation markers, while inducing the gene expression of proliferation transcription factors and proliferative markers. We also observed that MEK signaling pathway appeared to be the dominating pathway that was activated by EGF in epidermal keratinocyte. EGF signaled primarily through MEK and PKC signaling pathways to inhibit TFs that promote differentiation and induced those that promote proliferation primarily through MEK and PKC signaling pathways. When MEK signaling pathway was not involved, PKC signaling pathway appeared to mediate the up-regulation of some keratinocyte pro-differentiation TFs expression.

In summary, these findings provide a new mechanistic understanding of how EGFR activation controls gene expression at genome-wide level and uses signal transduction to repress keratinocyte differentiation and impair the epidermal barrier integrity, as well as shed light on the role of EGFR imbalance in skin pathogenesis.

MATERIALS AND METHODS

Keratinocyte Cell Cultures

Microarray 1

Neonatal foreskin NHEKs (Lonza Walkersville Inc.) were grown in basal keratinocyte-SFM (KSFM) (Invitrogen) supplemented with 5 ng/ml EGF and 50 µg/ml bovine pituitary extract (BPE). Fifth-passage NHEKs were grown to either 50% or 100% confluent cell density before treatment with basal medium, or medium containing EGF (10 ng/ml) or TGF-α (50 ng/ml) for 48 hr, with the treatment medium being replaced once at 24 hr (Sutter et al., 2009).

Microarray 2

Neonatal foreskin NHEKs were grown in the same condition as above. Fifth-passage NHEKs were grown to 100% confluent cell density. Cultures were treated with +/-EGF (10 ng/ml) after 48 hours of the last addition of complete medium. RNAs were then harvested at 1, 2, 4, 8, 16, and 24 hours after this initial treatment +/- EGF. The zero time (t_0) point controls were changed to basal medium, and RNAs were immediately harvested. For statistical analysis, there were 3 replicates at each time point. In order to control for variation in plating, cells were pooled from two individual plates before harvesting RNA.

RNA Extraction and cDNA Microarray

Microarray 1

Total RNA was isolated using RNA Stat-60 (Tel-Test). The mRNA levels were measured using the Affymetrix Human Gene 1.0 ST arrays according to the standard GeneChip® Whole Transcript Sense Target Labeling assay manual. Arrays were

washed and stained in the Affymetrix Fluidics Station 400. After hybridization, the arrays were scanned using the Affymetrix GeneChip Scanner 3000 7G. The microarray data have been submitted to the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus repository (GSE32217).

Microarray 2

Total RNA was isolated using RNA Stat-60 (Tel-Test). The mRNA levels were measured using the Affymetrix Human Genome U133 Plus 2.0 arrays according to the standard GeneChip® Expression assay protocol. Biotinylated cRNAs were prepared according to the standard Affymetrix protocol from 8 ug of total RNA (Enzo BioArray High Yield RNA Transcript Labeling Kit). Following fragmentation, 20 ug of cRNA were hybridized for 16 hr at 45ºC. Arrays were washed and stained as described above.

Array Data and Statistical Analysis

Microarray 1

The 16 Human Gene 1.0 ST arrays were preprocessed using dChip (Li and Hung Wong, 2001). Genes with at least 1.5 fold difference when comparing the 100% cell density untreated samples to the 50% cell density untreated samples were exported for further analysis. Two-way ANOVA was used to identify differentially expressed genes by either density or treatment factors using JMP Genomics 4.1 (SAS, Cary, NC). The multiple hypothesis problem was corrected by Benjamini-Hochberg false discovery rate control at the 0.05 level (Benjamini and Hochberg, 1995). Pair-wise comparisons were performed using the Tukey's Honestly Significant Difference test.

The 1,083 genes that are associated with the epidermis in the literature were identified using GeneIndexer (Homayouni et al., 2005). The keywords used for this

analysis were epidermal differentiation, epidermal barrier, skin, cornified envelope, keratinocyte, and epidermis, with a 0.1 score cut off. GeneIndexer utilizes Latent Semantic Indexing, a vector space model for information retrieval, to identify both explicit and implicit gene-to-keyword associations contained within titles and abstracts in Medline citations (Homayouni et al., 2005). Functional Annotation Clustering from DAVID (htt://david.abcc.ncifcrf.gov/) was used to obtain the 72 highly enriched genes associated with ectoderm development. Biological process and cellular component categories were extracted from the DAVID analysis results. Genes related to skin diseases were generated by combining the results obtained from Chilibot (Chen and Sharp, 2004) and IPA (Ingenuity® Systems, www.ingenuity.com). Figure 7 in Chapter 3 shows a detail flow chart of this data analysis.

Microarray 2

The GC-robust multiarray average (GC-RMA) (Wu et al., 2004) was used to adjust background intensity levels, and to normalize and combine the probe pair intensities into an estimate of gene expression for each probe set in the data. A function from Affymetrix Microarray Suite (MAS) 5.0 (Liu et al., 2002b) was used to detect the Present (P) and Absent (A) calls of the gene expression values. Only probe sets that had at least 2 P calls out of 3 replicates at any treatment time were selected for further analysis. The data was then filtered by keeping probe sets that were upregulated or downregulated with at least 2.6-fold change at any time point compared to its corresponding time point control. Probe sets that showed significantly differential expression profiles across all groups in the time-course experiment were identified at a 1 percent false discovery rate using maSigPro (Conesa et al., 2006). maSigPro controls for

false discovery rate by applying the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995). maSigPro first adjusts a general regression model for the data using least-square technique to identify differentially expressed genes. Then, the differences between experimental groups are identified using stepwise regression analysis. Each probe set was fitted to a polynomial regression of up to 3 degrees. The best regression model for each probe set was selected using a "*two.ways.backward*" stepwise regression. In the current Affymetrix technology, one gene can be represented by multiple probe sets. To avoid this redundancy in our data set, we selected only probe sets having the smallest regression model p-value. Figure 20 in Chapter 4 shows a detail flow chart of this data analysis.

Quantitative RT-PCR

Microarray 1

Total RNA (1 µg) was reverse transcribed using the SuperScript First-Strand cDNA Synthesis System (Invitrogen). Real-time PCR reactions were performed with Absolute Blue SYBR Green Fluorescein (Cat# AB-4219/B, Thermo Scientific) using four different samples in each group. The Ct values for all genes were normalized to that of tubulin, alpha 1C (*TUBA1C*), and the relative value for the control samples (no EGF and 50% cell density) was set as one arbitrary unit. Primer sequences are listed in Table 1. The Δ-Δ Ct method was used to analyze the relative changes in gene expression (Livak and Schmittgen, 2001).

Microarray 2

Total RNA (2 µg) was reverse transcribed using superscript first strand cDNA synthesis system (Invitrogen). Real time PCR reactions were performed with SYBR

Green Supermix (BioRad) using three different samples in each group. The Ct values for all genes were normalized to that of cyclophillin *PPIA*, and the relative value for the control samples (t_0) was set as one arbitrary unit. Primers sequences are listed in Table 2. The Delta-Delta Ct method was used to analyzed the relative changes in gene expression (Livak and Schmittgen, 2001).

Antibodies and Immunoblotting

Cell lysates were prepared with lysis buffer containing 62.5 mM Tris-HCl pH 6.8, 2% sodium dodecylsulfate SDS and 1% β-mercaptoethanol. After washing the plates twice with cold phosphate buffered saline (PBS), 250 µl of the lysis buffer was added to each plate on ice. The cells were then scraped into 1.5 ml tubes (Cat# 05-048-129, Fisher Scientific). The tubes were boiled for 5 minutes, centrifuged at 10,000 rpm at 4°C for 20 min using the Eppendorf Centrifuge 5415C. The supernatants were removed to new tubes and stored at -80°C. Protein samples were quantitated using the Micro BCA protein assay kit (Cat# 23235, Fisher Thermo Scientific) as follow:

1) Five hundred µl of 0.1 M iodoacetamide and 500 µl of 6.25 mM Tris HCl pH 6.8 were added to the cuvette.

2) Bovine serum albumin BSA (0, 2, 4, 8, 10, 15, and 20 µg) or protein samples (2 µl) were added to the above mix and incubated for 15 min at 37°C followed by a cooling process of 5 min at room temperature.

3) Pierce reagent mix (50% Micro BCA Reagent A, 48% Micro BCA Reagent B, and 2% Micro BCA Reagent C) was added to the above buffer and incubated for 60 min at 60°C.

4) The absorbance of each sample was recorded at 562 nm using the spectrophotometer. Water was used as the blank.

Protein samples were separated by 6% or 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride membranes (Millipore). Membranes were blocked with 5% nonfat dry milk in a mixture of Tris-Buffered Saline and 1% Tween 20 (TBS-T), incubated with primary antibodies, followed by incubation with horseradish peroxidase-conjugated goat anti-mouse (115-035-003), goat anti-rabbit (111-036-003), or donkey anti-goat (705-035-003) IgG (Jackson ImmunoResearch) and development by enhanced chemiluminescence (Pierce Fisher Thermo Scientific) on Kodak BioMax light film (Cat # Z370371). Antibodies used for this study are listed in Table 3. ImageJ (Abramoff et al., 2004) was used to quantitate each protein band.

Table 1. qRT-PCR Primers Used for Expression Validation of Microarray 1

Gene	Forward primer (5'-3')	Reverse complement primer (5'-3')	Melting Temperature (°C)
KRT1	tgtctggagaatgtgccccgaacg	ccgccgccacctccagaaccat	59
FLG	gacaccccggatcctctcacc	agctgccatgtctccaaactaaac	55
LAMA3	caagaggcctccccacaaacagc	tggccccaacaatacagagtgagc	55
LAMC2	ctggcctggaccctgagaag	ccggccggcaagtgatt	61
SPTLC3	tgggatgggattcgcaactaactca	ggggcagatgcacgatggaacct	57
KDSR	atgggccttttccgcactattg	agccacattcctgaagagcactg	57
SGPP2	agtggccccgtccctcctc	gacgccacccagcacatcc	57
SGPL1	cctgttgggctgccttgatgc	tccgggcgtgtagtaatgtgatgc	55
SPHK1	ggtgcccgacgaggactttg	ccgcccgcacgtagaacag	59
ASAH1	tgaaccgcaccagccaagaga	ggcagtcccgcaggtaagtttc	57
DEGS1	caggaggcggaggcagagg	aaagttcccccggtacccaagtta	55
DEGS2	gcgggtgtacaggctggcaaaaga	acaagggcagcagtccagagcaca	55
UGCG	cctgcgggagcgttgtc	ttgttgaggtgtaatcgggtgtag	55
GBA	atcccgatggctctgctgttgtg	gccctgctgtgccctctttagtca	57
SGMS2	aaggggagatccgtgggttgt	cattgggtggcagcagcagtgt	57
CERK	gcctgccccaaacccactaacaa	tgccccggaaatcagagcctatc	57

All cDNAs were amplified using the melting temperature at which they had similar efficiency to that of TUBA1C.

Table 1. qRT-PCR Primers Used for Expression Validation of Microarray 1 (cont.)

Gene	Forward primer (5'-3')	Reverse compliment primer (5'-3')	Melting Temperature (°C)
LASS3	gccccacaccgaccccacat	aacaaagcgagcccctgagaaagt	59
LASS4	ggagacgccggaaccaggat	aggccgcccacgaaggag	55
PTPLB	ggcacgcggaagaagaagg	cacccggctgtcatcacca	57
TECR	agccccacgccaccattg	gggccccgcgtactctgttag	59
IVL	ggccacccaaacataaataaccac	cacctagcggacccgaaataagt	55
LOR	caggggcaccgatgggcttagag	tgaggcactggggttgggaggtag	58
KRT10	tgaaaccgagtgccagaata	cgtagccgccgccgaaactt	59
CLDN1	cgatgaggtgcagaagatga	ccagtgaagagagcctgacc	53
TJP1	tggtcggaaaacatgctacaca	aggccatggaaccagtctcaca	59
GJA1	accaaccgctcccctctcg	tcccgcctgccccattc	55
ELOVL4	acgtgacgccggctgaggaga	cccgggagaaagacgaggaggtg	57
KLF4	ccggcgggaagggagaag	aggggcgccaggttgctac	59
GATA3	ggcccggcaggacgaga	gtagggcgggtaggtggtgatg	57
ABCA12	tctcgccgaagtatatgggatgtt	gcttcggggagatgtgattgg	55
ALOX12B	accccacctcgccacctcacc	caccgccccagttgcaaagtctct	55
PRDM1	tcccgaacatgaaaagacgataaa	ctctccgggataagggtagtgaag	57
BDNF	ggcccaatgaagaaaacaataagg	gcgggcagggtcagagtg	57
FBXW7	acgttaacagggcaccagtgg	cacccgttttcaagtcccatagtt	55
CST6	tgggcagcaacagcatctacta	cctcggggacttatcacatctg	57
TGM1	tccgcccacgacacagacacatc	gcaggggccgcagcagaaga	59
SLC27A4	aggcgggcggggtagga	gccgggtctcagcagggttta	55
DLX3	accgccgttccaagttcaaga	aggcggctgctgctgtaagtg	59
PIGA	acggggtgcctggactaata	tggcctcgctgatgtctgataagt	53
CDSN	agggcccatcgtctcgcactc	acccaccacctcgtagccaccata	59
OCLN	ggcagggtgtgggaagcaggac	gacgcgggagtgtaggtgtggtgt	59
CGN	ttcccctctttgccattcctacct	accagacccccggcactttatcat	59
CLDN16	gtcatactcagcccctcgcacaga	tgaaccaaaagccagggagaaaag	59
CLDN4	ccgcgccctcgtcatcatcag	ataaggccggccaacaggaacacc	59
TUBA1C	ctacccccgcatccacttcc	ggggcaccaatccacaaactg	53, 57, 59

All cDNAs were amplified using the melting temperature at which they had similar efficiency to that of TUBA1C.

Table 2. qRT-PCR Primers Used for Expression Validation of Microarray 2

Gene	Forward primer (5'-3')	Reverse compliment primer (5'-3')	Melting Temperature (°C)
ATF3	ttcatcggcccacgtgtattgtcc	ctcccgccttgatggttctctgct	60
BCL6	gaagccctatccctgtgaaatg	tctggcgcaagtgaagtcg	55
BMP2	gtgtccccgcgtgcttcttag	gctgggggtggggtctctgttt	59
BMP6	ggcgcccttgtctcagtcatt	ctagagccggcagtccagaagtta	57
BNC1	tcgacccttcacagttcccatcac	cggcggaccctgaagaacc	61
CDH1	ccatcaggcctccgtttct	gtggcaatgcgttctctatcc	57
CDKN1A	ggcggcagaccagcatgacagatt	gcagggggcggccagggtat	59
CDKN1B	cgtaggggcgctttgttttgttcg	ggctcgcctcttccatgtctctgc	59
CDKN2A	catggtgcgcaggttcttggtgac	cgtgagccgcgggatgtga	59
CTNNB1	gtaccggagcccttcacatc	gtcgccacaccttcattccta	57
E2F7	ggatcgggcctgtggacttca	ctgtacgggctgctcggttctg	60
EAF2	atagcgcagcgggattctcaca	ttttctagccgacattctccagtatca	57
EGR1	gcgcagtgccatccaacgacag	ttggcggcagggtaggcaggag	55
ELF1	aaccgtcagtgtggcttcctctcc	ctgtggctgctgctccgttttc	56
ELF5	agcgcctgccttctcttg	ggcgcttagtccagtattcag	55
ETS1	aactcgggggccaggactcttt	ctagggcagcagcaggaatgaca	59
ELK3	gaggagccgcccgaacacagc	tgagaagggtgaggcggatgaaata	61
FOS	cagcccgccctcgtctcct	ctgcgctcggcctcctgtc	59
FOSB	tcgccctccctcctcgctctgt	ccacccccctgtccccaaagtcac	61
FOSL1	acccccacactcatgaccacacc	gggggaaggggaggagacattg	59
FOXN1	caccaccccagccaccacctc	ccggggctgccaagtcacct	61
HES1	ctggagaggcggctaaggtgtttg	ggtgccgctgttgctggtgtag	57
ID2	cagtcctgtgaggtccgttagg	tggtgatgcaggctgacaatagtg	59
ID3	ggtgcgcggctggtacgag	ggggccatcagggggtccag	59
IRX3	acccgcaccccgccttctacc	ctcctcgcgctcgctcccataa	60
IRX5	gcccggcgtacagcaccag	ggggcgccgcgtaaggat	59
ITGA6	tttttggcgtggctgacttacatc	gcagcaggcccgaggttaggac	60.3
ITGB4	gccgccgcctggtaaaca	caggacacgccggatgacag	56
JUN	gacggcgcccccagtgtg	gccgcccctccccaacc	59
KLF6	tttgggggaaggggggttgttg	gaaggggctgaggtcggtgagtt	59
LBH	cagtggagagcggggagttgtgt	cggggcagtgaatggggaaata	57
MAF	cccccggcgatgagacg	tggagttggcgaaaggtgtgatac	56
MAFB	cggcggaccctgaagaacc	ccaggaccggccacgactc	56
MAFF	cgtgggccctgtcttcctctt	tctgccccatccccaacctac	59
MEIS1	gactcgggcgctttgcttca	gcgggtccccatacatcgtg	57
MITF	ctacggccacgggaacagga	ttacaaacaacaagcccaaaccact	55
MSX2	gaagggccaaggcgaaaagact	aggcgggatgggaagcacag	59
MXD1	acgggctcatcttcgcttgtgc	aggtgtcgctgctctcgctgaa	57
MYC	cggggctttatctaactcgctgta	gcccgcccgctgctatg	58
NFKB	gccgtccagcgccatctcact	agccctcagcaaatcctccaccac	59
NFKBIZ	aagggcccgattcgttgtctgat	tccccgggcgttggtgttt	59
PBX1	tccccctcccccctcctcatcct	cccccggctcttcctcttctgc	59
PIR	gagggtccggagaagcattgg	cccggcccgcagtcatc	59
POU2F3	gccaggtggagccaggaaatg	ggccgggaaccagcacaag	57

All cDNAs were amplified using the melting temperature at which they had similar efficiency to that of TUBA1C.

Table 2. qRT-PCR Primers Used for Expression Validation of Microarray 2 (cont.)

Gene	Forward primer (5'-3')	Reverse compliment primer (5'-3')	Melting Temperature (°C)
PPIA	gcagagggttaaggcgcagactac	taaggtgggcagagaaggggtttt	56, 57, 59
PRDM1	tcccgaacatgaaaagacgataaa	ctctccgggataagggtagtgaag	57
PRMT6	acaggcccgggaggtggtg	gtcgctgatgggggctatgaaga	59
RB1	cgtcatgccgcccaaaac	acctcccaatactccatccacaga	57?
RELA	ctccgcgggcagcatcc	atcccggcagtcctttcctacaa	57
RFX2	accgccgccgccatagagac	cctgctggggtacctgctgaac	59
SIN3A	gcccctgcccctcctgtgtat	ttgggtgatgatggctgctatgaact	57
SKIL	gtcggaggctgttcttactggtgtg	agggtcaatgcaatggtctggttt	59
SMAD1	cacccgtttcctcactctcccaatag	ataagcaaccgcctgaacatctcctc	59
SMAD3	aggcgtgcggctctactaca	ctccccagcctttgacgaa	55
SMAD7	tgacgcgggaggtggatgg	ctgatgaactggcgggtgtagca	57
TCF4	gaggcgggggaggtgttgagatt	gggaggggacggagggaagg	55
SOX2	tcggcggcggcaggat	ggcgggcgggggtgtc	59
SOX9	cagcactcgccgcagcagat	gtgtcggcgatgggggtgta	57
TGIF1	gattcttcgggattggctgtatga	acggcgggaaattgtgaactg	59
TP63	agtttcccgtccatctcccttag	aatgacagcccttgaccagaatg	57
THOC4	ccgtggcgctggaggttt	cacgcggatttgctggtctg	55
TRIM16	cttggcagggagacggaggaaca	atcagggcagcagaaggcagacagt	57
TWIST2	gcggcgctacagcaagaagtcg	cagagggcagcgtggggatgat	59
ZNF323	ccggggtttgcaataagggagtc	aaaggccggaaatgcgtcagc	59

All cDNAs were amplified using the melting temperature at which they had similar efficiency to that of TUBA1C.

Transepithelial Electrical Resistance (TER) and Paracellular Permeability

The TER was determined on confluent monolayers of NHEKs grown on polycarbonate Transwell™ filters (0.4-µm pore size, 12-mm diameter, 1.12 cm^2; Cat # 3401; Corning, Inc.) with the Endohm device in the resistance mode (World Precision Instruments) at 48, 72, and 96 h after the transfer into high Ca^{2+} (1.8 mM) with or without EGF (10 ng/ml). TER values (Ohms) were calculated by subtracting the blank values from the bare filter with medium and multiplying by the surface area of the filter. Before the TER measurement, the Endohm device was equilibrated with the culture medium at room temperature for 15 min.

Table 3. Antibodies Used for Immunoblotting and Immunofluorescence Assays

Primary Antibody				Secondary Antibody		
	Catalog Number	Source	Dilution		Source	Dilution
A. Immunoblotting						
KRT1	NLC-K1	Leica Microsystems	1:4000	Goat anti-mouse	Jackson ImmunoResearch	1:10,000
FLG	NCL-Filaggrin	Leica Microsystesms	1:300	Goat anti-mouse	Jackson ImmunoResearch	1:10,000
DSG1	27B2	Molecular Probes	1:330	Goat anti-mouse	Jackson ImmunoResearch	1:10,000
TJP1	ZO1-1A12	Molecular Probes	1:400	Goat anti-mouse	Jackson ImmunoResearch	1:10,000
GRHL1	HPA005798	Sigma-Aldrich	1:400	Goat anti-rabbit	Jackson ImmunoResearch	1:15,000
CLDN1	MH25	Molecular Probes	1:250	Goat anti-rabbit	Jackson ImmunoResearch	1:15,000
KLF4	sc-20691	Santa Cruz	1:250	Goat anti-rabbit	Jackson ImmunoResearch	1:15,000
FOXN1	ARP30053_T100	Avia System Biology	1:200	Goat anti-rabbit	Jackson ImmunoResearch	1:15,000
POU2F3	ARP32537_P050	Avia System Biology	1:2000	Goat anti-rabbit	Jackson ImmunoResearch	1:15,000
ELF5	sc-9645	Santa Cruz	1:200	Donkey anti-goat	Jackson ImmunoResearch	1:30,000
B. Immunofluorescence						
CLDN1	MH25	Molecular Probes	1:15	AlexaFluor 488 goat anti-rabbit IgG H+L	Molecular Probes (A11008)	1:1000
TJP1	ZO1-1A12	Molecular Probes	1:40	AlexaFluor 594 goat anti-mouse IgG H+L	Molecular Probes (A11005)	1:1000

Paracellular flux assays were performed on confluent monolayers of NHEKs grown on polycarbonate Transwell™ filters (0.4-µm pore size, 12-mm diameter, 1.12 cm^2, Cat # 3401; Corning, Inc.). Frozen P3 NHEKs were grown in KSFM supplemented with 5 ng/ml EGF and 50 µg/ml BPE. When cells were approximately 80% confluent, they were trypsinized and 9.4 x 10^4 NHEKs in 500 µl were plated on the filters and left overnight to attach. The flux was measured 48, 72, and 96 h after the transfer into high Ca^{2+} medium (1.8 mM) with or without EGF (10 ng/ml). Two different tracers were used, a 3 kDa FITC-dextran (Cat # D3305; Molecular Probes, Inc.), and a 40 kDa Texas Red-dextran (Cat# D1829; Molecular Probes, Inc.). The tracers were suspended in P buffer (10 mM Hepes, pH 7.4, 1 mM sodium pyruvate, 10 mM glucose, 3 mM $CaCl_2$, 145 mM NaCl). Media in the apical and basal compartments of the keratinocyte sheet grown on the Transwell™ filters were replaced with 164 µl or 600 µl of P buffer, respectively. The plates were incubated in 37°C to equilibrate for at least 30 min. Next, 36 µl of P buffer containing either one of the tracers was added to the apical compartment at a final concentration of 1 mg/ml. Cells were incubated at 37°C for 3 h. The basal compartment media was diluted 1:7 (v/v) with the P buffer before equal-volume aliquots (100 µl) were collected for measurement. The amounts of 3 kDa and 40 kDa dextrans were determined in a fluorometer (FLx800™; BioTek Instruments, Inc.) on a flat-bottom 96-well plate (Cat# 353296, BD Falcon). The amount of diffusible dextrans was calculated from a titration curve of known concentration of the tracers.

Organotypic Cultures

The sources for reagents and materials used for this experiment are listed in Table 4. Neonatal Dermal Fibroblasts (ND Fibroblasts, Lonza) (seventh passage) were maintained

Table 4. Sources and Catalog numbers for Reagents and Materials Used in the Organotypic Culture Experiment

Item	Source	Cat #
Adenine	Sigma	A2786-5G
strontium chloride	Sigma	439665-5G
L- serine	VWR/Ameresco Solar	S4311-25G
choline chloride	Sigma	C7527-100G
Ethanolamine	Sigma	E0135-100ML
O-phosphoryl –ethanolamine	Sigma	P0503-1G
selenious acid	Sigma	211176-10G
Triiodothyronine	Sigma	T0281-10MG
Hydrocortisone	Sigma	H0888-1G
Progesterone	Sigma	P8783-1G
Transferring	Invitrogen	11107018
calcium chloride	Sigma	C4901-100G
bovine insulin	Sigma	I0516 or I6634
linoleic acid/bovine serum albumin mixture	Sigma	L9530-5ML
fibroblasts normal human dermal neonatal (CI-1-F)	Lonza	CC-2509
Ham's F12	Fisher/Hyclone	10-080-CV
Fetal bovine serum	Fisher/Hyclone	SH30070
DMEM with 4.5 g/l glucose	Fisher/Media Tech	MT-15-017-CM
L-Glutamine	Sigma	G7513-20ML
sodium pyruvate	Sigma	S8636-100ML
penicillin/streptomycin (10,000 units/10,000 µg, 100mls)	Fisher	SV30010
Transwell inserts (3.0µm polycarbonate membrane insert, 6 well)	Corning	3414
sterile cotton pads	Fisher	19-064-585
type 1 collagen	BD Biosciences	354236
ascorbic acid	Acros Organics	401475000
HEPES	Sigma	H4034

in DMEM with 4.5 g/l glucose supplemented with 4 mM L-Glutamine, 0.11 mg/ml sodium pyruvate, 10% FBS with 100 units of penicillin and 100 µg of streptomycin/ml. This medium was defined as fibroblast growth medium. Fifth passage NHEKs were maintained in KSF medium supplemented with 5 ng/ml EGF and 50 µg/ml bovine pituitary extract.

Organotypic cultures were prepared as described previously (Chen et al., 1995b). Specifically, cultures were grown on polycarbonate Transwell™ filters (3 µm pore size, 24 mm diameter) as follows:

1) Type 1 collagen (Cat# 354236, BD Biosciences) was mixed with DMEM which had been supplemented with 10% FBS and 50 µg/ml ascorbic acid. The final collagen concentration was 1.25 mg/ml. This was mixed on ice to prevent early gelation.

2) One ml of the collagen/media mix was put into each insert to cover the entire bottom. This layer was allowed to harden for approximately 30 min at 37°C until the collagen turned pink.

3) Type 1 collagen was mixed with ~20,000 fibroblasts/ ml to a final collagen concentration of 1.0 mg/ml in DMEM supplemented with 10% FBS and 50 µg/ml ascorbic acid. The collagen was added last and slowly to the mix. Everything was mixed on ice to prevent early gelation.

4) Three ml of the collagen/fibroblast mix was put into each well.

5) The collagen was allowed to polymerize at 37°C for 2 h (Gangatirkar et al., 2007).

6) Two ml of fibroblast growth media (as described above) was placed in the bottom well.

7) The following day, 2.5 ml and 1.5 ml fresh fibroblast growth media was placed in the bottom well and the insert, respectively (this volume was recommended by Corning). By this time, the cellular collagen layers had contracted some (note: the more fibroblast the more contraction).

8) The gel was allowed to contract for 5-7 d after initial plating in a incubator at 37°C. After 6 d of incubation at 37°C, the gel was washed and NHEKs (1.5×10^5 cells in 50 µl) were seeded on top using micro pipette tip. The inserts were incubated for 5 min at room temperature to allow medium to soak into the collagen.

9) The inserts were then transfered to the incubator and the cells were allowed to attach for 2 h.

10) Plating medium (Chen et al., 1995b) was added to cover the gel (2.5 ml in the well and 0.5 ml in the insert).The plating medium was DMEM/Ham's F12 (3:1) Mediatech/Hyclone) supplemented with a final concentration of 1.9 mM calcium chloride (Sigma), 7.25 mM L-Glutamine (Sigma), 0.18 mM adenine (Sigma), 1 mM strontium chloride (Sigma), 1 mM L-serine (VWR), 0.64 mM choline chloride (Sigma), 0.1 mM ethanolamine (Sigma), 0.1 mM O-phosphoryl-ethanolamine (Sigma), 2 µg/ml linoleic acid/bovine serum albumin mixture (ratio 1:100, Sigma), 53 nM selenous acid (Sigma), 5 µg/ml insulin (Sigma), 5 µg/ml transferrin (Invitrogen), 20 pM triiodothyronine (Sigma), 0.4 µg/ml hydrocortisone (Sigma), 10 nM progesterone (Sigma), 50 mM Hepes, 10 ng/ml EGF and 100 units penicillin and 100 µg/ml streptomycin (Hyclone).

11) This medium was changed every 2-3 d for the next 5 d.

12) The cultures were kept submerged for 5 d. The organotypic cultures were then raised to the air-liquid interface using cotton filter pads.

13) Two ml of cornification medium (Chen et al., 1995a) with or without EGF (20 ng/ml) was put into the bottom well. The cornification medium used for this period was DMEM/Ham's F12 (1:1) with all supplements as in plating media, but without the EGF and progesterone.

14) The medium was changed every 2-3 d. Only 1.5 ml of media was used from this point forward. Cotton pads were changed every 1 week. At the time the cotton pads were changed, 2 ml of cornification media was used. After that, 1.5 ml of media was used at every meida change. Topical treatment of 30 µl 1x PBS with or without EGF (20 ng/ml) was done at every media change throughout the air-exposure period. The cultures were harvested after 14 d of air exposure.

Histology

Cultures were fixed with buffered formalin (10%) obtained from the histology lab for at least 1-2 hour at room temperature. Since the tissues were very fragile, they were embedded in agar (1%) mixed with buffered formalin. The 1% agar was made as follow:

1) Combine 90 ml ddH_2O + 1 g agarose
2) Weigh the flask with agarose to be able to adjust for water evaporated during boiling.
3) Boil gently until thoroughly dissolved.
4) Add water to weight measured in 2).
5) Add 10 ml of 10% neutral buffered formalin in a 15 ml tube and mix well.
6) Put the quantity to be used immediately into a small bottle with a dropper top. Store this in either a 60°C oven (keeping the bottle tightly stoppered), or while in use in a

60°C water bath in a chemical safety hood if formalin is used. To melt, put a 15 ml tube in a beaker containing water that had been boiled using a microwave.

7) Pour the rest of the agar into a sterile 100 ml bottle. Allow to solidify. Store at room temp (expiration 2 months). When needed, warm the bottle in a microwave (loosen the cap of the bottle), then aliquot the required amount.

8) The tissues were embedded in agar as follows:

a) Working on a clean piece of glass, place a small amount of the agar on the glass. Observe the change in its consistency. When it begins to solidify, place the tissue in the agar. Orient the tissue as you would if you were embedding into a paraffin block. Drop more agar over the tissue. Continue to do this until the tissue is surrounded by the agar and a small mound of agar is formed. If the agar is allowed to solidify too much between applications the layers will peel away from each other. Large bubbles should also be avoided during this process.

b) When the mounded agar is solid, trim the excess away with a scalpel to form a roughly square shape. Slide the scalpel under the agar/tissue mound and gently lift it into a processing cassette. Close the cassette and placed in 70% alcohol. Take to the histology lab for further processing.

Transepidermal Water Loss (TEWL) Measurement

The TEWL measurement protocol was adapted from Urtti et al., 2008. The TEWL (g/m^2h) was measured using a VapoMeter (Delfin Technologies Oy) with the nail adapter (4.5 mm in diameter). Organotypic cultures grown on filters were removed from the inserts by moving the scalpel carefully and gently around the edges of the insert. The cultures were placed on Whatman filter paper soaked with 1x PBS and allowed to

equilibrate with ambient air for 15 min at room temperature before TEWL measurements were taken. TEWL measurements were carried out at 24-25°C and 27-35% humidity.

Indirect Immunofluorescence and Confocal Microscopy

Fifth passage NHEKs (17,000 cells in 500 µl) were plated in each chamber of glass culture slides (Cat # 354118, BD Falcon) that had been coated with fetal bovine serum for 1 h. Last feed was given to the cells at 100 % confluent cell density. Forty eight hour after the last feed, a pre-treatment with basal medium with or without EGF (10 ng/ml) in the presence of 1.8 mM Ca^{2+} was given to the cells. After the 24 h pre-treatment, the medium was changed to basal medium containing the exact same treatment of EGF (10 ng/ml) and 1.8 mM Ca^{2+} as in the pre-treatment medium. After 96 h of EGF treatment, cells were fixed in 4% paraformaldehyde (in PBS) for 15 min and then washed with PBS 3 times (5 min/wash). Slides were blocked with 5% normal goat serum and 0.1% NP-40 in PBS for 30 min, followed by 30 min of incubation with CLDN1 (MH25) and TJP1 (ZO1-1A12, Molecular Probes) antibodies diluted in blocking solution (16.67 µg/ml and 12.5 µg/ml, respectively). Cells were then washed 3 times with PBS (10 min/wash) and incubated for 30 min with secondary antibodies: 1:1,000 AlexaFluor 488 goat-anti-rabbit IgG H+L (A1108, Molecular Probes), and 1:1,000 AlexaFluor 594 goat-anti-mouse IgG H+L (A11005, Molecular Probes). Before removing the chambers, cells were rinsed with PBS 3 times (10 min/rinse). The glass slides were then mounted with Prolong® Gold antifade reagent with DAPI (Cat# P36931; Molecular Probes) (use one small drop/chamber, regardless of whether the chamber was empty). To help the mounting media spread evenly, slides were warmed at 37°C for 10-25 min and left overnight in the dark to dry.

Fluorescent images were captured using the Zeiss LSM 710 laser scanning confocal microscope with the ZEN 2009 software (Zeiss). During image acquisition, all images were taken with a 40x objective lens at identical settings (pixel dwell, 12.6 µs; master gain, 823; digital gain, 1.00; digital offset, -157.41; pinhole, 90 µm; and 2% laser power). For presentation, brightness and contrast levels were adjusted across the entire images using Adobe Photoshop.

Cornified Envelope Assay

Fifth passage NHEKs were grown to confluence and pretreated with vehicle (0.1% DMSO) or PD153035 (300 nM) 2 h before treatment. Basal medium with or without EGF (10 ng/ml) was added in the presence of vehicle or PD153035 (300 nM) for 24 h. The medium was replaced with fresh medium containing the same treatments. After 72 h, the cell envelope competence assay (n = 3) was performed as previously described (Cline and Rice, 1983) with modifications.

1) Cells were washed twice with 5 ml 1 x PBS containing 0.02% EDTA (Cat # E5134-500G, Sigma) and then trypsinized with 1 ml 0.05% Trypsin/EDTA solution (Cat # 25300054, Invitrogen) for 3 min at 37°C. Plates were shaken gently until most of the cells came off the plates.

2) Two ml of trypsin neutralizing solution (Cat # CC5002, Lonza) was added to each plate and the cells were resuspended and transferred to a 15 ml tube.

3) Two ml of PBS were used to collect the remaining cells on the plates, which were added to the same 15 ml tube. Cells were counted by using 2 x 10 µl of cell suspension directly with a hemocytometer, using phase contrast (do not add trypan blue) to determine the total number of cells both alive and dead.

4) Cells were centrifuged at 200 x g for 10 min using the IEC CL31R Multispeed centrifuge (Thermo Scientific).

5) Cells were then resuspended vigorously in serum-free media (3 ml for 100 mm plate, 2 ml for 60 mm plate, 1 ml per 6-well dish well).

6) They were incubated with 10 µg/ml of calcium ionophore A23187 (C7522, Sigma-Aldrich) for 5 h at 37°C in a gentle Hybaid rocker with speed of rotation was set to 12 (National Labnet Company).

7) Cells were centrifuged at 2,500 x g for 5 min using the IEC CL31R Multispeed centrifuge (Thermo Scientific).

8) Cells were suspended in 10mM Tris/HCL pH 7.5 + 1% SDS and 1% 2-mercaptoethanol by strong vortexing and pipetting (use 400 µl for 100 mm plates, 250 µl for 60 mm plates, and 125 µl for each 6-well dish well).

9) The remaining cornified envelopes were then counted immediately.

10) The percentage cornified envelopes was estimated by dividing the number of envelopes counted by the initial cell number.

Lipid Extraction

Fifth passage NHEKs were maintained in KSFM supplemented with 5 ng/ml EGF and 50 µg/ml bovine pituitary extract. Pre-treatment in basal medium with or without EGF (10 ng/ml) began 48 h after the last feed with complete medium at confluence. After 24 h of pre-treatment, the medium was changed to basal medium with or without EGF (10 ng/ml) in the presence of 1.8 mM Ca^{2+}. After 48 h of treatment, cell pellets were extracted by the Bligh-Dyer method (Bligh and Dyer, 1959), and split into the aqueous and organic phases. The organic phases were dried under nitrogen gas, redissolved in

chloroform (Cat # 650498, Sigma)/methanol (Cat # L6804, Fisher) (1:1) and stored at -20°C until used. The 3.5 ml aqueous phase was discarded as no further analysis was required of this phase.

High Performance Thin-layer Chromatography (HPTLC)

Extracted lipids from the organic phase were separated using one-dimensional HPTLC on 10 cm x 10 cm silica plates using the "ceramide development system" as previously described (Ponec and Weerheim, 1990). Specifically, ceramides were separated as follows:

1) Cleaning Plates. To remove possible impurities that could interfere with lipid separation, plates were washed in 60:40 solution of methanol/ethyl acetate (Cat # 33000000, Pharmco-AAPER), followed by 30:20:50 solution of chloroform/ethyl acetate/diethyl ether. Then, the plates were air dried.

2) Activation. After evaporation of all solvents, the plates were activated for 15 min at 130°C in the Isotemp oven (Fisher Scientific).

3) Sample Application. Each lane was approximately 1cm wide. Three µl of a standard mixture containing linoleic acid, Cholesterol, Cer(NS), Cer(AP), and Glucosylceramide was applied to each plate. Each of these standards had a final concentration of 0.2 µg/µl. Standards for cholesterol and linoleic acid, ceramide NS and GC, and ceramide AP, were from Sigma-Aldrich, Avanti Polar Lipids, and Evonik Industries, respectively. Then, 28 µl of the - EGF sample and 28 µl of the + EGF sample were added to the next two spots on the plate, respectively.

NOTE: Application of sample drops was done on a 6 mm streak, as opposed to a dot.

4) Development. All developments were carried out at room temperature. Plates were placed “face down” in the Latch-Lid Chromatotank chamber (General Glass Blowing Company)

5) Ceramide development system. Plates were put in the chamber with 25 ml of

a) chloroform (Cat # 650498, Sigma). By capillary action, chloroform rose on the plate. When it reached a 15 mm height of the TLC plate, the plate was removed from the chamber and dried under an air stream at 40°C on the heat block of the Reacti-Therm III Heating Module for 10 minutes. During this time, the solvent was removed from the chamber. When the plate was dried, 25 ml of the next solvent was poured into the chamber.

b) chloroform/acetone (Cat # 179124, Sigma)/methanol (Cat # L6804, Fisher) (18:2:4). When the solvent reached a 10 mm height of the TLC plate, the plate was removed from the chamber and processed as described in a) above.

c) chloroform/methanol/hexyl acetate (AC14850-0010, Acros Organics)/acetone (21.5:1:0.5:2.5). When the solvent reached a 70 mm height of the TLC plate, the plate was removed from the chamber and processed as described in a) above.

d) chloroform/acetone/methanol (19:1:5) When the solvent reached a 20 mm height of the TLC plate, the plate was removed from the chamber and processed as described in a) above.

e) chloroform/methanol/diethyl ether/ethyl acetate/hexyl acetate/acetone (18:1:1:1:0.5:4). When the solvent reached a 75 mm height of the TLC plate, the plate was removed from the chamber and processed as described in a) above.

f) hexane (Cat#34859, Sigma)/diethyl ether/ethyl acetate (Cat # 33000000, Pharmco-AAPER) (20:4:1). When the solvent reached a 90 mm height of the TLC plate, the plate was removed from the chamber and dried under air stream at 40°C on the heating block of the Reacti-Therm III Heating Module for 10 minutes.

6) Staining. After final drying, plates were heated for 5 min at 130°C using the Isotemp Oven (Fisher Scientific). Plates were submerged into a staining solution containing acetic acid, H_2SO_4, H_3PO_4, H_2O (5:1:1:95) and 3.0% copper (II) sulfate for 10 sec.

7) After staining, the plates were dried at 60°C on a heating block of the Reacti-Therm III Heating Module for and then charred at 180°C for 10 min. A FOTO/Analyst® Investigator/Eclipse workstation was used to estimate the quantities of each band relative to each other on a plate, while ImageJ was used to get a more precise density measurement on a 100 megabytes image of the plate.

Assignment of lipid bands. Our separation method closely follows the "ceramide development system" presented by Ponec & Weerheim (Ponec et al., 2003). This method separates the lipids according to their polarity with the least polar lipids at the top of the HPTLC plate. Cer9 presents just below ceramide Cer2 (Jungersted et al., 2010; Ponec and Weerheim, 1990). Cer2 presents as two bands, a large upper band and a smaller, lower band (Breiden et al., 2007; Ponec and Weerheim, 1990). Jungersted and colleagues do not show the second band of Cer2. This led to initial concern as to how to assign the bands seen on our plates. However, Ponec and Weerheim show that Cer9 presents as a much lighter band than Cer2. This matched our data best, and we agreed the much lighter band below the second band of Cer2 as Cer9. Cer8 appears below

ceramide Cer5 in (Breiden et al., 2007; Ponec and Weerheim, 1990). Hence, we assigned our bands accordingly.

Inhibition of EGFR Signaling Pathways Studies

Chemicals used to inhibit PI3K, MEK, p38, PKC, and Rac1 were wortmannin (Cat # 681675, Calbiochem), U0126 (Cat # 662005, Calbiochem), SB20358 (Cat # 559389, Calbiochem), Gö6983 (Cat # 365251, Calbiochem), NSC23766 (100 µM, Cat # 553502, Calbiochem), respectively. Fifth passage NHEKs were grown in KSFM supplemented with 5 ng/ml EGF and 50 µg/ml bovine pituitary extract. After 48 hours of the last addition of complete media, plates were randomized and pre-treated 60 min in basal media with either wortmannin (400 nM), U0126 (10 µM), SB20358 (20 µM), Gö6983 (10 µM), or 0.1 % DMSO (D2650-5X5ML, Sigma). Cells were then treated with or without EGF (10 ng/ml) for 4 h or 24 h. For statistical analysis, 3 replicates were isolated at each time point. Another separate experiment was done with NSC23766 (100 µM, Cat # 553502, Calbiochem). NHEKs were grown as previously described. After 48 h of the last addition of complete media, plates were randomized and pre-treated for 16 h in basal media with NSC23766. Then, cells were treated with or without EGF (10 ng/ml) for 4 or 24 h. For statistical analysis, 3 replicates were isolated at each time point.

Statistical Analysis

The statistical tests that were used to determine significance between groups were carried out using Prism 3.0 or Microsoft Excel 2007. The specific test used for each analysis is listed in each figure legend.

EGFR REGULATION OF EPIDERMAL BARRIER FUNCTION

EGF Affects Keratinocyte Cell Fate

Cell density is a major determinant of keratinocyte cell fate (Poumay and Pittelkow, 1995), acting through intercellular signaling mechanisms to affect the expression of thousands of genes (Gazel et al., 2003; Radoja et al., 2006). However, the role of EGF in regulating these genes has yet to be described. Our interest was to determine the genome-wide effects of EGFR activation on epidermal barrier function. Using microarray technology, we identified 4,685 density-dependent genes (Figure 7). Of these genes, 2,676 genes were found to be regulated by EGF. Contrary to the effects of density, EGF repressed the expression levels of 91% of the density-induced genes and elevated the level of expression of 96% of the density-repressed genes. The former observation suggested that EGF plays a critical role in inhibiting keratinocyte differentiation. Specifically, EGF affected 1,039 genes that had explicit, as well as implicit, relationships to epidermal differentiation, based on the literature. In order to display the microarray profiles of the epidermis associated genes, we prioritized 72 genes of the 1,039 genes that were highly annotated to epidermal differentiation (Figure 7). A heat map of the 72 genes is shown in Figure 8A. Among the 72 genes, EGF down-regulated 83% of the density-induced genes and up-regulated all of the density-repressed genes. To validate this observation, we performed qRT-PCR on well-established suprabasal (*KRT1* and *FLG*) and basal (*LAMA3* and *LAMC2*) expressed genes (Figure 8B). As qRT-PCR results confirmed this relationship and the genes in these clusters appeared to associate with the differentiating or proliferating cell compartments of the epidermis, we further explored this association for all 72 genes. An intriguing finding regarding the 72 genes was that

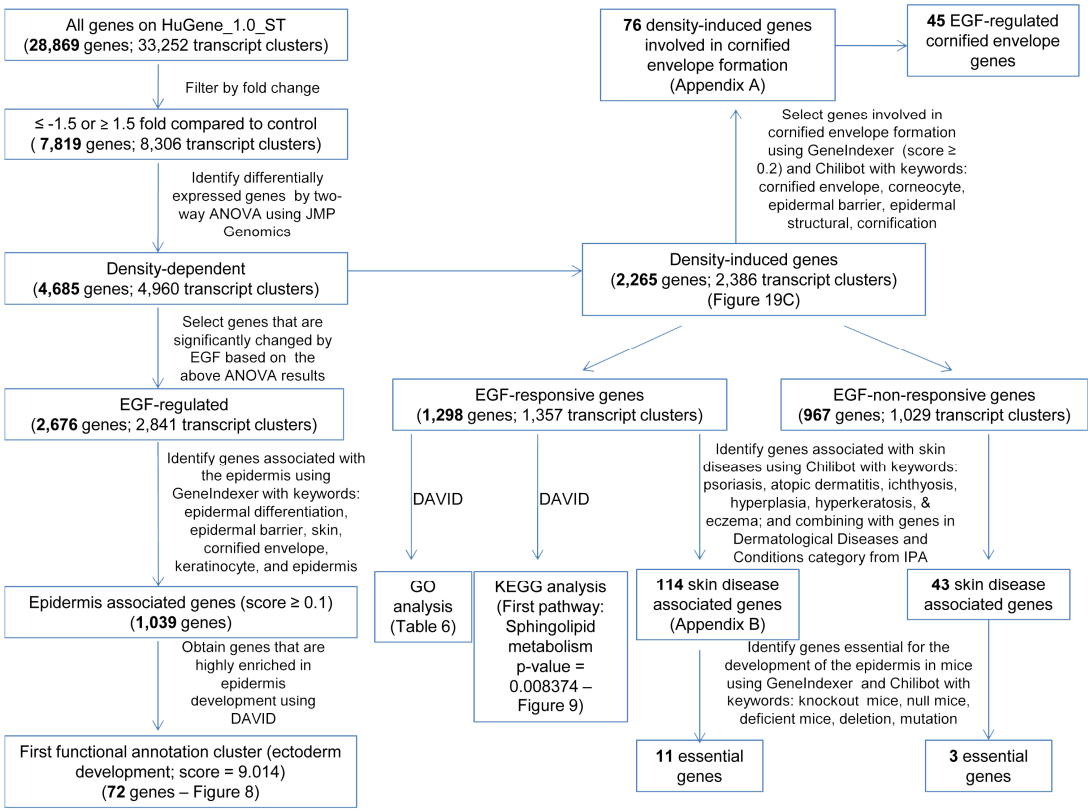

Figure 7. Diagram of the Gene Expression Data Analysis Workflow for Microarray 1

On Human Gene 1.0 ST arrays, probes are grouped into transcript clusters. A known or putative gene can be represented by one or more transcript clusters. Contrary to the effects of density, EGF repressed the expression levels of 91% of the density-induced genes and elevated the levels of expression of 96% of the density-repressed genes. This observation suggested that EGF plays a critical role in inhibiting keratinocyte differentiation. Hence, we used GeneIndexer to find 1,039 genes that had explicit, as well as implicit, relationships to epidermal differentiation. In order to display a visible heatmap of the microarray profile of the epidermis associated genes, we reduced the 1,039 genes to 72 genes that were highly annotated to epidermal differentiation. Our interest was to determine the biological effects of EGF on keratinocyte differentiation. During keratinocyte differentiation, most of the differentiation markers and suprabasal genes are induced (Table 5). Hence, our analysis was focused on the density-induced genes. From the list of density-induced genes, we identified genes associated with skin diseases, as dysregulation of the EGFR signaling pathways have been associated with skin diseases. We also performed gene ontology, KEGG, and bioinformatics analyses on the density-induced EGF-responsive genes.

most of the density-upregulated genes are expressed in the suprabasal layers (spinous, granular, and cornified layers) whereas most of the density-downregulated genes are expressed in the basal layer of the epidermis (Table 5). This observation suggests that EGF may affect the mRNA levels of genes expressed not only in the differentiating keratinocytes but also in the proliferative keratinocytes; thus, affecting the homeostasis of the epidermis. To determine the physiological relevance of this observation, we compared the effects of EGF and TGF-α, an endogenous ligand expressed in human keratinocytes (Coffey et al., 1987) on mRNA level of epidermal keratinocyte genes. We observed that they produced almost identical responses (Figure 8C), supporting the use of EGF to study the action of EGFR signaling in our study.

Activation of EGFR signaling in NHEKs is shown to be essential for the G1/S cell cycle progression and for the inhibition of CE formation and differentiation-related genes (Kobayashi et al., 1998; Sun and Green, 1976). Inhibition of EGFR signaling opposes these effects and promotes terminal differentiation (Peus et al., 1997; Sutter et al., 2009). Recently, a study using organotypic cultures indicated that DSG1 is required to suppress the sustained activation of EGFR/ERK signaling in the granular layer and acts together with other desmosomal proteins such as DSG2 and DSC3 to support a signaling network that balances keratinocyte proliferation and differentiation to maintain epidermal tissue homeostasis (Getsios et al., 2009). Here, EGF decreased RNA and protein levels of DSG1 and atranscription factor known to regulate its expression, GRHL1 (Figure 8D). Mice lacking *Grhl1* develop hair loss and plamoplantar keratoderma as observed in humans with *DSG1* mutations (Wilanowski et al., 2008). This cross-regulation between

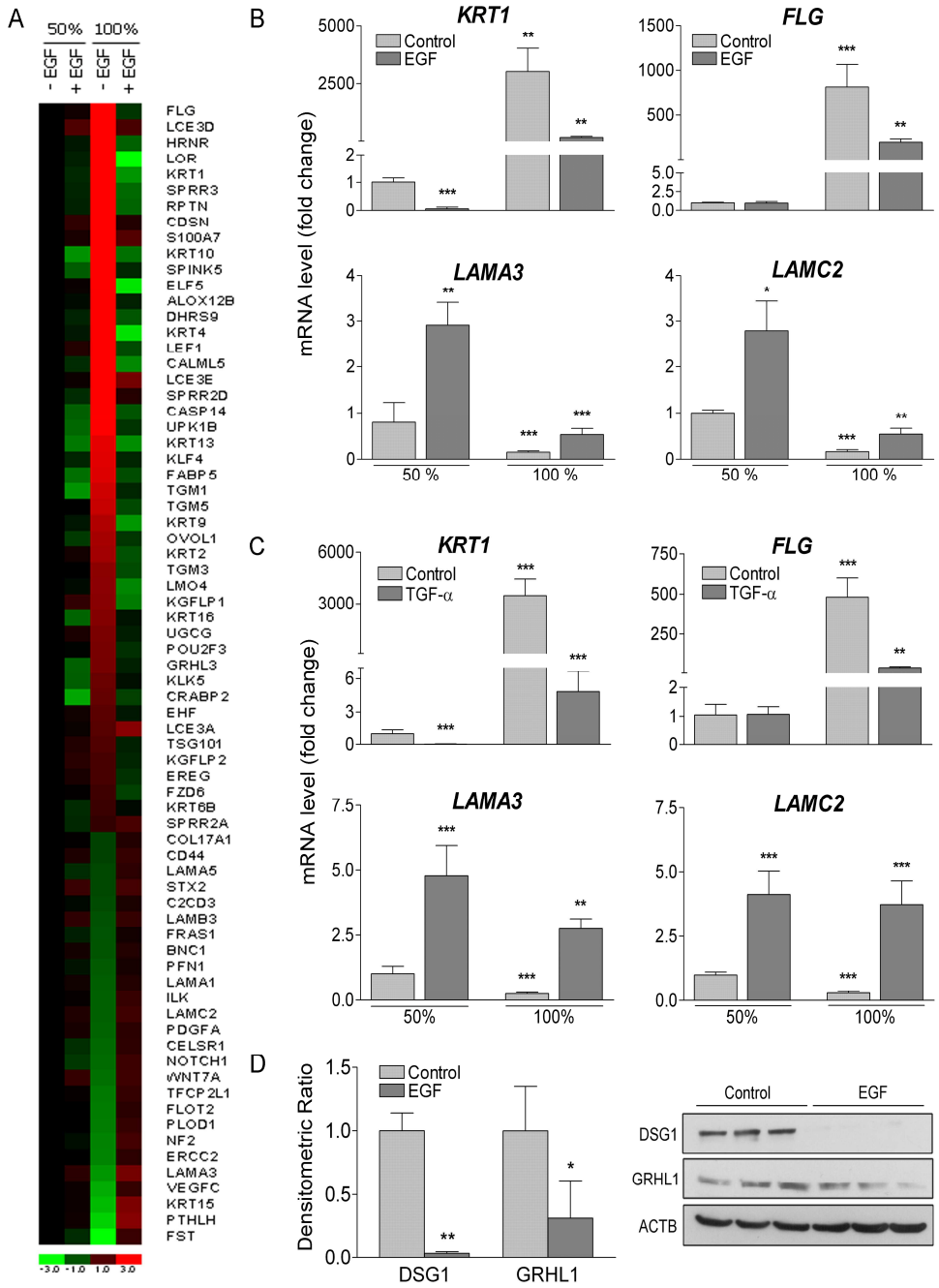

Figure 8. EGFR Regulation of Keratinocyte Cell Fate

(A) Heat map profile of $\log_2$ ratio of 72 genes identified by GeneIndexer and DAVID as highly enriched in ectoderm development (Figure 7). NHEKs were grown to either 50% or 100% confluent cell density before treatment with basal medium, or medium containing EGF (10 ng/ml) for 48 h, with the treatment medium being replaced once at 24 h. The ratios of the means are color-coded to show relative RNA expression. Samples with no EGF and 50% confluent cell density are set as control and color-coded in black. Green indicates down-regulation while red indicates up-regulation of relative gene expression compared to control. Samples grown to 100%

Figure 8. EGFR Regulation of Keratinocyte Cell Fate (cont.)
density with EGF (10 ng/ml) were compared to those without EGF in the same cell density condition, in order to visualize the effects of EGF on density-dependent gene expression (n = 3-4).
(B) Validation by qRT-PCR (n = 3-4) of EGF effects on genes known to be expressed in the suprabasal (*KRT1* and *FLG*) and basal (*LAMA3* and *LAMC2*) layers of the epidermis. *p < 0.017, **p< 0.01.
(C) The effects of TGF-α (50 ng/ml) on genes expressed in the suprabasal (*KRT1* and *FLG*) and basal (*LAMA3* and *LAMC2*) layers as measured by qRT-PCR (n = 4). *p < 0.017, **p< 0.01.
(D) Densitometry (left panel) of protein immunoblots (right panel) for DSG1 and GRHL1 (n = 3). Values are normalized to the loading control ACTB. Cells were grown to 100 % confluent cell density before switching to basal medium with or without EGF (10 ng/ml). The media was replaced with fresh basal media containing the same treatements after 24 h. Cell lysates were harvested 24 h after the last media change.
All bars represent means ± SD. Student's t-test was used to evaluate for statistical significance. *p < 0.05, **p< 0.01, ***p < 0.001. Ŝidák-Bonferonni corrections were used in (B) and (C) with p-value cut off indicated.

DSG1 and EGFR provides a specific example of a mechanism for regulating epidermal homeostasis.

EGF Affects All Major Processes of Epidermal Differentiation

One goal of this research was to determine the biological effects of EGF on keratinocyte differentiation. As shown in Figure 8A, during skin differentiation, most of the genes known to be expressed in the suprabasal layers of the epidermis are upregulated. Therefore, we used 1,298 density-upregulated EGF-responsive genes to uncover aspects of epidermal differentiation that are regulated by EGF. Gene ontology (GO) analysis was performed to identify biological processes and cellular components overrepresented among the 1,298 genes. In Table 6, only nonredundant categories with the largest number of genes and p-value < 10^{-3} or p-value < 10^{-2} are shown for biological process and cellular component, respectively. Since our goal was to understand how EGF regulates the epidermal barrier function, we focused on these categories: lipid biosynthesis, cornified envelope, and cell-cell junction, as they play critical roles in the formation of epidermal barrier. Our next task was to explore these aspects in detail.

Table 5. Literature Support for the Granular- or Basal Stratum-specific Expression of the 72 Genes in Ectoderm Development

Transcript ID	Gene Symbol	Gene Name	Reference*
A. Granular Expressed Genes			
7920165	FLG	filaggrin	Radoja et al, 2006 ;Toulza et al, 2007
7920185	LCE3D	late cornified envelope 3D	Marshall et al, 2001
7920155	HRNR	hornerin	Makino et al, 2001
7905563	LOR	loricrin	Radoja et al, 2006; Toulza et al, 2007
7963491	KRT1	keratin 1	Toulza et al, 2007
7905548	SPRR3	small proline-rich protein 3	Mischke et al, 1996
7920146	RPTN	repetin	Boehnke et al, 2007
8124862	CDSN	corneodesmosin	Radoja et al, 2006; Toulza et al, 2007
7920252	S100A7	S100 calcium binding protein A7	Eckert et al, 2006
8015104	KRT10	keratin 10	Grone et al, 2004
8109001	SPINK5	serine peptidase inhibitor, Kazal type 5	Radoja et al,2006; Galliano et , 2005
7947481	ELF5	E74-like factor 5 (ets domain transcription factor)	Oettgen et al, 1999
8012309	ALOX12B	arachidonate 12-lipoxygenase, 12R type	Radoja et al, 2006; Toulza et al, 2007
8046124	DHRS9	dehydrogenase/reductase member 9	Everts et al, 2007
7963534	KRT4	keratin 4	Wanner et al, 1996
8102232	LEF1	lymphoid enhancer-binding factor 1	Merrill et al, 2001
7931859	CALML5	calmodulin-like 5	Toulza et al, 2007
7920182	LCE3E	late cornified envelope 3E	Marshall et al, 2001
7920196	SPRR2D	small proline-rich protein 2D	Katou et al, 2003
8026398	CASP14	caspase 14, apoptosis-related cysteine peptidase	Toulza et al, 2007
8081826	UPK1B	uroplakin 1B	Lobban et al, 1998
8015323	KRT13	keratin 13	Murakami and Saito, 1990
8163002	KLF4	Kruppel-like factor 4 (gut)	Radoja et al, 2006
7948420	FABP5	fatty acid binding protein 5 (psoriasis-associated)	Radoja et al, 2006; Oettgen et al,1999
7978222	TGM1	transglutaminase 1	Toulza et al, 2007
7988050	TGM5	transglutaminase 5	Radoja et al, 2006
8015357	KRT9	keratin 9	Knapp et al, 1986
7941401	OVOL1	ovo-like 1(Drosophila)	Nair et al, 2006
7963479	KRT2	keratin 2	Virtanen et al, 2010
8060432	TGM3	transglutaminase 3	Radoja et al, 2006
7902810	LMO4	LIM domain only 4	Sugihara et al, 1998 (basal)
8067839	KGFLP1	keratinocyte growth factor-like protein 1	
8015376	KRT16	keratin 16	Le et al, 1998
8157216	UGCG	UDP-glucose ceramide glucosyltransferase	Radoja et al, 2006; Sutter et al, 2009
7944537	POU2F3	POU class 2 homeobox 3	Toulza et al, 2007
7898916	GRHL3	grainyhead-like 3 (Drosophila)	Yu et al, 2006
8038670	KLK5	kallikrein-related peptidase 5	ishida-Yamamoto et al, 2005
7921099	CRABP2	cellular retinoic acid binding protein 2	Radoja et al, 2006
7939314	EHF	ets homologous factor	Tugores et al, 2001
7920191	LCE3A	late cornified envelope 3A	Marshall et al, 2001
8161478	KGFLP2	keratinocyte growth factor-like protein 2	
8095728	EREG	epiregulin	
8147766	FZD6	frizzled homolog 6 (Drosophila)	Romanowska et al, 2009 (basal)
7963406	KRT6B	keratin 6B	Wojcik et al, 2000
7920205	SPRR2A	small proline-rich protein 2A	Radoja et al, 2006; Mischke et , 1996
7947015	TSG101	tumor susceptibility gene 101	Oh et al, 2002

*The literature was manually collected from Pubmed.

Table 5. Literature Support for the Granular- or Basal Stratum-specific Expression of the 72 Genes in Ectoderm Development (cont.)

Transcript ID	Gene Symbol	Gene Name	Reference*
B. Basal Expressed Genes			
7936144	COL17A1	collagen, type XVII, alpha 1	Radoja et al, 2006
7939341	CD44	CD44 molecule (Indian blood group)	Kaya et al, 1997
8067409	LAMA5	laminin, alpha 5	Tateishi et al, 2010
7967685	STX2	syntaxin 2	Butt et al, 1996
7950336	C2CD3	C2 calcium-dependent domain containing 3	
7924029	LAMB3	laminin, beta 3	Radoja et al, 2006
8095907	FRAS1	Fraser syndrome 1	Pavlakis et al, 2008
7991080	BNC1	basonuclin 1	Tseng, 1998
8011759	PFN1	profilin 1	ubiquitously expressed
8022176	LAMA1	laminin, alpha 1	Miner et al, 2004
7938154	ILK	integrin-linked kinase	Lorenz et al, 2007
7908072	LAMC2	laminin, gamma 2	Meng et al, 2003
8137670	PDGFA	platelet-derived growth factor alpha polypeptide	Radoja et al, 2006
8076757	CELSR1	cadherin, EGF LAG seven-pass G-type receptor 1	Devenport and Fuchs, 2008
8165217	NOTCH1	Notch homolog 1, translocation-associated (Drosophila)	Baldi et al, 2004 (suprabasal)
8085475	WNT7A	wingless-type MMTV integration site family, member 7A	Radoja et al, 2006
8054872	TFCP2L1	transcription factor CP2-like 1	
8013788	FLOT2	flotillin 2	Sasaki et al, 2008 (lipid rafts)
7897803	PLOD1	procollagen-lysine 1, 2-oxoglutarate 5-dioxygenase 1	Radoja et al, 2006
8072242	NF2	neurofibromin 2 (merlin)	Stamenkovic and Yu, 2010
8037537	ERCC2	excision repair cross-complementing, group 2	
8020551	LAMA3	laminin, alpha 3	Radoja et al, 2006
8103822	VEGFC	vascular endothelial growth factor C	
8015337	KRT15	keratin 15	Radoja et al, 2006
7962000	PTHLH	parathyroid hormone-like hormone	Grone et al, 1994
8105302	FST	follistatin	Mukhopadhyay et al, 2006

*The literature was manually collected from Pubmed.

Table 6. Significant Gene Ontology Terms Associated with the 1,298 Density-upregulated EGF-responsive Genes

Category	Number of genes	p-value
Biological Process		
ectoderm development	46	7.88E-14
epidermal cell differentiation	21	1.44E-08
keratinization	13	8.67E-06
lipid biosynthesis process	39	3.06E-04
Cellular Component		
cornified envelope	8	3.63E-04
intermediate filament cytoskeleton	24	7.89E-04
late endosome	12	8.83E-04
cell-cell junction	23	5.90E-03

EGF Decreases Free Fatty Acid (FFA) and Ceramide Biosynthesis

Lipid organization is essential for the epidermal barrier function. It plays an important role in cohesion and desquamation of the SC as well as in prevention of excess water loss from the human body and penetration of unwanted influences from the environment (Elias and Menon, 1991; Ponec and Weerheim, 1990). The major lipid components isolated from the cornified epidermal layers are ceramides (Cer), cholesterol, and free fatty acids (FFA). The biosynthesis of these lipid classes requires the presence of many enzymes. Hence, we studied the effect of EGF not only on all lipid classes in cultures of NHEKs, but also associated this effect with the changes in expression of genes encoding lipid biosynthetic enzymes. The lipid biosynthesis process was enriched in the GO analysis (Table 6). Moreover, using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, the sphingolipid metabolism pathway was also overrepresented (p-value = 0.008374) (Figure 6 and 8). This indicates that EGF may alter lipid components in epidermal keratinocytes. We explored this possibility by mining and validating the microarray expression profiles of genes encoding enzymes in the sphingolipid and free fatty acid biosynthetic pathways. Figure 9 highlights the effects of EGF from the microarray data on the expression of density-dependent genes in the KEGG sphinglolipid metabolism pathway, with 71% of the RNAs encoding enzymes in this pathway being significantly altered by EGF. Cers found in the SC are generated via two pathways: (1) *de novo* synthesis which builds Cers from less complex molecules, and (2) the salvage pathway which breaks down more complex sphingolipids to release sphingosine, which is then re-acylated to form Cers (Figure 10). These Cers are then packaged into lamellar bodies (LB) as glucosylCer (GlcCer) and sphingomyelin (SM).

Following extrusion of the LB at the intersection of SG and SC, the GlcCer and SM are hydrolyzed back to Cers (Uchida and Holleran, 2008). We observed that EGF decreased expression of enzymes not only in the *de novo* pathway, but also in the salvage pathway. In the *de novo* synthesis, EGF decreased the expression of *SPTLC3*, a gene encoding for an enzyme catalyzing the rate limiting step of the pathway, *LASS3*, the gene encoding for the major epidermal dihydroceramide synthase, and *DEGS2*, the gene encoding for the major epidermal dihydrocermide desaturase/4-hydroxylase that produces the phytoceramides. In the salvage pathway, EGF decreased the expression of *SGPP2*, *ASAH1*, *ACER1*, and *LASS3* (Figure 10 and 11). The expression of *UGCG* and *SGMS2*, enzymes which make GlcCer and SM from Cer, respectively (Uchida and Holleran, 2008), was down-regulated by EGF (Figure 10 and 11). Treatment with EGF showed no significant effect on the expression levels of *GBA* and *SMPD3* (Figure 10). qRT-PCR validated all microarray expression profiles of other genes (*SPHK1* and *SPGL1*) in the sphingolipid biochemical pathway (Figure 10), except the expression of *SPGL1*. These results suggest that ceramide production is significantly reduced as EGF regulates many biosynthetic enzymes including those catalyzing the rate limiting steps.

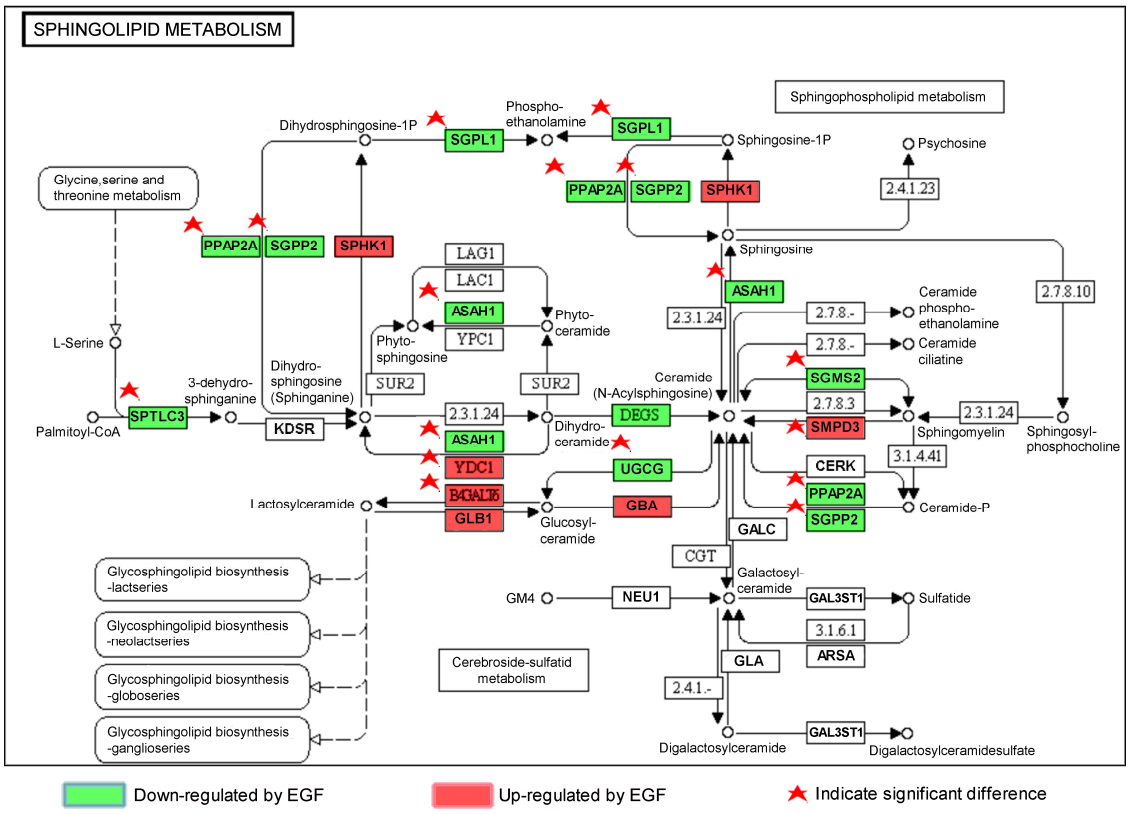

Figure 9. Sphingolipid Metabolism from the KEGG Pathway Database
An overlay of genes in the list of 1,298 density-upregulated EGF-responsive genes on the KEGG sphinglolipid metabolism pathway (Figure 7). Transcripts for biosynthetic enzymes that are significantly affected by density from the microarray were selected to be studied for the effects of EGF. Light green indicates down-regulation by EGF, while light red indicates up-regulation by EGF. The star indicates a significant difference between control and EGF treated samples at 100% confluent cell density, using the Tukey-Kramer honestly significant difference test.

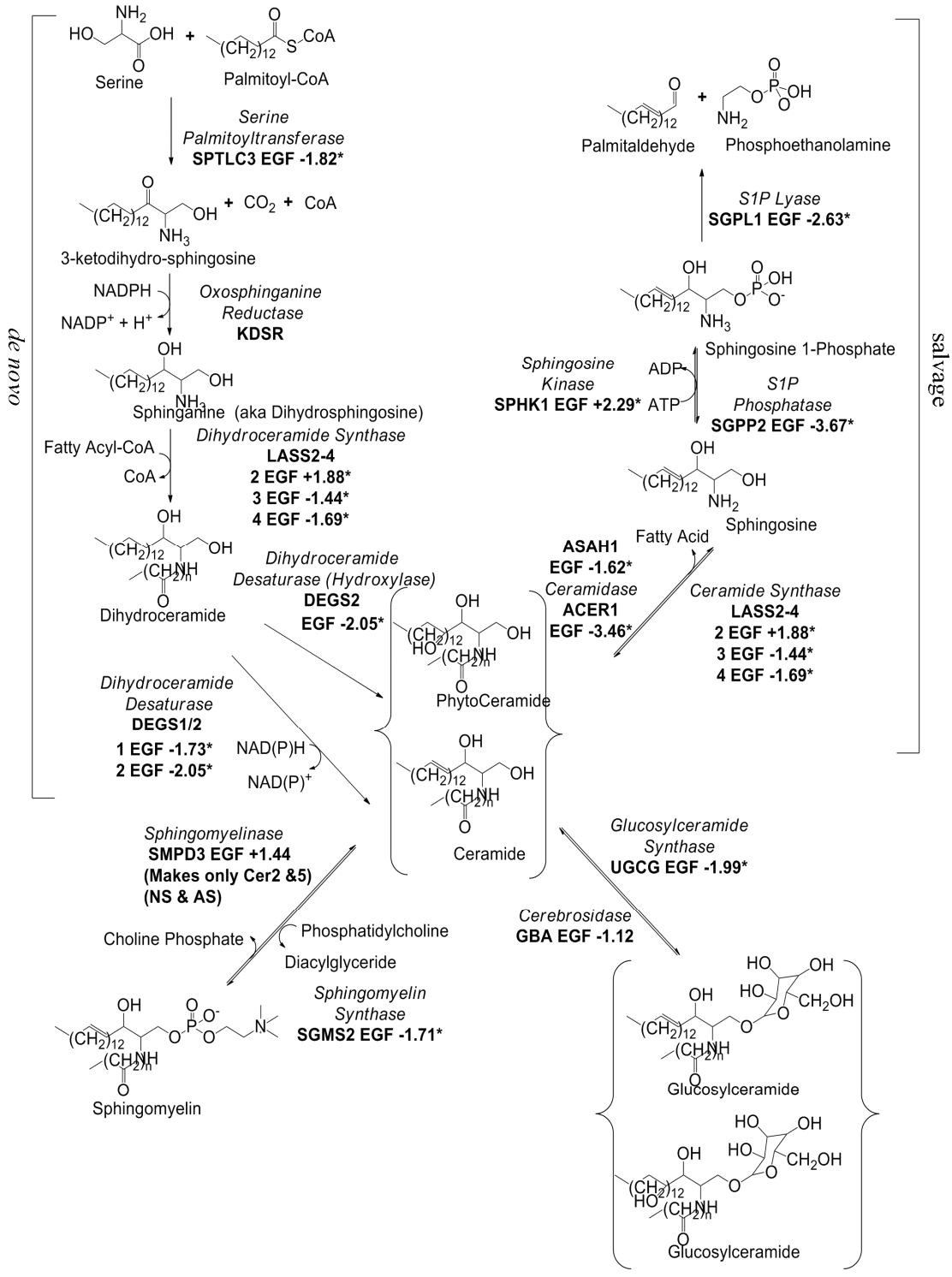

Figure 10. Sphingolipid Biochemical Pathways

Figure 10. Sphingolipid Biochemical Pathways (cont.)
This pathway was constructed based on (Uchida and Holleran, 2008) and (Feng and Prestwich, 2005). The initial condensation, catalyzed by SPTLC, is also the rate-limiting step for *de novo* synthesis, forming 3-ketodihydro-sphingosine (Hornemann et al., 2009). This product is then rapidly reduced to sphinganine, which is then acylated by a member of the LASS family of enzymes to form dihydroceramide. LASS3 is the most relevant isozyme for ceramides of the epidermal barrier due to its fatty acyl-CoA chain length specificity (Stiban et al.). Dihydroceramide is then desaturated by DEGS1/2 or hydroxylated by DEGS2 (exclusively), to form ceramides and phytoceramides, respectively. Cer5 and Cer2 may also be formed via the salvage pathway by LASS acylation of sphingosine that is already present in the cell. These reactions occur in the endoplasmic reticulum. At this point, the pool of newly formed ceramides and phytoceramides are trafficked to the Golgi apparatus. UGCG glycosylates all ceramides and phytoceramides to form glucosylceramides. SGMS catalyzes the addition of choline phosphate (from phosphatidylcholine) to Cer5 and Cer2 to form sphingomyelin. These two products (glucosylceramides and sphingomyelin) are packaged into the lamellar bodies, which are extruded at the intersection of the stratum granulosum and stratum corneum.
Effects of EGF (relative fold changes) determined by qRT-PCR are shown. * indicates that the effects of EGF are significant by t-test ($p < 0.05$).

We then investigated the next lipid component, FFA. We first built the FFA biochemical pathway based on the KEGG fatty acid biosynthesis pathway (Figure 12). The microarray expression of all enzymes in the FFA biochemical pathway was validated by qRT-PCR. EGF inhibited 3 out of 4 major steps of fatty acid elongation as it down-regulated the RNA levels of *ELOVL3*, *ELOVL4*, and *ELOVL6* (condensation), *PTPLB* (dehydration), and *TECR* (reduction) significantly (Figure 13). The down-regulation of *PTPLB* and *TECR* expression would lead to less stearic acid formation, which in turn restricts the production of oleic acid. These acids contribute about 50% to the fatty acid composition in the SC (Lampe et al., 1983). Based on these results, we hypothesized that EGF could decrease total ceramide and FFA production in epidermal keratinocytes.

To test our hypothesis, we used high performance thin-layer chromatography (HPTLC). We observed that levels of cholesterol were unchanged, while levels of FFA

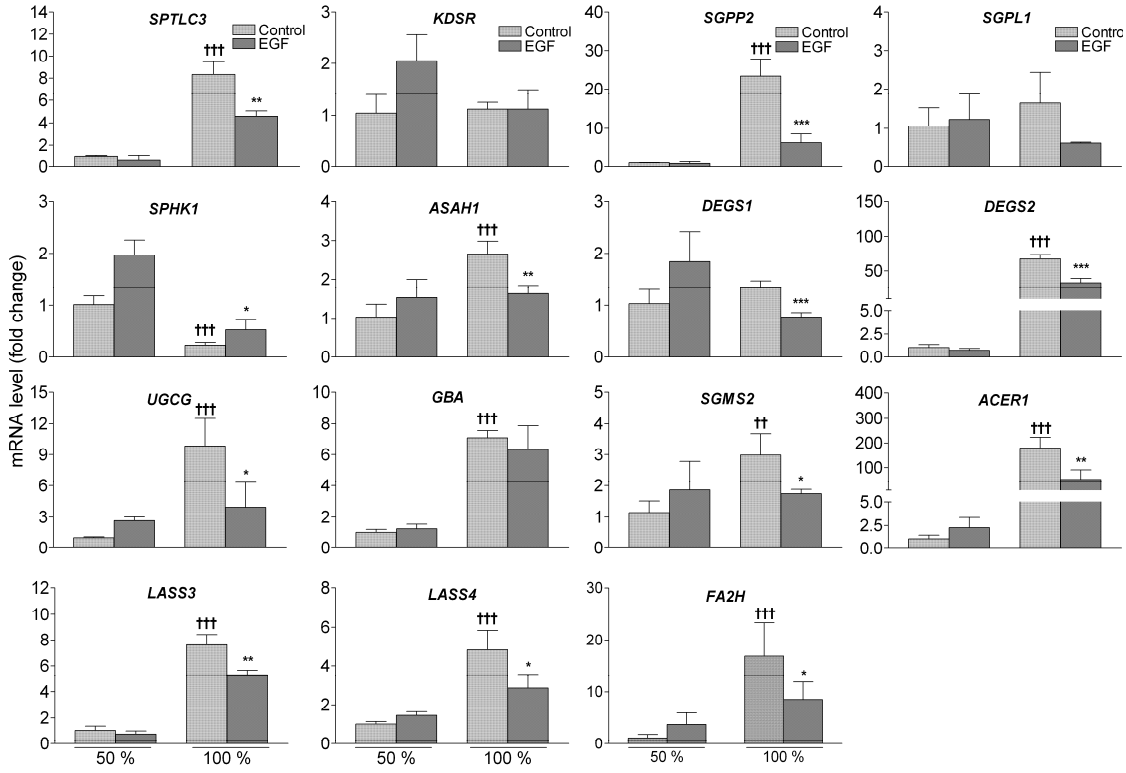

Figure 11. qRT-PCR Analysis of Transcripts Encoding Sphingolipid Biosynthetic Enzymes

Validation of microarray expression profiles of genes encoding sphingolipid biosynthetic enzymes. NHEKs were grown to either 50% or 100% confluent cell density before treatment with basal medium, or medium containing EGF (10 ng/ml) for 48 h, with the treatment medium being replaced once at 24 h . † denotes comparison between the untreated samples (confluent density effect). * denotes the comparison between control and EGF at 100% confluent cell density. All bars indicate means ± SD (n = 3-4). Student's t-test with Ŝidák-Bonferonni correction was used (where applicable) to evaluate statistical significance. $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, $*p < 0.0253$ (Ŝidák-Bonferonni correction p-value cut off), $**p < 0.01$, $***p < 0.001$.

were reduced by 57%. Several Cer species were also significantly decreased. Inhibition of genes encoding for enzymes in the *de novo* and salvage ceramide pathways by EGF, as shown in the qRT-PCR results (Figure 10 and 11), corresponds to a decrease in some 6-hydroxylated Cers, i.e. Cer7 and Cer8 (Figure 14). EGF also reduced the production of acylGC, a product associated with expression levels of the Cer and GlcCer synthases

(*LASS3*, *LASS4* and *UGCG*, respectively). The 11% reduction in acylGC synthesis likely led to a decrease in Cer1 (Figure 14), a VLCFA containing Cer and the most abundant acylCer in the epidermis. Cer3 and Cer6 production were also diminished, corresponding with the inhibition by EGF of the expression of *DEGS2* (Figure 11 and 14), the enzyme that makes phytoceramides (Ternes et al., 2002). Our microarray and qRT-PCR suggests that the reduction of Cer7 is possibly due to the down-regulation of FA2H (Figure 11), the enzyme that produces the 2-hydroxylated ceramides (Uchida et al., 2007).

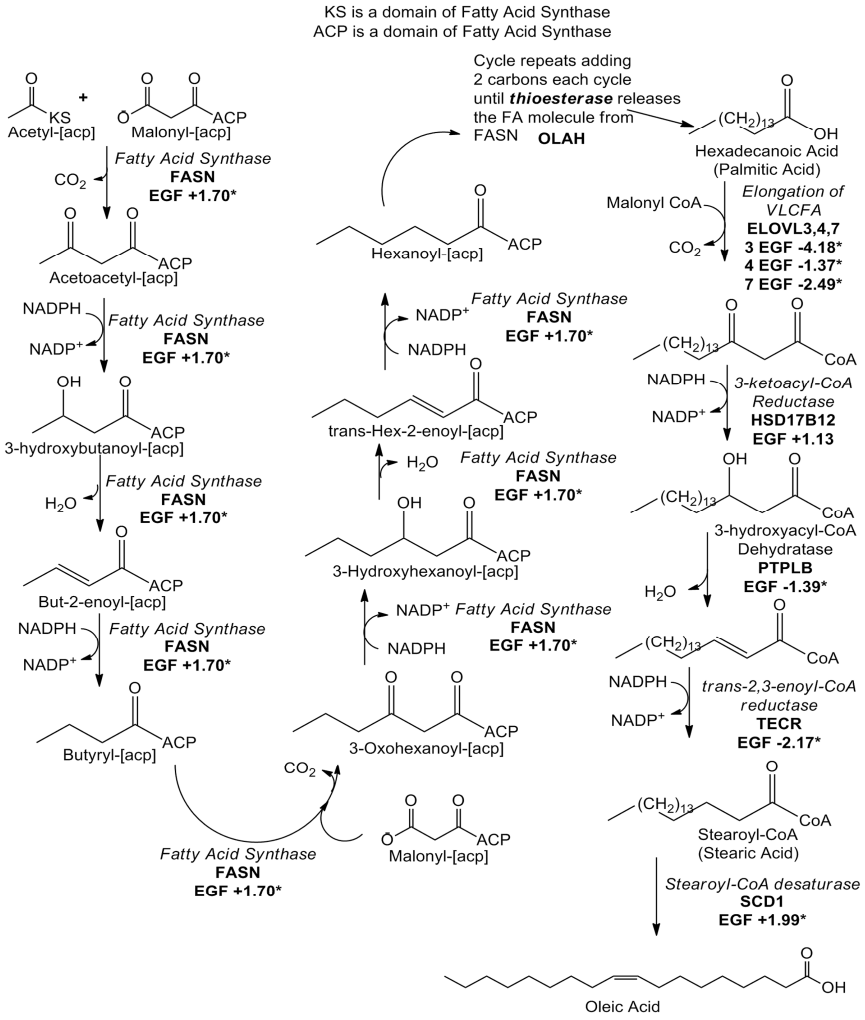

Figure 12. The Free Fatty Acid Biosynthetic Pathway

Figure 12. The Free Fatty Acid Biosynthetic Pathway (cont.)
This pathway was constructed based on the KEGG pathway for FFA synthesis combined with one cycle through the four enzymes that catalyze the four consecutive reactions of fatty acid elongation (Jakobsson et al., 2006). Initial fatty acid synthesis, catalyzed by FASN occurs in the cytosol; elongation occurs in the ER. The initial step of elongation is catalyzed by the ELOVL family of enzymes, and is also the rate-limiting step (Jump, 2009). The majority of the fatty acids present in the epidermal barrier are elongated by ELOVL3 and ELOVL6. ELOVL1 and ELOVL4 catalyze the elongation of the very long chain fatty acid present in the ceramides of the epidermal barrier (Ohno et al.). Additionally, FA2H (not shown in this figure) hydroxylates FFA to form 2-OH FFA. These 2-OH FFA are part of the pool of FFA that are acylated to sphingoid bases to form ceramides as discussed in Figure 10. Ceramides containing 2-OH FFA include Cer7, Cer6, and Cer5, and are critical for proper formation of the epidermal barrier (Uchida et al., 2007). Effects of EGF (relative fold change) determined by qRT-PCR are shown. KS, keto acylsynthase; ACP, acyl carrier protein. * indicates that the effects of EGF are significant by t-test ($p < 0.05$).

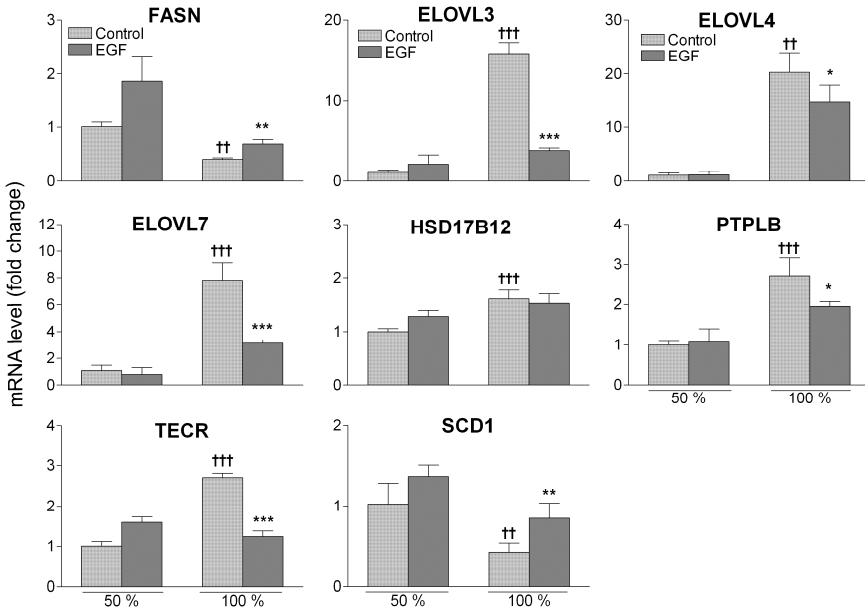

Figure 13. qRT-PCR Analysis of Transcript Encoding FFA Biosynthetic Enzymes
Validation of microarray expression profiles of genes encoding FFA biosynthetic enzymes. NHEKs were grown to either 50% or 100% confluent cell density before treatment with basal medium, or medium containing EGF (10 ng/ml) for 48 h, with the treatment medium being replaced once at 24 h. † denotes comparison between the untreated samples (confluent density effect). * denotes the comparison between control and EGF at 100% confluent cell density. All bars indicate means ± SD (n = 3-4). Student's t-test with Ŝidák-Bonferonni correction was used (where applicable) to evaluate statistical significance. ††$p < 0.01$, †††$p < 0.001$, *$p < 0.0253$ (Ŝidák-Bonferonni correction p-value cut off), **$p < 0.01$, ***$p < 0.001$.

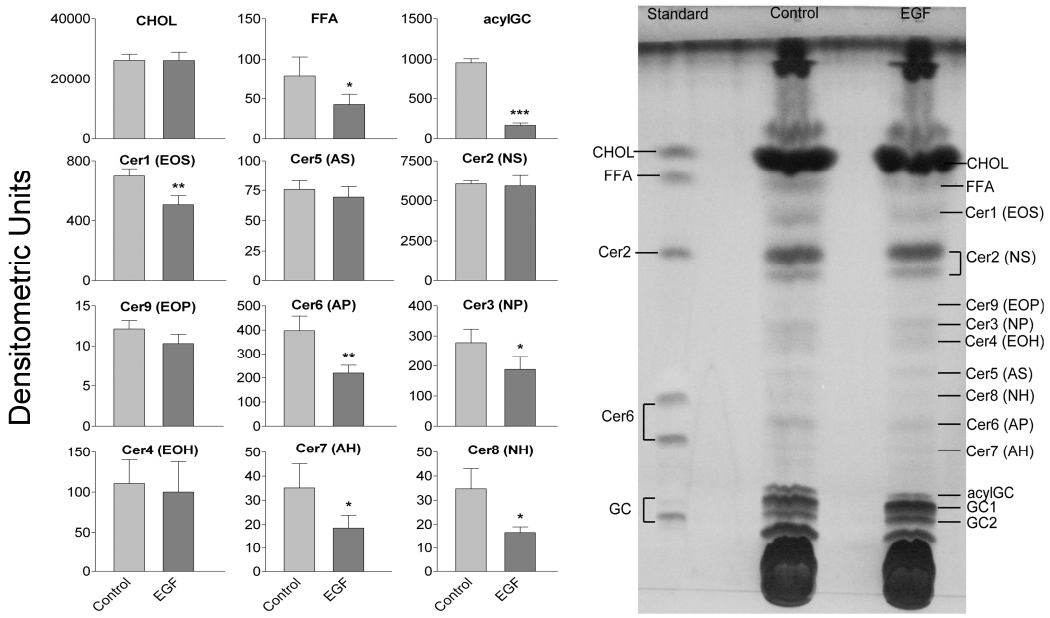

Figure 14. EGF Decreases FFA and Total Ceramide Amount
Densitometric measurements (left) of TLC plates (right). NHEKs were grown to 100% confluent cell density before basal medium or medium with EGF (10 ng/ml) was added. The media was replaced with fresh basal media containing the same treatments after 24 h in the presence of 1.8 mM Ca^{2+}. Lipids were extracted 48 h after the last media change. CHOL, cholesterol; FFA, free fatty acid. The ceramide structures are classified according to the sphingoid base (S: sphingosine; P: phytosphingosine; H: 6-hydroxysphingosine) and the *N*-acyl fatty acid (A: α-hydroxy group, O: ω-hydroxy group, E: acylated in the ω-OH position). All bars indicate means ± SD (n = 3). * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$. Standards (0.2 µg/µl each) (right panel) for cholesterol (CHOL) and linoleic acid (FFA), ceramide NS (Cer2), ceramide AP (Cer6), and glucosylceramide (GC) were used. Assignment of lipid bands is described in details in Chapter 2. On the TLC plate, STD, standard; - EGF, control; + EGF, treatment with EGF (10 ng/ml). Note: semi-synthetic enanpiomeric mixtures were used as standards for Cer6 and for GC.

EGFR Signaling Inhibits Cornified Envelope Competence

The CE structure is formed beneath the plasma membrane of the corneocytes, the terminally differentiated keratinocytes. These structures are covalently bound to Cer lipids in order to provide the effective physical and water barrier functions of the skin. CE formation requires deposition of many proteins that are catalytically cross-linked by the TGM1 enzyme (Elias et al., 2000). The CE was the first cellular component enriched

by GO analysis (p-value = 3.64E-04) (Table 6). This observation led us to investigate the effect of EGF on genes in this category as well as other genes encoding proteins involved in the CE formation. We identified 76 density-upregulated genes that contributed to CE synthesis in epidermal keratinocyte, based on the published literature (Appendix A). Of these genes, EGF significantly altered mRNA levels of 45 of the identified genes including genes encoding *TGMs*, *SPRRs*, late cornified envelope (*LCEs)*, and *S100s*. These proteins are known to be the primary proteins participating in the synthesis of CE (Candi et al., 2005). The expression profiles of *TGM1*, *LOR*, *KRT10*, *KRT1*, and *FLG* were validated by qRT-PCR (Figure 8B and 15). The results of this analysis were consistent with previous studies (Drozdoff and Pledger, 1993; Marchese et al., 1990; Poumay and Pittelkow, 1995), suggesting a reliable effect of EGF on the 40 novel genes identified as EGF regulated in this study.

We further confirmed our microarray data by protein immunoblotting analysis. Cornified envelopes consist of keratins that are enclosed in insoluble proteins that are grouped into bundles by FLG (Candi et al., 2005). Hence, we wanted to determine whether EGF activated the EGFR to lower the protein levels of KRT1 and FLG by using PD153035, a specific and potent EGFR tyrosine kinase inhibitor. In concordance with the microarray and qRT-PCR data, EGF significantly reduced the levels of proFLG, a precursor of FLG, and KRT1 proteins (Figure 16A). This effect was attenuated by the EGFR inhibitor, PD153035. The significant induction of these two proteins in the presence of the inhibitor alone indicates the presence of basal EGFR signaling (Figure16A), possibly activated by TGF-α, an endogenous ligand of EGFR in

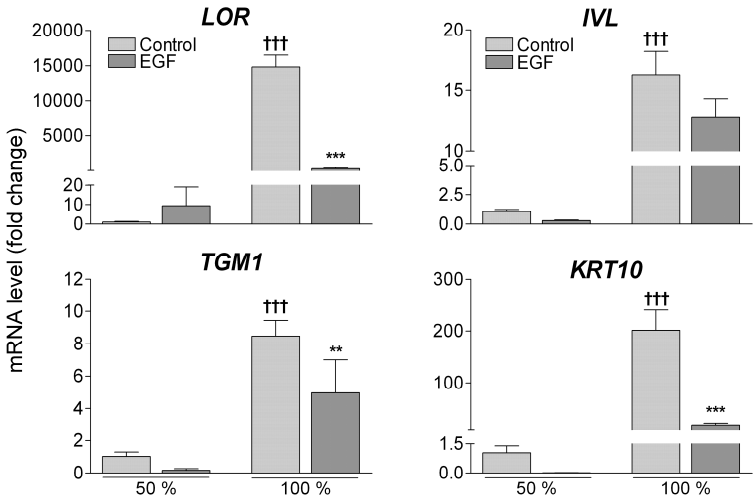

Figure 15. qRT-PCR of Selected Genes Encoding CE Proteins
Validation of microarray expression profiles of some well known CE genes. NHEKs were grown to either 50% or 100% confluent cell density before treatment with basal medium, or medium containing EGF (10 ng/ml) for 48 hr, with the treatment medium being replaced once at 24 hr (n = 3-4). Results are expressed as relative expression from values obtained in response to samples grown to 50% confluent cell density without EGF normalized to values obtained with TUBA1C. Bars represent means ± SD (n = 3-4). † denotes comparison between the untreated samples. * denotes the comparison between control and EGF at 100% cell density. *$p < 0.0253$ (cuf off p-value after Ŝidák-Bonferroni correction), **$p < 0.01$, $^{\dagger}p < 0.0253$ (cut off p-value after Ŝidák-Bonferroni correction), $^{\dagger\dagger}p < 0.01$, ††† $p < 0.001$ by t-test.

keratinocytes (Coffey et al., 1987). Since activation of EGFR remarkably affected the level of proteins forming the CE, we examined this signaling effect on CE competence. Consistent with other studies (Monzon et al., 1996; Sun and Green, 1976), EGF caused a significant decrease in the percentage CE (Figure 16B). However, this reduction was reversed by PD153035, indicating that EGF worked through EGFR signaling to inhibit CE synthesis. These data together with the expression and protein analysis results strongly support the idea that EGFR activation inhibits CE formation by altering levels of enzymes and structural proteins essential for the synthesis of this differentiated structure.

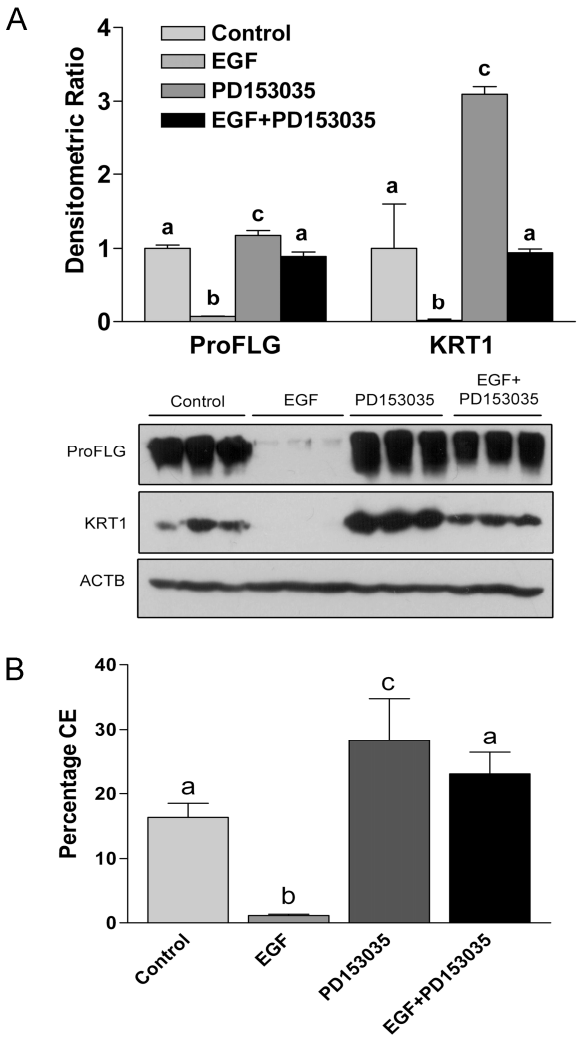

Figure 16. EGFR Signaling Inhibits CE Competence
The effects of EGFR signaling on CE proteins and competence.
(A) Densitometry (above) of immunoblots (below) of Profilaggrin (ProFLG) and keratin 1 (KRT1). ACTB is a loading control. NHEKs were grown to confluence and pretreated with 0.1% DMSO or PD153035 (300 nM) 2 hours before treatment. Basal medium with or without EGF (10 ng/ml) was added in the presence of 0.1% DMSO or PD153035 (300 nM). The media was replaced with fresh basal media containing the same treatments after 24 h. Cell lysates were harvested 24 h after the last media change (n = 3).
(B) EGF effects on CE competence. NHEKs were grown and treated as described in (A). CEs were isolated 3 days after the last media change (n = 3).
All bars denote mean ± SD. One-way ANOVA followed by Tukey's Multiple Comparison Test was performed. If there are different letters in two groups of a comparison, the means of these two groups are significantly different.

EGF Disrupts Tight Junction (TJ) Barrier Function

Cell-cell adhesion was enriched by GO analysis (p-value = 0.0059) (Table 6). In this category we found desmosomal, gap junction, and tight junction (TJs) genes. Recently, evidence has been presented that components of intercellular junctions, *i.e.* TJs, are crucial for the development of the barrier function in the skin. Defects in TJ are observed in patients with AD (De Benedetto et al., 2011). However, to our knowledge there has not been a study of the effects of EGF on tight junction barrier function in NHEKs. First, we validated the mRNA levels of TJ genes by qRT-PCR and found that EGF significantly suppressed the expression of *CLDN1*, *CLDN16*, and *TJP1* (Figure 17). Mice lacking the *CLDN1* gene show rapid postnatal lethality due to impaired TJ barrier function, leading to excessive transepidermal water loss (TEWL) across the skin (Furuse et al., 2002). TJP1, the first TJ component identified, is an intracellular membrane scaffolding protein important for TJ structure and assembly (Stevenson et al., 1986). We therefore investigated the effect of EGF (10 ng/ml) on protein levels of CLDN1 and TJP1 by immunoblot. Treatment with EGF caused a significant decrease in the levels of these two proteins (Figure 18A). This result was confirmed using indirect immunofluoresence. In untreated cultures, we observed that CLDN1 and TJP1 distributed around the circumference of each cell at the apex of lateral membranes. CLDN1 colocalized with TJP1 at areas of cell-cell contact. The staining of these two proteins was reduced substantially in cultures treated with EGF (Figure 18B). These results indicate that EGF inhibits the formation of the TJs, suggesting an increase in paracellular permeability of the barrier.

To determine the effect of EGF on TJ barrier function, we measured transepidermal electrical resistance (TER) and paracellular tracer flux in NHEKs. The TER reflects the transepithelial permeability of water-soluble ions and is a sensitive measure of barrier integrity. A higher TER indicates a lower permeability across a membrane. The TER of the control cultures was measured at 48, 72, and 96 h. The resistance increased 3-fold at 72 h compare to 48 h and remained unchanged at 96 h from the 72 h level (Figure 18C), suggesting a much improved and stabilized permeability barrier function starting at 72 h. However, when EGF was present, this permeability barrier function was significantly disrupted, as indicated by lower TER readings at every time point (Figure 18C). Consistent with the TER results at each time point, a significant increase in the flux of dextrans was observed in the EGF-treated NHEKs compared to untreated ones (Figure 18D), indicating that EGF treatment results in a leaky barrier. The paracellular permeability of TJs exhibits a strong size selectivity, which distinguishes the paracellular route from the transcytosis route. Hence, different sizes of dextran molecules, 3 kDa and 40 kDa, were used to demonstrate the size selectivity of the TJ barrier. We demonstrated that fewer 40 kDa molecules diffused across the membrane compared to the 3 kDa dextran molecules, and that EGF increased the flux of both sizes of dextran molecules across the TJ barrier (Figure 18D). Together, these results indicate that EGF disrupts the permeability barrier function by inhibiting the levels of membrane proteins that form the TJs in cultures of NHEKs.

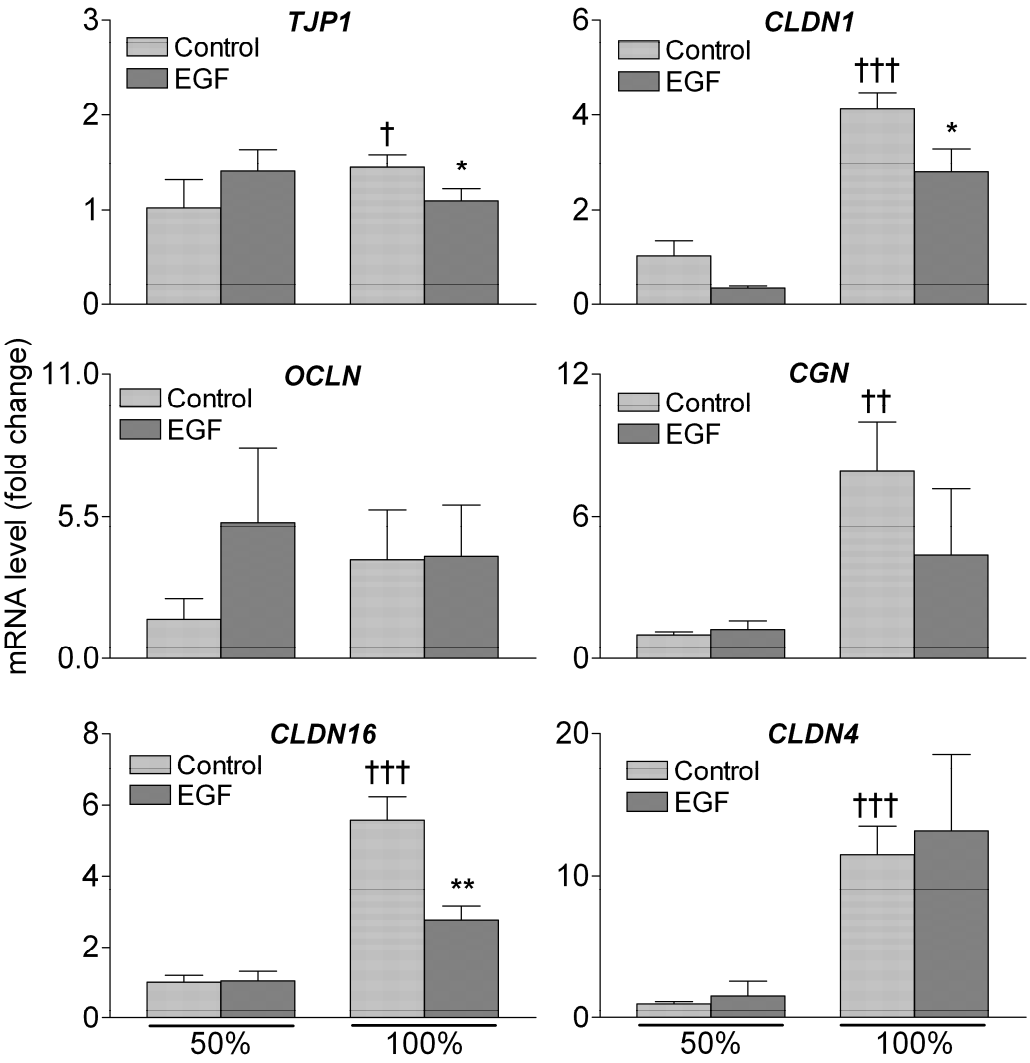

Figure 17. qRT-PCR of Genes Encoding Tight Junction Proteins
Validation of microarray expression profiles of genes encoding TJ proteins. NHEKs were grown to either 50% or 100% confluent cell density before treatment with basal medium, or medium containing EGF (10 ng/ml) for 48 hr, with the treatment medium being replaced once at 24 hr. Results are expressed as relative expression from values obtained in response to samples grown to 50% confluent cell density without EGF, and normalized to the values obtained with tubulin alpha (TUBA1C). Expression of mRNAs from the control is set to 1. Bars represent means ± SD (n = 3-4). † denotes comparison between the untreated samples. * denotes the comparison between control and EGF at 100% confluent cell density. *$p < 0.0253$ (cut off p-value after Ŝidák-Bonferroni correction), **$p < 0.01$, $^{\dagger}p < 0.0253$ (cut off p-value after Ŝidák-Bonferroni correction), $^{\dagger\dagger}p < 0.01$, ††† $p < 0.001$ by t-test.

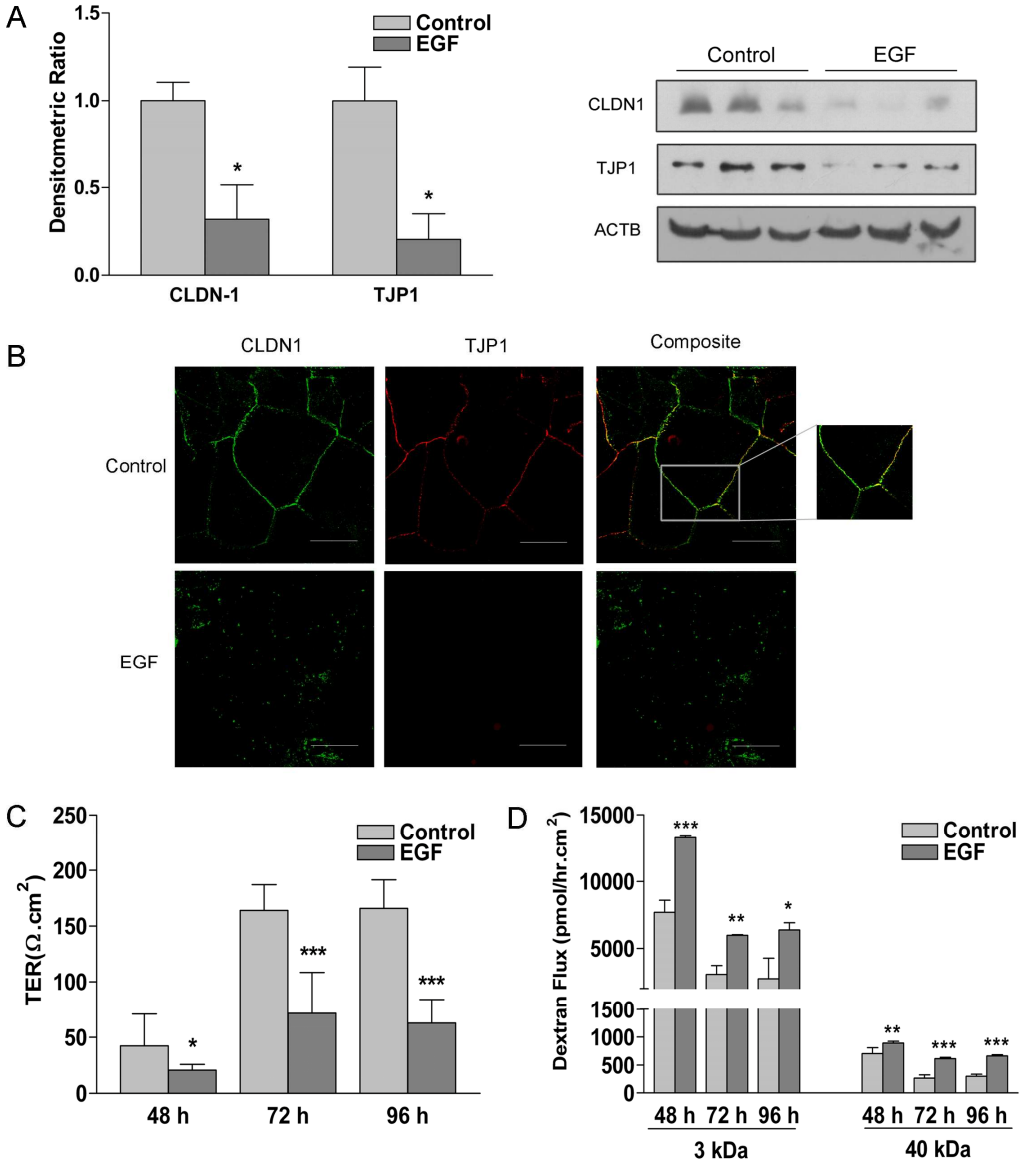

Figure 18. EGFR Signaling Disrupts Tight Junction Barrier Function in Epidermal Keratinocytes

The effects of EGF on TJ barrier function.

(A) Densitometric measurements (left) of junctional protein immunoblots for CLDN1 and TJP1 (right). ACTB is a loading control. NHEKs were grown to confluence before switching to basal medium with or without EGF (10 ng/ml) in the presence of 1.8 mM Ca^{2+}. The media was replaced with fresh basal media containing the same treatments after 24 h. Cell lysates were harvested 72 h after the last media change (n = 3).

(B) Indirect immunofluorescence of CLDN1 and TJP1 in the presence or absence of EGF (n = 3). NHEKs were grown as described in (A). Representative immunofluorescent micrographs of the control and EGF (10 ng/ml) treated monolayers are shown. Scale bar, 50 µm.

Figure 18. EGFR Signaling Disrupts Tight Junction Barrier Function in Epidermal Keratinocytes (cont.)
(C) Transepithelial electrical resistance (TER) of keratinocytes grown on Transwell® filters. NHEKs were seeded at confluence (94,000 cells/insert) and incubated overnight before switching to basal medium or medium with EGF (10 ng/ml) in the presence of 1.8 mM Ca^{2+}. The media was replaced with fresh basal media containing the same treatments after 24 h. TER was measured 48, 72, and 96 h after EGF treatment (n = 6). (D) Paracellular permeability as measured by 3- and 40-kDa dextran flux across the samples in (C) above.
All bars represent means ± SD. *p < 0.05, **p < 0.01, ***p < 0.001. Student's t-test was used to evaluate statistical significance in (A)-(C).

EGF Impairs the Epidermal Barrier Integrity and Preferentially Regulates Genes Associated with Skin Diseases

One of the most important functions of the epidermal barrier is to prevent excessive water loss. To acquire this function, the epidermis requires a permeability barrier together with competent cornified envelopes that are covalently bound to a well organized lipid matrix. To demonstrate the effect of EGF on epidermal barrier function as a whole, we measured TEWL in organotypic cultures. The TEWL of control cultures was 64 ± 7.4 $g/m^2 \cdot h$, similar to what was reported previously (Nolte et al., 1993). EGF caused a 29% increase in the rate of TEWL compared to control cultures (Figure 19A). This increase was accompanied by changes in epidermal morphology. Large basal cells and a less stratified SC with substantial nuclear retention were observed in the EGF-treated cultures (Figure 19B), consistent with a previous study (Chen et al., 1995b). These results demonstrate that EGF impairs the epidermal barrier integrity and function, significantly increasing TEWL.

Defective epidermal differentiation and disruption of epidermal barrier function are primary features of many skin diseases. EGFR signaling plays a relevant role in the control of skin inflammation. Abnormally high levels of the EGFR are observed in

chronic inflammatory skin disorders including psoriasis (Sergi et al., 2000), atopic dermatitis and allergic contact dermatitis (Mascia et al., 2003). Hence, we hypothesized that EGF would alter expression of genes that are mis-regulated in skin disorders. Using Ingenuity Pathway Analysis, Chilibot, and GeneIndexer, we identified 114 EGF-regulated genes associated with skin diseases from the list of 1,298 density-upregulated EGF-responsive genes (Appendix B and Figure 7). In parallel, we identified 43 EGF-nonregulated genes associated with skin diseases from the list of 967 density-upregulated EGF-nonresponsive genes (Figure 18C). Hence, we tested the null hypothesis that there is no assocication between treatments (control versus EGF) and the number of skin diseases genes regulated by these treatments. In order to test this hypothesis, the Fisher's exact test was used and the two tail p-value obtained from the test was 5.31E-5. This p-value indicates that the null hypothesis should be rejected and that there is a strong association between known skin disease genes and the different treatments. In addition, the association tends to lie in the 114 known skin diseases and the set of EGF-responsive genes (Figure 18C). Among the 114 skin disease genes, we found 11 genes that are essential for the development of epidermal barrier function in mice (Appendix B). Figure 19D shows qRT-PCR results of the two transcription factors (*KLF4* and *GATA3*) and two ichthyosis genes (*ALOX12B* and *ABCA12*) that are in the list of essential genes. Consistent with the expression data, the protein level of KLF4 was significantly inhibited by EGF (Figure 19E). qRT-PCR validated the expression profiles of 91% of the 11 essential genes (Figure 19D and Figure 20), affirming the preferential effect of EGF on important skin disease-related genes. Taken together, these results indicate that EGF impairs epidermal barrier function, thus making the skin more prone to many types of

disease by altering the expression of genes and proteins that contribute to these skin diseases.

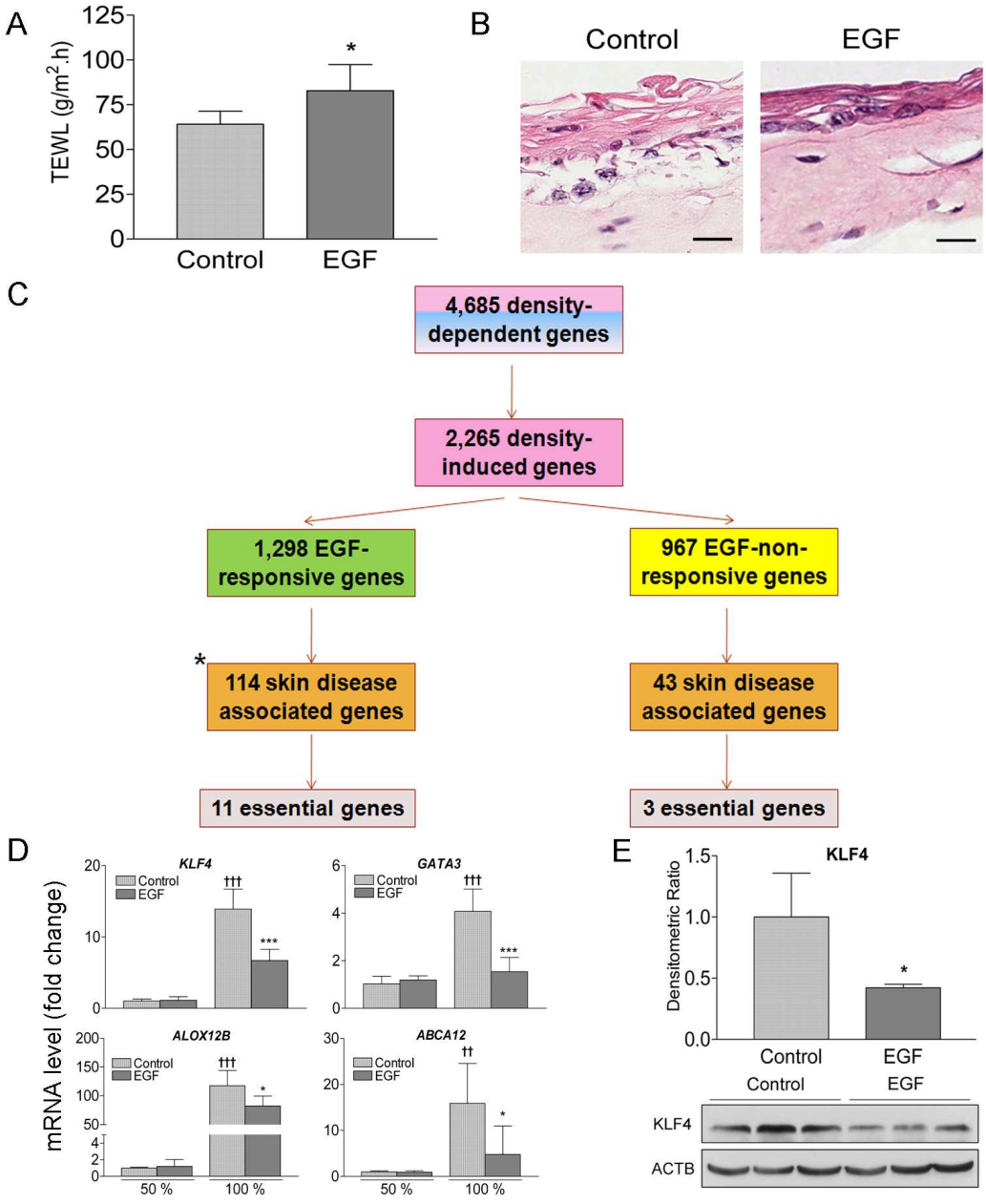

Figure 19. EGF Impairs Epidermal Barrier Integrity and Preferentially Regulates Genes Involved in Skin Diseases

Figure 19. EGF Impairs Epidermal Barrier Integrity and Preferentially Regulates Genes Involved in Skin Diseases (cont.)
Effects of EGF on epidermal barrier integrity as a whole.
(A) EGF increases TEWL of organotypic skin cultures (n = 6). Cultures were incubated with or without EGF (20 ng/ml) throughout the 14 days of air exposure.
(B) EGF induces abnormal keratinocyte morphology as shown by histology (hematoxylin and eosin) of the organotypic cultures from (A). Scale bar, 20 µm.
(C) EGF preferentially regulates genes known to be associated with skin diseases. *The Fisher's exact test was used to determine if there was a significant association between genes related to skin diseases and EGF (p-value = 5.31E-5).
(D) EGF decreases mRNA levels of genes that are essential for the development of epidermal barrier function in mice (n = 3-4). Results are expressed as relative expression from values obtained in response to samples grown to 50% confluent cell density without EGF normalized to values obtained with *TUBA1C*. † denotes comparison between the untreated samples. * denotes the comparison between control and EGF at 100% confluent cell density. Two-way ANOVA followed by Bonferroni post-tests were used to evaluate statistical significance. Additional essential genes are shown in Figure 20.
(E) Densitometry (above) of KLF4 immunoblot (below) (n = 3). Densitometry of control samples are set to 1. ACTB is a loading control. NHEKs were grown to confluence before basal medium or medium with EGF (10 ng/ml) was added. The media was replaced with fresh basal media containing the same treatments after 24 h. Cell lysates were isolated 24 h after the last media change (n = 3).
All bars represent means ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Student's t-test was used to evaluate statistical significance in (A) and (E).

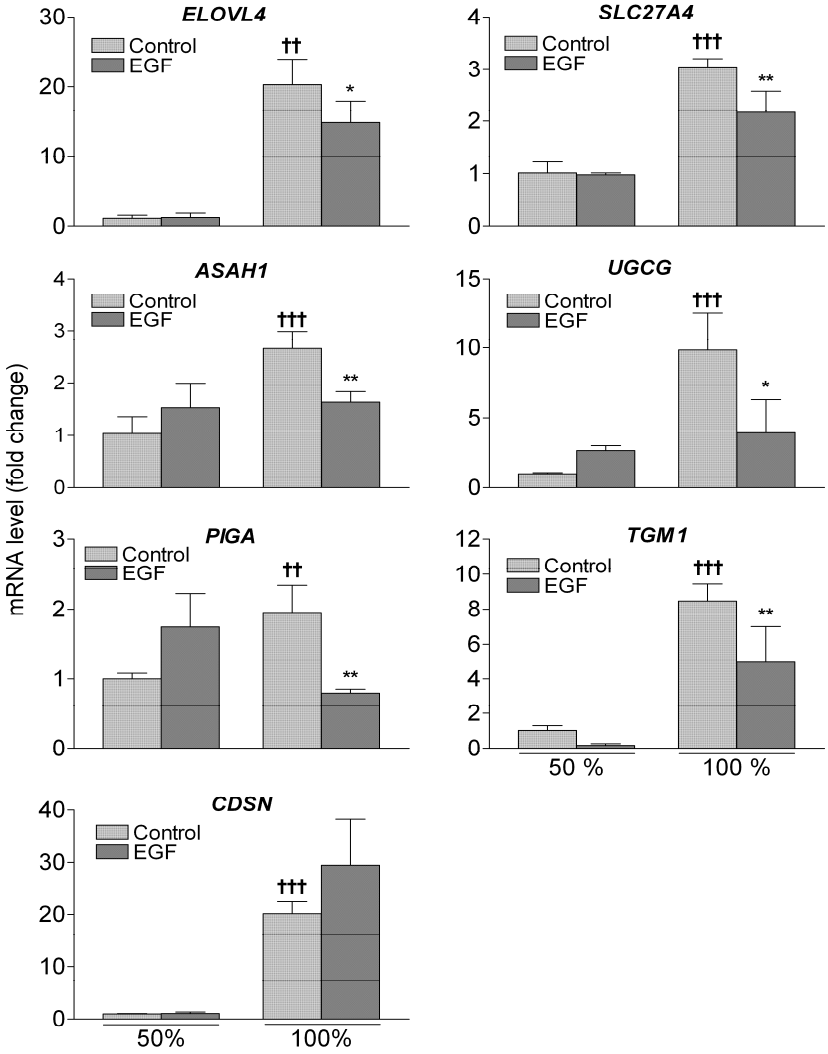

Figure 20. qRT-PCR of Genes that are Essential for the Development of Epidermal Barrier Function

Validation of microarray expression profiles of genes that are essential for the development of epidermal barrier function in mice. NHEKs were grown to either 50% or 100% confluent cell density before treatment with basal medium, or medium containing EGF (10 ng/ml) for 48 h, with the treatment medium being replaced once at 24 h (n = 3-4). Results are expressed as relative expression from values obtained in response to samples grown to 50% confluent cell density without EGF normalized to values obtained with tubulin alpha (*TUBA1C*). Bars represent means ± SD. † denotes comparison between the untreated samples. * denotes the comparison between control and EGF at 100% confluent cell density. Two-way ANOVA followed by Bonferroni post-tests were used to evaluate statistical significance. *$p < 0.05$, **$p < 0.01$, ††$p < 0.01$, ††† $p < 0.001$.

IDENTIFICATION OF EGFR-DIRECTED TRANSCRIPTIONAL NETWORKS THAT REGULATE THE HOMEOSTASIS OF EPIDERMAL KERATINOCYTES

Microarray Analysis and Data Mining

Our previous studies of NHEKs, demonstrated by genome-wide microarray, functional, biochemical, and morphological data that the presence of EGF impairs epidermal differentiation and barrier function via disrupting lipid biosynthesis, CE, and TJ formation. In this second study, we wanted to further explore the molecular mechanisms underlying these inhibitory effects of EGFR signaling in NHEKs. We performed global gene expression analysis of EGF treated cells in a time series (t_0, 1, 2, 4, 8, 16 and 24 h post EGF treatment) using the Affymetrix GeneChip™ Human Genome U133 Plus 2.0 chip, which contains approximately 38,500 annotated genes. In this analysis, 7,819 genes were up- or down-regulated by EGF by at least 2.6-fold (Figure 21). Among these, EGF significantly alters the levels of RNA of 3,033 genes (about 39% of those selected). These genes were then clustered using mFuzz, a soft clustering algorithm (Kumar and Matthias, 2007). mFuzz produced 9 different clusters (Figure 22). Genes in these clusters were then grouped into 2 groups: intermediate and late responsive genes. Intermediate responsive genes were those that were affected by EGF at 4 and/or 8- 16 h, but not at 24 h (cluster 1, 4, 6, and 7 in Figure 22). Genes that have a sustained EGF effect until 24 h are considered late responsive genes (cluster 2, 3, 5, 8, and 9 in Figure 22). In this dataset, we found 1,238 intermediate responsive genes, 1,795 late responsive genes, and did not identify any early genes.

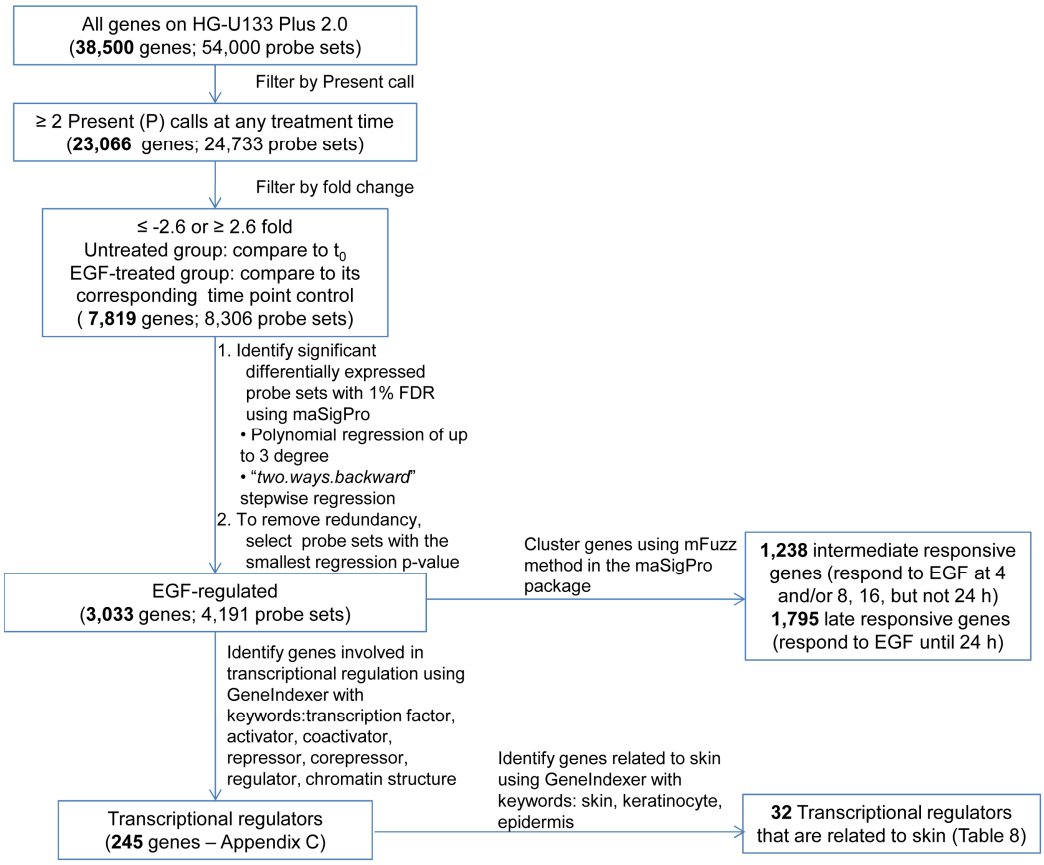

Figure 21. Diagram of the Gene Expression Data Analysis Workflow for Microarray 2

On the HG-U133 Plus 2.0 arrays, a gene can be represented by one or more probe sets. This diagram describes in detail the statistical analysis for the microarray dataset and other bioinformatics analyses used to identify transcriptional regulators. To determine the regulatory network underlying EGF effects, we identified genes encoding for proteins involved in the transcriptional regulation using GeneIndexer analysis with keywords such as "transcription factor, activator, coactivator, repressor, corepressor, coactivator, regulators, and chromatin structures". This allowed us to identify 245 transcriptional regulators. Among these 245 genes, we identified 32 transcriptional regulator genes known to be associated with the epidermis, using GeneIndexer (Homayouni et al., 2005). In order to cluster the 3,033 EGF-regulated genes, we used the mFuzz method that is implemented in the maSigPro package (Conesa et al., 2006).

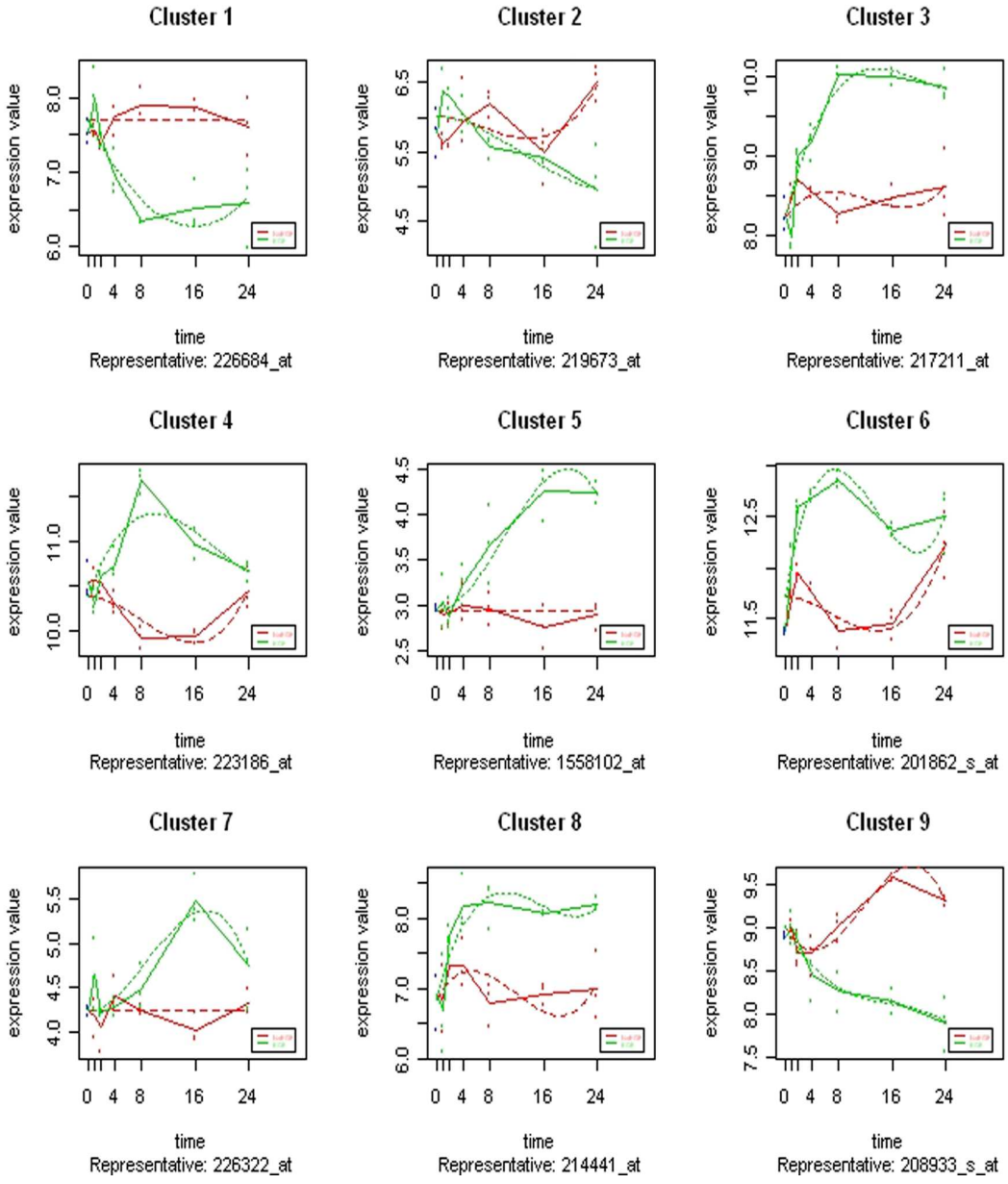

Figure 22. Clusters Produced by mFuzz Algorithm
The 3,033 significant genes were clustered using mFuzz. Probe sets in clusters 1, 4, 6, and 7 are considered to be intermediate responsive probe sets. The genes in these clusters respond maximally to EGF at 4 or 8 h, but do not respond to EGF at 24 h. Probe sets in clusters 2, 3, 5, 8, and 9 are considered to be late responsive probe sets. The genes in these clusters respond to EGF starting at 8 h and continue to respond to the treatment up to 24 h. The effect of the control and EGF conditions is shown in red and green, respectively. Dashed lines indicate best fitted curve for the data, while straight lines show the connection of the mean at each time point. The expression value is log base 2.

EGF Influences Keratinocyte Cell Fate Through a Complex Transcriptional Regulatory Network

Our previous work provides preliminary evidence that EGF controls keratinoctye cell fate by affecting the expression of genes responsible for the basal and suprabasal cell phenotypes via the regulation of multiple pro-differentiation TFs and signaling molecules (Chapter 3). Consistent with our previous microarray (Figure 23) and qRT-PCR (Figure 24) results, EGF down-regulated the RNA levels of differentiation markers that are well established to be expressed in the suprabasal layers (*KRT1* and *FLG*) and up-regulated RNA levels of proliferation markers that are expressed in the basal layer (*ITGB4* and *BNC1*). To determine the regulatory network underlying these EGF effects, we identified genes encoding for proteins involved in the transcriptional regulation using GeneIndexer analysis with the keywords "transcription factor, activator, coactivator, repressor, corepressor, coactivator, regulators, and chromatin structures" (Figure 21). This allowed us to identify 245 transcriptional regulators (Appendix C) from the list of 3,033 significant genes. Table 7 shows a subset of potentially important transcriptional regulators in keratinocyte differentiation, based on the observation that their expression levels were up- or down-regulated by EGF by at least 10-fold. We believe that these genes may have important biological roles in keratinocyte differentiation, as they responded strongly to the EGF treatment. Interestingly, the majority of these genes responded most effectively to EGF starting at 8 h or beyond, suggesting that they may be regulated by additional immediate early genes. Among these 245 genes, we identified 32 genes whose functions are known to be involved in the proliferation or differentiation of keratinocytes (Figure 21 and Table 8). Intriguingly, EGF down-regulated the RNA

levels of 86% of the differentiation regulators and up-regulated the expression of 80% of the known regulators

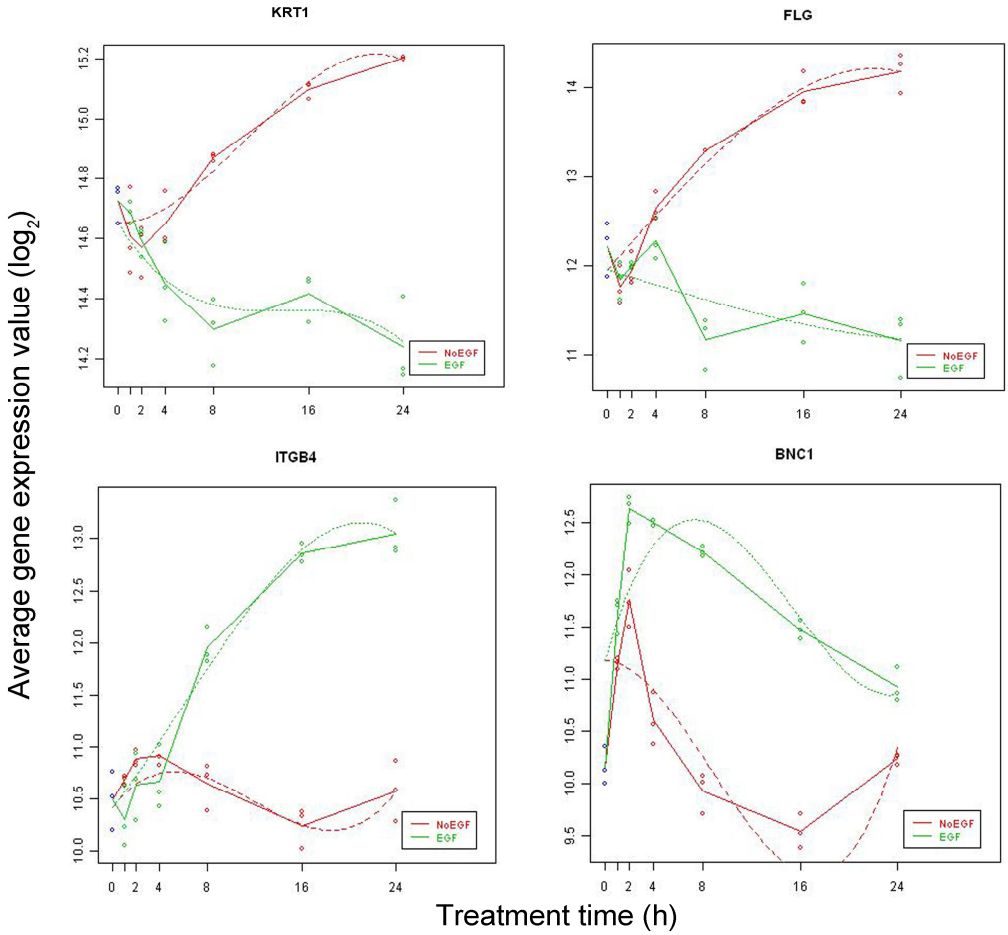

Figure 23. Microarray Profiles of EGF-regulated Genes that are Responsible for the Basal and Suprabasal Cell Phenotypes
Microarray profiles of suprabasal genes (*KRT1* and *FLG*) and basal genes (*ITGB4* and *BNC1*). NHEKs were grown to 100 % confluent cell density before receiving a last feed. After 48 h of last feed, cells were treated with or without EGF (10 ng/ml) in basal medium for 0, 1, 2, 4, 8, 16, and 24 h. Red indicates control samples while green indicates EGF treated samples (n = 3).

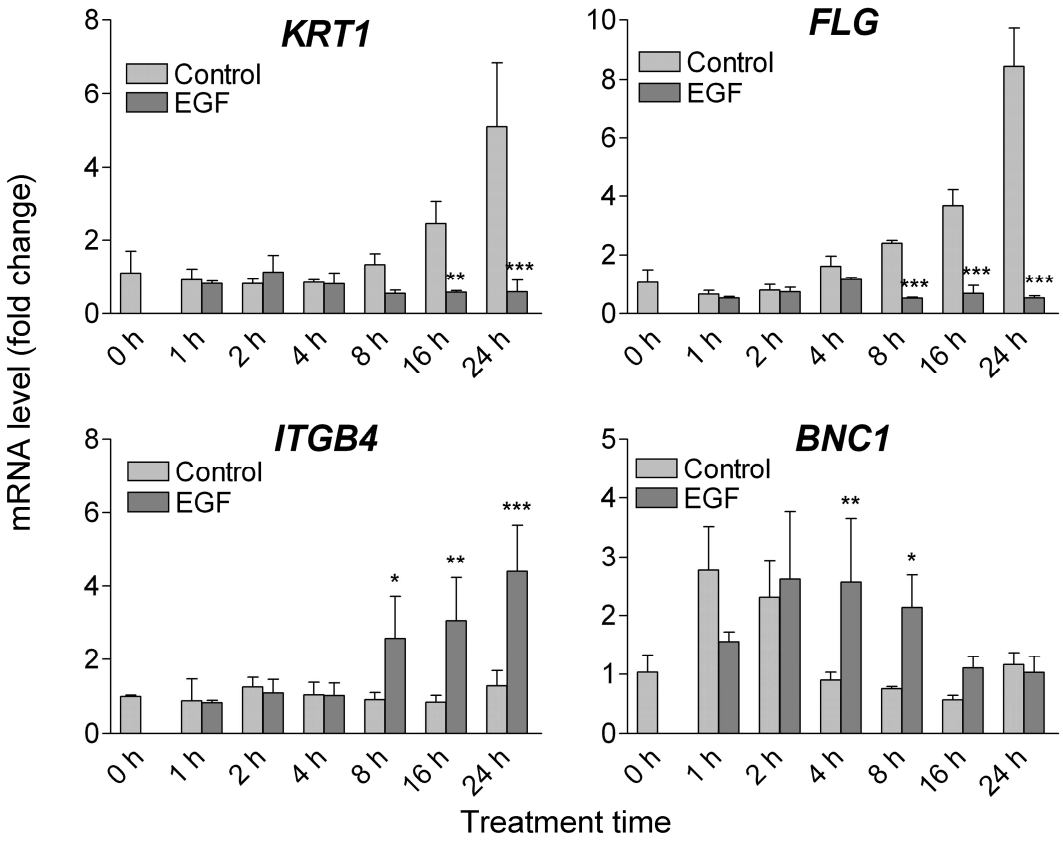

Figure 24. EGF Affects the Expression of Genes Responsible for the Basal and Suprabasal Cell Phenotypes
Validation of microarray expression profiles of the genes in Figure 23. These genes were selected to confirm the effects of EGF on the expression of basal and suprabasal-specific genes identified in microarray experiment 1. NHEKs were grown to 100 % confluent cell density before receiving a last feed. After 48 h of last feed, cells were treated with or without EGF (10 ng/ml) in basal medium for 0, 1, 2, 4, 8, 16, and 24 h. Results are expressed as relative expression from values obtained in response to the t_0 sample. Bars represent means ± SD (n = 3). Two-way ANOVA with Bonferroni post-tests was used to evaluate significant difference. *p < 0.05, **p < 0.001, ***p < 0.0001.

of proliferation (Table 8, Figure 25-27 and Figure 29-31). qRT-PCR results validated the expression profiles of 78% of the 32 transcriptional regulators (Figure 28 and 32). Based on the mFuzz clustering results, these genes were grouped into intermediate and late genes. We noticed that there was an equal amount of pro-differentiation and proliferation genes in each cluster (Table 8), indicating that EGF affects keratinocyte proliferation and

differentiation simultaneously. Based on these results, we improved a proposed dynamic model of epidermal homeostasis (Okuyama, 2004) to form an integrated model that reveals the regulatory action of EGF in this process (Figure 33). In this model, EGF suppresses genes (in green) that promote differentiation and induces genes (in red) that promote proliferation of keratinocytes. These results support and expand on the recognition of EGFR signaling as a key regulator of keratinocyte cell fate, affecting the transition between growth arrested reversible cells and transit amplifying cells, affecting k_{-2} in Figure 33 by inhibiting and inducing the transcription factors (TFs) that regulate differentiation and proliferation, respectively.

Table 7. Literature Supporting the Transcriptional Regulatory Roles of Genes That Are Up- or Down- Regulated by EGF by At Least 10 Fold at Any Time Point

Probe Set	Gene Symbol	Type	Role in cell development	EGF MAX FC[1]	EGF MIN FC[2]	Hr MAX FC[3]	Hr MIN FC[4]	Reference
221011_s_at	LBH	activator		159.74		16 h		Briegel and Joyner, 2001
204420_at	FOSL1	TF		77.30		8 h		Finzer et al, 2000
228964_at	PRDM1	repressor	Differentiation	34.29		4 h		Magnusdottir et al, 2007
202768_at	FOSB	TF		33.03		2 h		Ulery et al, 2006
228033_at	E2F7	repressor	Proliferation	30.66		8 h		Endo-Munoz, et al, 2009
206877_at	MXD1	repressor		29.95		4 h		Grandori et al, 2000
202935_s_at	SOX9	TF		27.98		16 h		Pan et al, 2008
206127_at	ELK3	repressor		24.38		16 h		Wasylyk et al, 2005; Chen et al, 2003
209189_at	FOS	TF	Proliferation	24.33		4 h		Ulery et al, 2006
209878_s_at	RELA	TF	Inhibit proliferation	17.64		16 h		Ghosh and Karin, 2002
36711_at	MAFF	TF	Proliferation	15.58		8 h		Motohashi et al, 2004
229404_at	TWIST2	repressor	Differentiation	13.90		16 h		Lee et al, 2003; Isenmann et al, 2009

[1]EGF Max FC: maximum fold change observed by EGF over the entire treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the entire treatment time.
[3]Hr Max FC: the time point at which the maximum EGF fold change was observed
[4]Hr Min FC: the time point at which the minimum EGF fold change was observed.

Table 7. Literature Supporting the Transcriptional Regulatory Roles of Genes That Are Up- or Down- Regulated by EGF by At Least 10 Fold at Any Time Point (Cont.)

Probe Set	Gene Symbol	Type	Role in cell development	EGF MAX FC[1]	EGF MIN FC[2]	Hr MAX FC[3]	Hr MIN FC[4]	Reference
226319_s_at	LOC644811 /// THOC4	coactivator		12.26		16 h		Mertz et al, 2007
211834_s_at	TP63	Activator repressor		11.58		16 h		Yang et al, 1998; Senoo et al, 2007; Zhu et al, 2007
224833_at	ETS1	TF	Proliferation	11.13		8 h		Jung et al, 2005
220625_s_at	ELF5	activator	Differentiation		-29.70		24 h	Choi and Sinha, 2006; Metzger et al 2008
210239_at	IRX5	TF	Differentiation		-15.05		8 h	Kerschenstei ner et al, 2008
212148_at	PBX1	TF	Differentiation		-50.88		24 h	Van Dijk et al, 1993
201566_x_at	ID2 /// ID2B	repressor	Proliferation		-11.54		4 h	Murphy et al, 2004
219551_at	EAF2	activator	Differentiation		-11.40		8 h	Xiao et al, 2006; Jiang et al, 2007; Maurus et al, 2005
207826_s_at	ID3	corepressor	Differentiation		-13.90		4 h	Deed et al, 1993
229638_at	IRX3	TF	Differentiation		-10.58		8 h	Bilioni et al, 2005; Gan et al, 2007
204069_at	MEIS1	TF	Differentiation		-15.96		8 h	Wang et al, 2006
207469_s_at	PIR	TF			-11.73		24 h	Pang et al, 2004
223275_at	PRMT6	chromatin structure			-15.03		24 h	Miranda et al, 2005; Hyllus et al, 2007
226872_at	RFX2	TF	Differentiation		-15.30		8 h	Horvath et al, 2004
228038_at	SOX2	activator	Differentiation		-11.43		8 h	Sharov et al, 2008
222146_s_at	TCF4	TF	Differentiation		-16.36		8 h	Nguyen et al, 2009
222016_s_at	ZNF323	TF			-26.86		16 h	Pi et al, 2002

[1]EGF Max FC: maximum fold change observed by EGF over the entire treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the entire treatment time.
[3]Hr Max FC: the time point at which the maximum EGF fold change was observed
[4]Hr Min FC: the time point at which the minimum EGF fold change was observed.

Table 8. Literature Supporting the 32 Transcriptional Regulators Known to Be Associated with Keratinocyte

Probe Set	Gene Symbol	EGF MAX FC[1]	EGF MIN FC[2]	Type of transcript-tional regulation	Role in keratinocyte development	Reference[3]
209878_s_at	RELA	17.645	-4.628	TF	Inhibits proliferation	Seitz et al, 2000
210993_s_at	SMAD1	-1.049	-3.739	TF/activator	Inhibits proliferation	He et al, 2001
203313_s_at	TGIF1	3.5119	1.4239	corepressor of SMAD2	Inhibits proliferation	Wotton et al, 1999; (Bartholin et al., 2008; Lo et al, 2001)
203140_at	BCL6	1.095	-5.005	TF/repressor	Differentiation	Yoshida et al, 1996; (Shen et al, 2008)
212420_at	ELF1	1.3569	-2.755	TF/activator	Differentiation	(Oettgen et al, 1997); Oettgen et al, 1996
220625_s_at	ELF5	-1.026	-29.7	TF/activator	Differentiation	(Oettgen et al, 1999); Choi and Sinha, 2006; Metzger et al 2008
	FOXN1			TF	Differentiation	Janes et al, 2004; Schlake et al, 2000
203394_s_at	HES1	2.4871	-1.526	TF/repressor	Differentiation	Nguyen et al, 2006; (Sang et al, 2008)
210239_at	IRX5	-1.148	-15.05	TF	Differentiation	Houweling et al, 2001; (Kerschensteiner et al, 2008)
223218_s_at	NFKBIZ	1.2235	-3.005	TF/activator	Differentiation	Oonuma et al, 2007; Shiina et al, 2004; (Kitamura et al, 2000; Trinh et al, 2008)
218902_at	NOTCH1	1.1415	-2.735	TF	Differentiation	Nguyen et al, 2006
212148_at	PBX1	1.2079	-50.88	TF/activator	Differentiation	Komuves et al, 2000; (Lu et al, 1994)
207109_at	POU2F3	3.4036	-4.888	TF	Differentiation	Sugihara et al, 2001; Shiina et al, 2004; Beck et al, 2007; Cabral et al, 2003
228964_at	PRDM1	34.286	2.1633	TF/repressor	Differentiation	Magnusdottir et al, 2007; (Martins et al, 2008)
218284_at	SMAD3	2.6209	-1.12	TF/activator	Differentiation	Ashcroft et al, 1999; Descargues et al, 2008; Flanders et al, 2002
204341_at	TRIM16 /// TRIM16L	2.6824	1.0004	coactivator	Differentiation	Beer et al, 2002; Raif et al, 2009

[1]EGF Max FC: maximum fold change observed by EGF over the entire treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the entire treatment time.
[3]The literature was manually collected from Pubmed.

Table 8. Literature Supporting the 32 Transcriptional Regulators Known to Be Associated with Keratinocyte (cont.)

Probe Set	Gene Symbol	EGF MAX FC[1]	EGF MIN FC[2]	Type of transcript-tional regulation	Role in keratinocyte development	Reference[3]
210319_x_at	MSX2	-1.075	-6.691	TF/repressor	Differentiation (Overexpression of MSX2 causes the skin to become hyperkeratotic)	Jiang et al, 1999
1554980_a_at	ATF3	3.2036	1.001	corepressor, repressor of Nrf2	Proliferation	Wang et al, 2007; (Zhang et al, 2002)
1552487_a_at	BNC1	4.8369	1.3962	TF	Proliferation	Tseng and Green, 1994; (Zhang and Tseng, 2007)
1554411_at	CTNNB1	7.1994	1.281	coactivator	Proliferation	Zhu and Watt, 1999
228033_at	E2F7	30.665	1.142	TF/repressor	Proliferation	Endo-Munoz et al 2009; (Li et al, 2008)
224833_at	ETS1	11.131	1.3979	TF	Proliferation	Nagarajan et al, 2009
209189_at	FOS	24.328	-2.458	TF	Proliferation (c-Fos is assumed to be absent in late stages of keratinocyte differentiation)	Mehic et al, 2005; Mils et al, 1997
201566_x_at	ID2 /// ID2B	1.0944	-11.54	TF/repressor	Proliferation	Murphy et al, 2004; (Boos et al, 2007; Moskowitz et al, 2007)
201465_s_at	JUN	3.1353	1.1422	TF	Proliferation	Shinoda and Huang, 1995
1555832_s_at	KLF6	9.6018	1.4543	TF/activator	Proliferation	Fitsialo et al, 2007; (Warke et al, 2003)
36711_at	MAFF	15.576	1.6971	TF/activator	Proliferation	Motohashi et al, 2004; Ye et al, 2006
226066_at	MITF	1.5963	-4.607	TF/activator	Proliferation	Gleason et al, 2008
206877_at	MXD1	29.952	1.0001	TF/repressor	Proliferation, Differentiation	Vastrik et al, 1995; Grandori et al, 2000; (Rottmann et al, 2008; Lee et al, 2006)
202431_s_at	MYC	1.9483	-1.03	TF/corepressor?	Proliferation	Murphy et al, 2004
206675_s_at	SKIL	3.9287	1.2144	TF/repressor	Proliferation	Fitsialo et al, 2007; (Levy et al, 2007)
204790_at	SMAD7	6.0035	1.1128	TF	Proliferation	Liu et al, 2003

[1]EGF Max FC: maximum fold change observed by EGF over the entire treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the entire treatment time.
[3]The literature was manually collected from Pubmed.

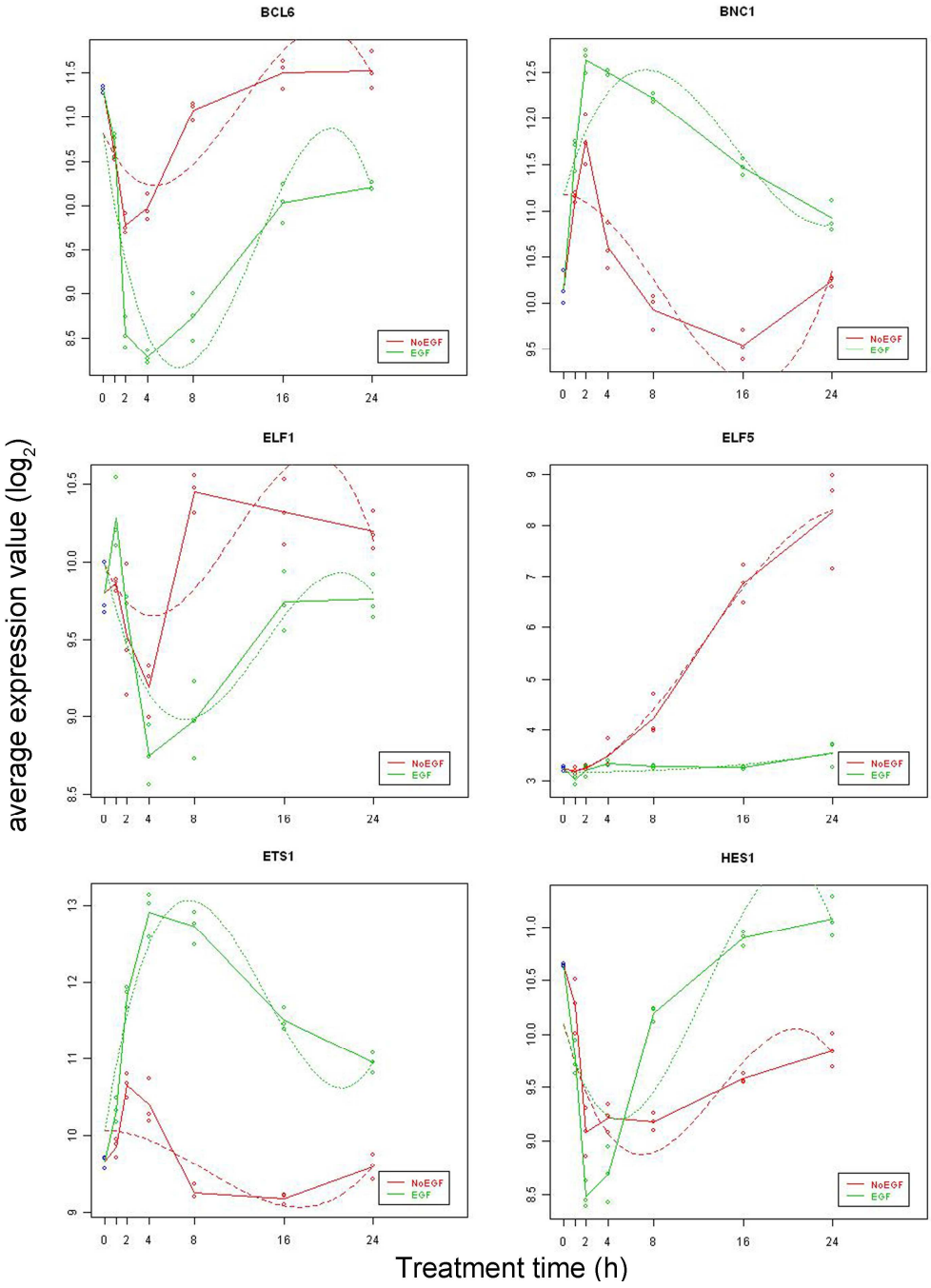

Figure 25. Microarray Expression Profiles of Intermediate Transcriptional Regulators (*BCL6, BNC1, ELF1, ELF5, ETS1, HES1)* (Related to Table 8)
Microarray profiles of *BCL6*, *BNC1*, *ELF1*, *ELF5*, *ETS1*, *HES1*. NHEKs were grown to 100 % confluent cell density before receiving a last feed. After 48 h of last feed, cells were treated with or without EGF (10 ng/ml) in basal medium for 0, 1, 2, 4, 8, 16, and 24 h. These graphs are organized in alphabetical order. Red dots indicate control samples. Green dots indicate EGF treated samples.

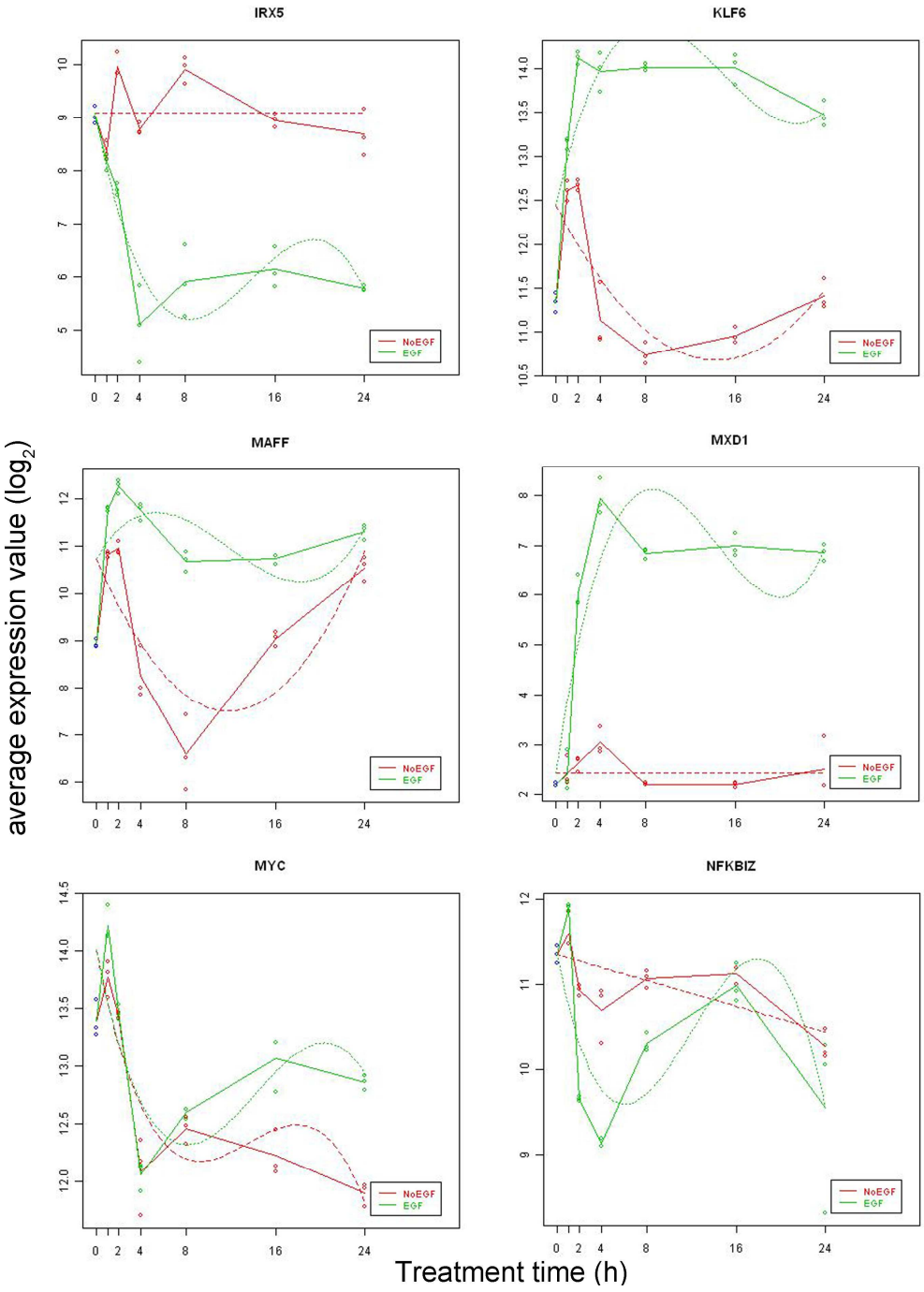

Figure 26. Microarray Expression Profiles of Intermediate Transcriptional Regulators (*IRX5, KLF6, MAFF, MXD1, MYC, NFKBIZ)* (Related to Table 8)
Microarray profiles of *IRX5, KLF6, MAFF, MXD1, MYC, NFKBIZ.* NHEKs were grown to 100 % confluent cell density before receiving a last feed. After 48 h of last feed, cells were treated with or without EGF (10 ng/ml) in basal medium for 0, 1, 2, 4, 8, 16, and 24 h. These graphs are organized in alphabetical order. Red dots indicate control samples. Green dots indicate EGF treated samples.

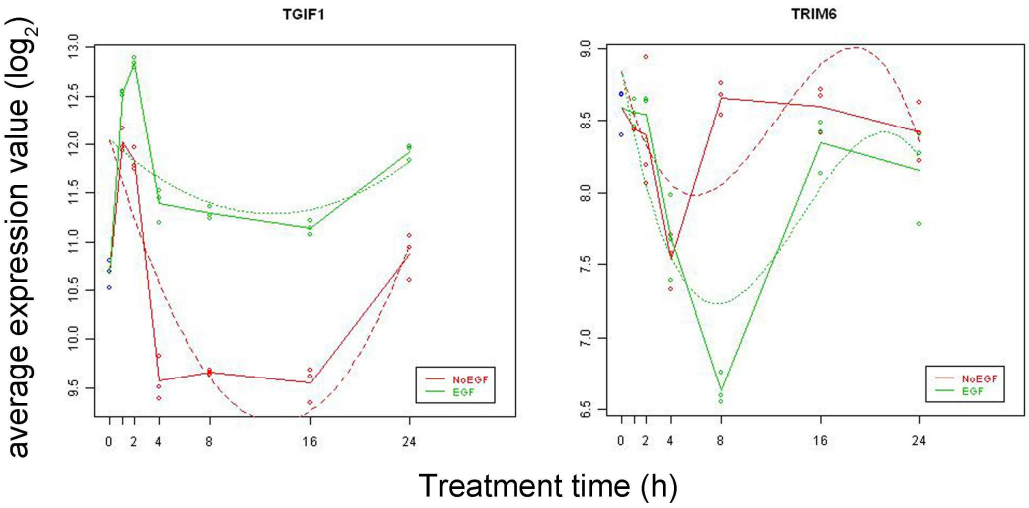

Figure 27. Microarray Expression Profiles of Intermediate Transcriptional Regulators (*TGIF1* and *TRIM16)* (Related to Table 8)
Microarray profiles of *TGIF1* and *TRIM16.* NHEKs were grown to 100 % confluent cell density before receiving a last feed. After 48 h of last feed, cells were treated with or without EGF (10 ng/ml) in basal medium for 0, 1, 2, 4, 8, 16, and 24 h. These graphs are organized in alphabetical order. Red dots indicate control samples. Green dots indicate EGF treated samples.

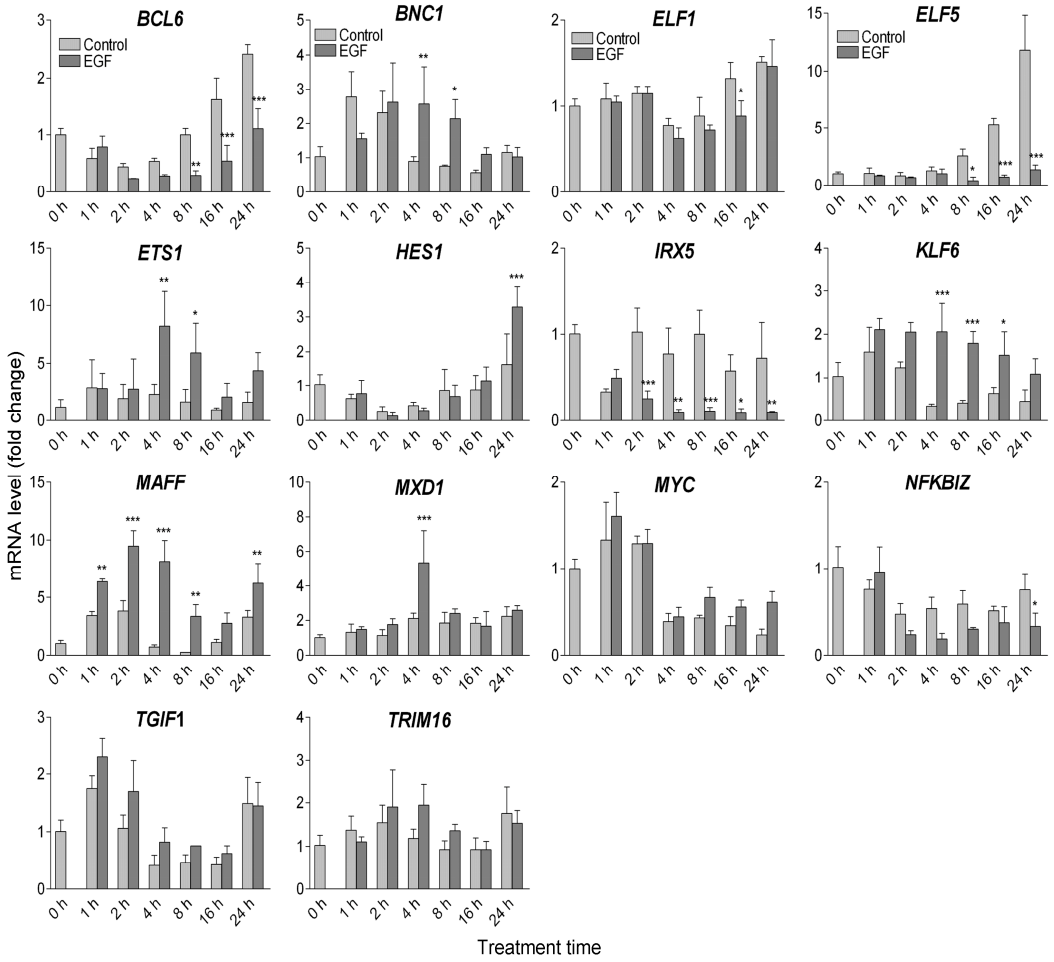

Figure 28. qRT-PCR Expression Profiles of Intermediate Transcriptional Regulators (Related to Figures 25-27)

Validation of microarray expression profiles of the genes in Figures 25-27. NHEKs were grown to 100 % confluent cell density before receiving a last feed. After 48 h of last feed, cells were treated with or without EGF (10 ng/ml) in basal medium for 0, 1, 2, 4, 8, 16, and 24 h. These graphs are organized in alphabetical order. Results are expressed as relative expression from values obtained in response to t_0 samples. Bars represent means ± SD (n = 3). Two-way ANOVA with Bonferroni post-tests was used to evaluate significant difference. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

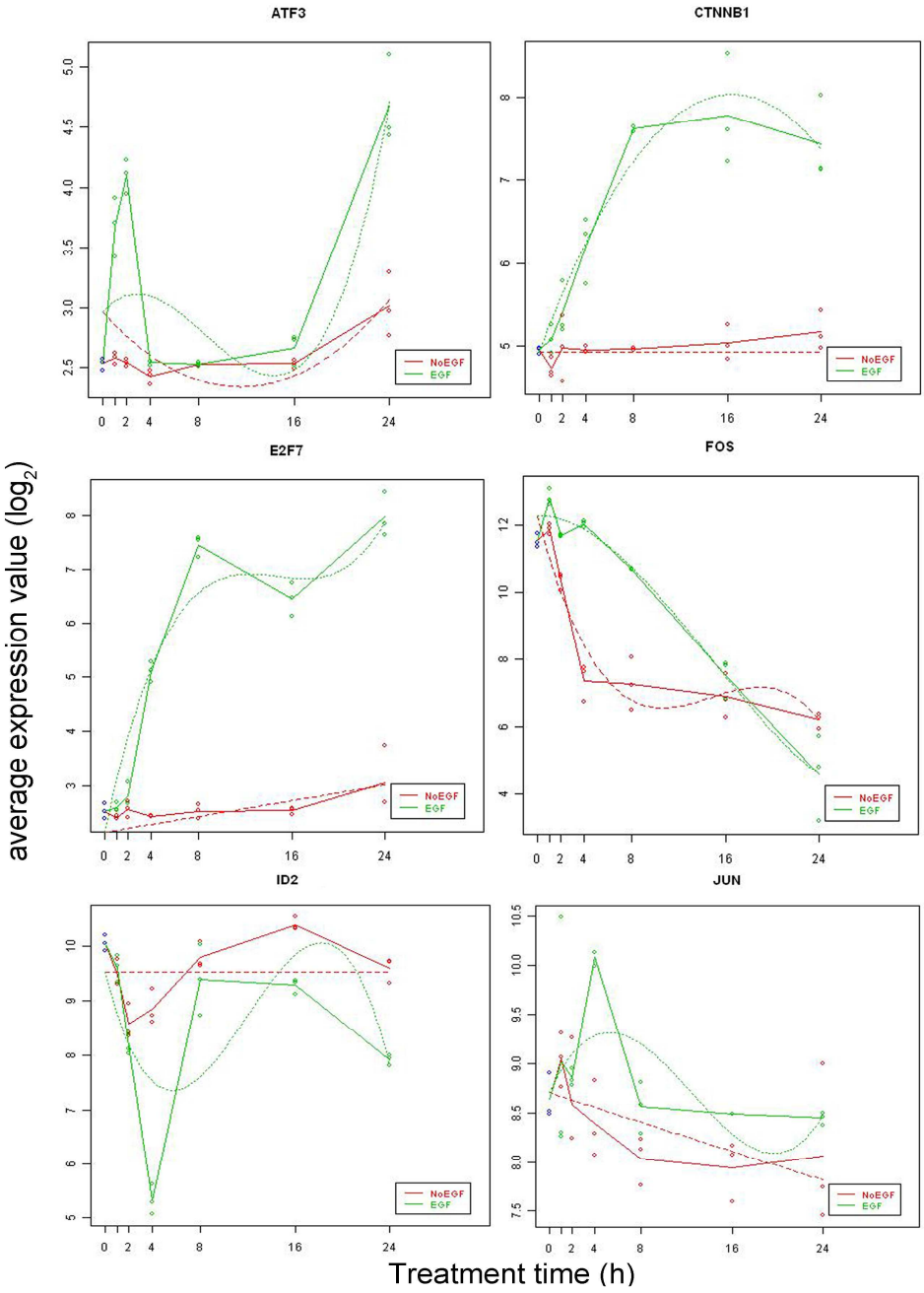

Figure 29. Microarray Expression Profiles of Late Transcriptional Regulators (*ATF3, CTNNB1, E2F7, FOS, ID2, JUN*) (Related to Table 8)
Microarray profiles of *ATF3*, *CTNNB1*, *E2F7*, *FOS*, *ID2*, *JUN*. FOXN1 microarray profile is not shown as it was detected as absent. NHEKs were grown to 100 % confluent cell density before receiving a last feed. After 48 h of last feed, cells were treated with or without EGF (10 ng/ml) in basal medium for 0, 1, 2, 4, 8, 16, and 24 h. These graphs are organized in alphabetical order. Red dots indicate control samples. Green dots indicate EGF treated samples.

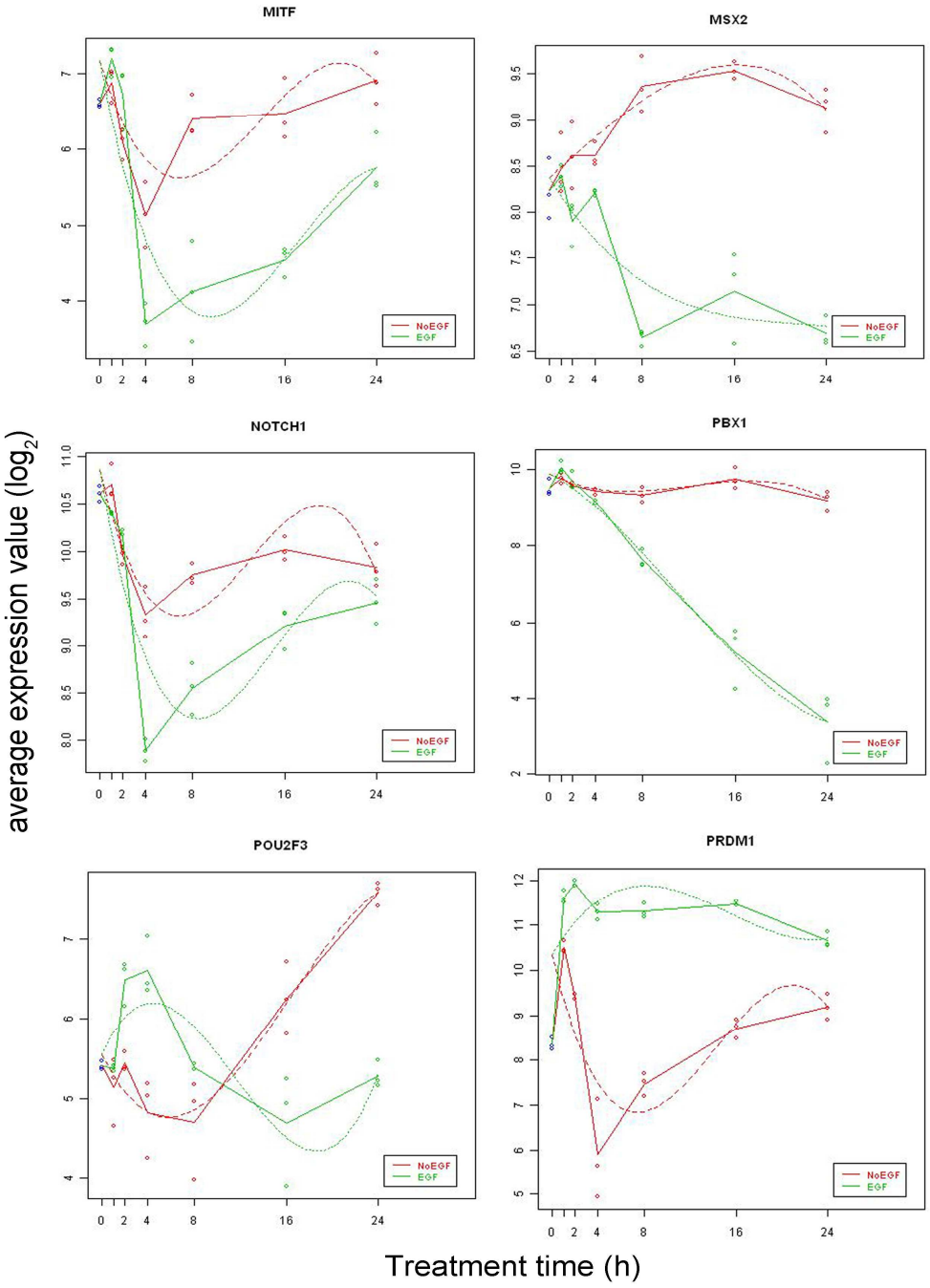

Figure 30. Microarray Expression Profiles of Late Transcriptional Regulators (*MITF, MSX2, NOTCH1, PBX1, POU2F3, PRDM1)* (Related to Table 8)

Microarray profiles of *MITF*, *MSX2*, *NOTCH1*, *PBX1*, *POU2F3*, *PRDM1*. NHEKs were grown to 100 % confluent cell density before receiving a last feed. After 48 h of last feed, cells were treated with or without EGF (10 ng/ml) in basal medium for 0, 1, 2, 4, 8, 16, and 24 h. These graphs are organized in alphabetical order. Red dots indicate control samples. Green dots indicate EGF treated samples.

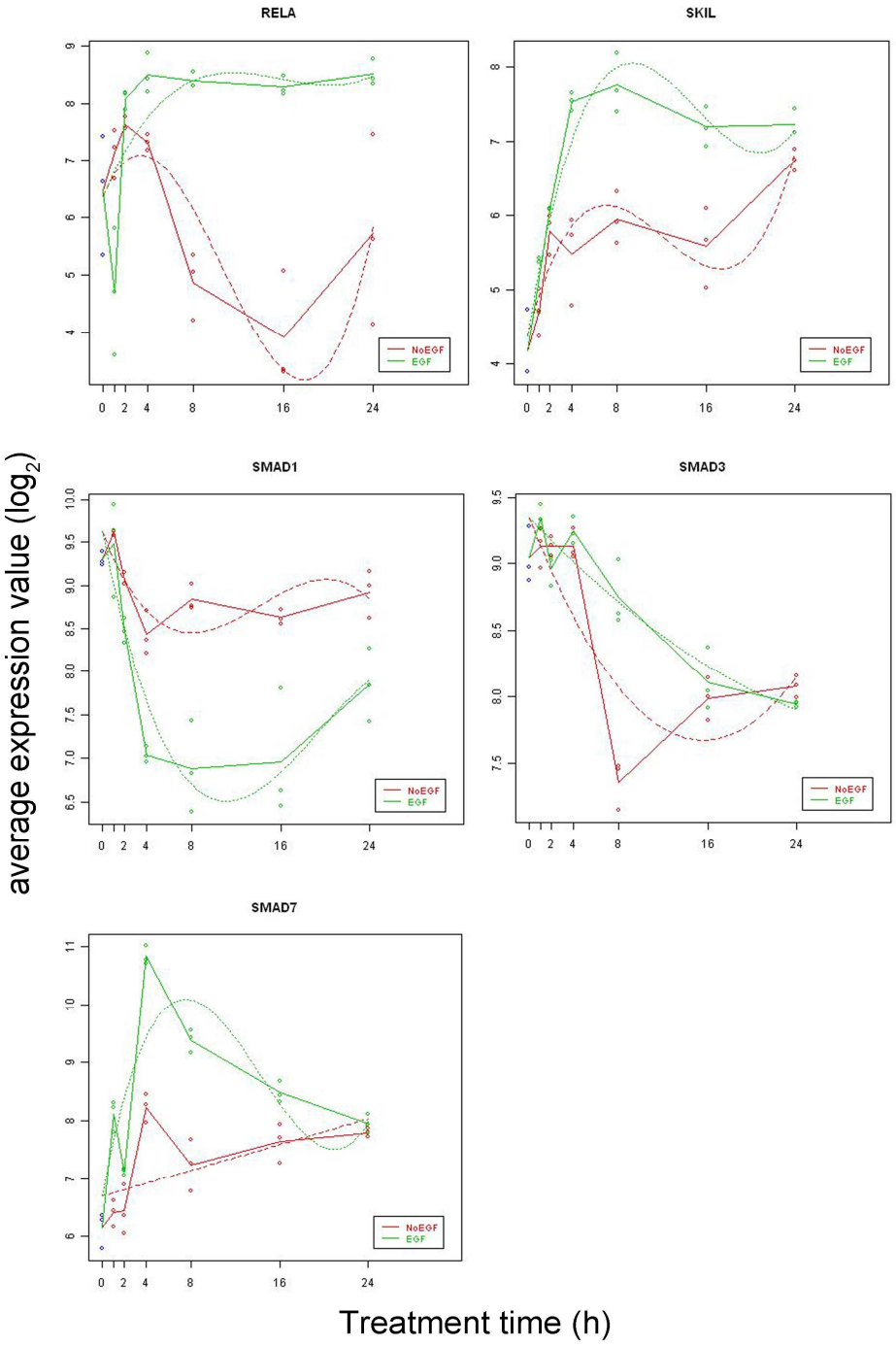

Figure 31. Microarray Expression Profiles of Late Transcriptional Regulators (*RELA, SKIL, SMAD1, SMAD3, SMAD7*) (Related to Table 8)
Microarray profiles of *RELA*, *SKIL*, *SMAD1*, *SMAD3*, *SMAD7*. NHEKs were grown to 100 % confluent cell density before receiving a last feed. After 48 h of last feed, cells were treated with or without EGF (10 ng/ml) in basal medium for 0, 1, 2, 4, 8, 16, and 24 h. These graphs are organized in alphabetical order. Red dots indicate control samples. Green dots indicate EGF treated samples.

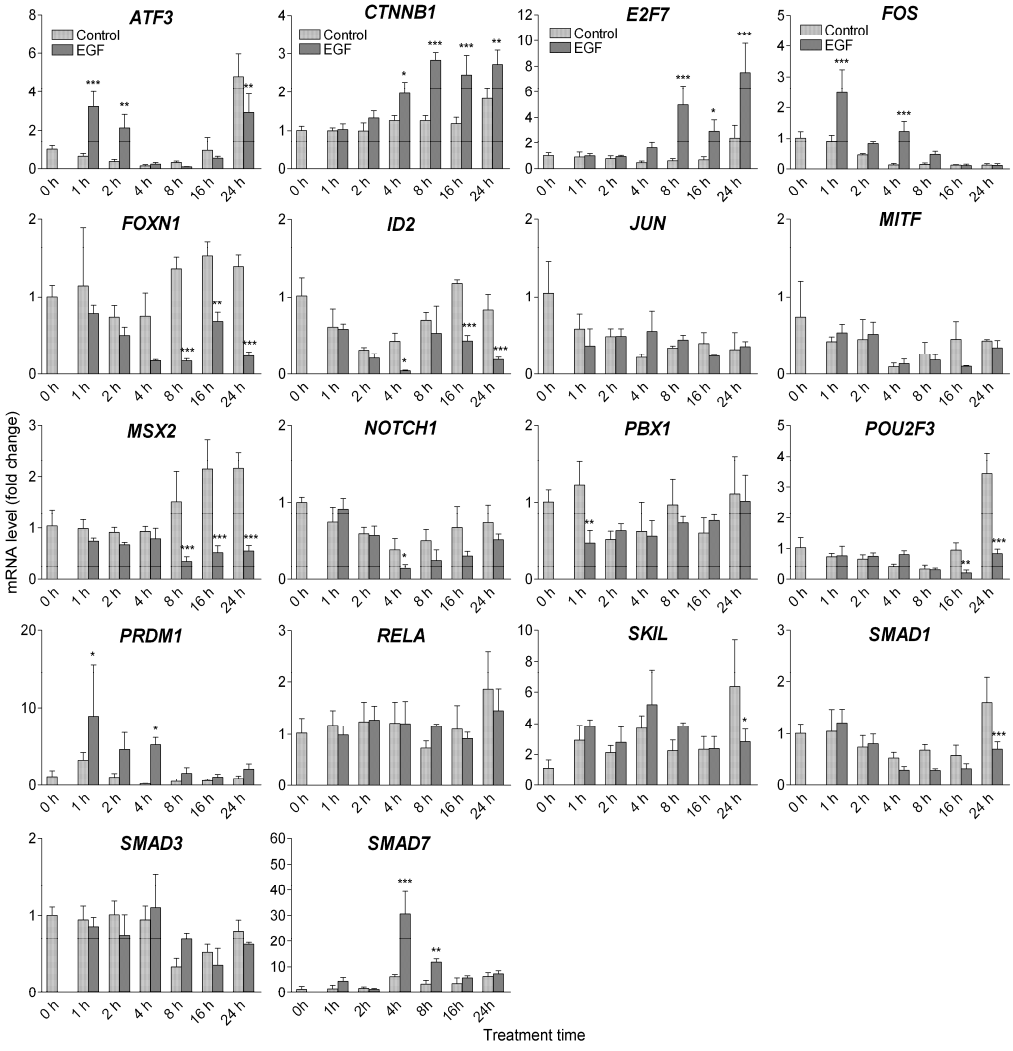

Figure 32. qRT-PCR Analysis of Late Transcriptional Regulators (Related to Figures 29-31)

Validation of microarray expression profiles of the genes in Figures 29-31. NHEKs were grown to 100 % confluent cell density before receiving a last feed. After 48 h of last feed, cells were treated with or without EGF (10 ng/ml) in basal medium for 0, 1, 2, 4, 8, 16, and 24 h. These graphs are organized in alphabetical order. Results are expressed as relative expression from values obtained in response to t_0 samples. Bars represent means ± SD (n = 3). Two-way ANOVA with Bonferroni post-tests was used to evaluate significant difference. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

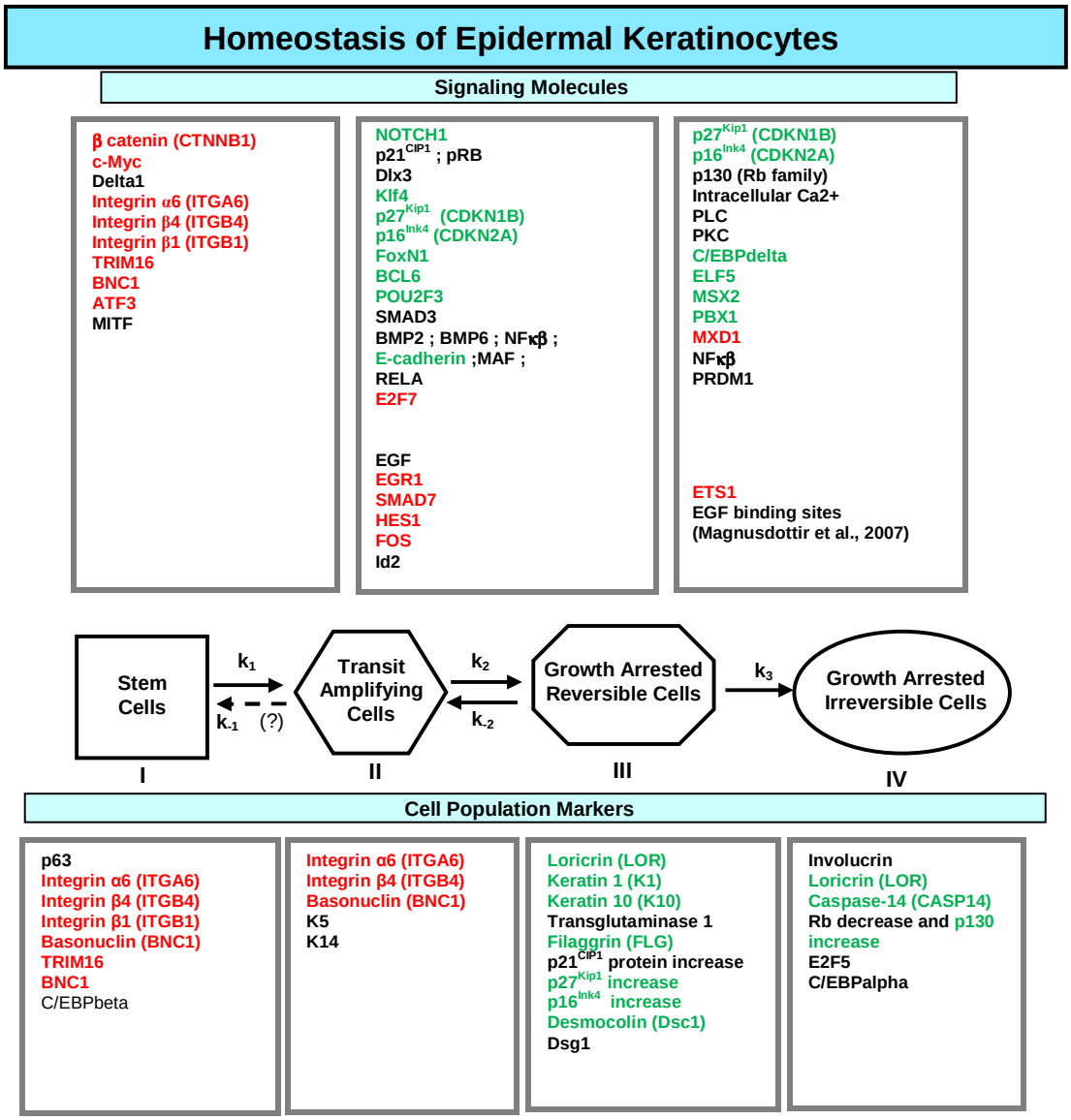

Figure 33. An Integrated Model of Homeostasis of Epidermal Keratinocytes
A dynamic model of epidermal homeostasis (Okuyama et al., 2004) has been proposed. In this model, there is an equilibrium between the stem cell, transit amplifying cell populations, and cells that have withdrawn reversibly versus irreversibly from the cell cycle. Boxes that are above the dynamic model list signals regulating the identified forward rate constants; those below the ---- line identify reverse constants). Boxes that are below the dynamic model identify cell markers that are currently used to discriminate the different compartments. EGF suppresses the expression of genes that are highlighted in green and induces those that are in red. The expression of these genes have been validated by qRT-PCR. Most of them are found to be significant by our microarray data.

Immunoblot analysis was performed on several pro-differentiation TFs including FOXN1, POU2F3, and ELF5. Consistent with the expression data, the protein levels of these TFs were significantly inhibited by EGF at 48 h (Figure 34). This indicates that EGF inhibits keratinocyte differentiation by down-regulating TFs involved in the terminal differentiation of keratinocytes.

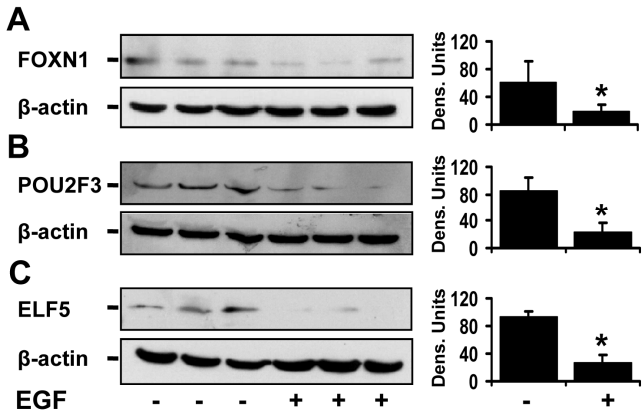

Figure 34. EGF Inhibits Pro-differentiation Transcription Factors. EGF decreases protein levels of the pro-differentiation TFs FOXN1, POU2F3, and ELF5. NHEKs were grown to 100 % confluent cell density before receiving a last feed. After 48 h of last feed, cells were treated with or without EGF (10 ng/ml) in basal medium for 24 h. Then, they were switch to basal medium containing with or without EGF (10 ng/ml) in the presence of 1 mM Ca^{2+}. Cell lysates were harvested 24 h after the last media change. ACTB is a loading control (n = 3). Bars represent means ± SD. Student's t-test was used to evaluate statistical significance ($p < 0.05$).

MEK and PKC Appear to be Primary EGFR Signaling Pathway that Affects Keratinocyte Differentiation

To identify the EGFR-dependent signaling pathways that mediate the EGF effects in epidermal keratinocytes, we treated NHEKs with wortmannin (400 nM), U0126 (10 µM), SB20358 (20 µM), Gö6983 (10 µM), or NSC23766 (100 µM) to specifically inhibit PI3K, MEK, p38, PKC, and RAC, respectively. We performed qRT-PCR on the genes

identified in Table 7 and Table 8 after 4 and 24 h of treatment with these 4 chemicals. We noticed that there was a significant overlap in the signals that altered the expression of individual genes. The MEK signaling pathway appeared to be a dominating pathway that was activated by EGF in epidermal keratinocytes. EGF signaled primarily through the MEK and PKC signaling pathways to decrease the expression of the TFs that promote differentiation and increase the expression of those that promote proliferation. When the MEK signaling pathway was not involved, the PKC signaling pathway appeared to mediate the up-regulation of some keratinocyte pro-differentiation TFs expression (Tables 9 and 10).

Table 9. Rank Order of Inhibitor Effects on EGF-downregulated Transcriptional Regulators Based on qRT-PCR Analysis

Gene Symbol	Molecular function	Role in cell development	Rank order of inhibitor release	Observed time point[1]
MITF	TF/activator	Proliferation	PI3K > PKC	24 h
ID2	repressor	Proliferation	MEK	4 h
NOTCH1	TF	Differentiation	MEK > Rac1	4 h
			MEK	24 h
IRX5	TF	Differentiation	MEK	4 h
FOXN1	TF	Differentiation	MEK > PKC > PI3K	4 h
POU2F3[2,3]	TF	Differentiation	PI3K	24 h
BCL6[2]	TF/repressor	Differentiation	PI3K	4 h and 24 h
			MEK	24 h
ELF5[2]	TF/activator	Differentiation	Rac1	4 h and 24 h
IRX3	TF	Differentiation	Block PKC, EGF suppressed IRX3 even more	4 h and 24 h
RFX2	TF	Differentiation	PI3K	
SOX2	TF	Differentiation	MEK > PKC > PI3K	4 h
ID3	TF	Differentiation	MEK > PKC	4 h and 24 h
MEIS1	TF	Differentiation	PI3K > Rac1	4 h
PIR[4]	TF	Differentiation	MEK > PKC > Rac1	24 h
PRMT6[3]	chromatin structure		PKC	24 h
NFKBIZ	TF/activator	Differentiation	PKC> MEK	24 h
TCF4	TF	Differentiation	not validated[5]	
ELF1	TF/activator	Differentiation	not validated**	
PBX1	TF/activator	Differentiation	not validated**	
SMAD1	TF/activator	Antiproliferation	not validated**	
EAF2	TF/activator	Differentiation	not validated**	
MSX2	repressor	Differentiation	not validated**	

[1]Time point where the suppression by EGF was released by an inhibitor.
[2]Block PKC, EGF suppressed the mRNA of the genes even more.
[3]Block Rac1, EGF suppressed the mRNA of the genes even more.
[4]Block PI3K, EGF suppressed the mRNA of the genes even more.
[5]not validated: qRT-PCR results using samples from microarray and inhibitor studies did not validate the microarray expression profile for EGF.

Table 10. Rank Order of Inhibitor Effects on EGF-upregulated Transcriptional Regulators Based on qRT-PCR Analysis

Gene Symbol	Molecular function	Role in cell development	Rank order of inhibitor suppression	Observed time point[1]
E2F7	repressor	Proliferation	MEK = PI3K	4 h
			PI3K > MEK	24 h
ETS1	TF	Proliferation	MEK > PKC = PI3K > Rac1	4 h
			PKC > MEK = PI3K	
KLF6	activator	Proliferation	MEK > Rac1	4 h
			MEK > PKC	24 h
CTNNB1	coactivator	Proliferation	PKC	4 h
SMAD7	TF/activator	Proliferation	MEK > PKC > Rac1	4 h
BNC1	TF	Proliferation	MEK	4 h
			PI3K > MEK	24 h
ATF3	corepressor, repressor of Nrf2	Proliferation	PKC = PI3K	4 h
FOS	TF	Proliferation	MEK > PKC > PI3K	4 h and 24 h
TGIF1	corepressor of SMAD2	Inhibit proliferation	MEK ~ PKC; PI3K	4 h
			PI3K	24 h
PRDM1	repressor	Differentiation	MEK > PKC > PI3K	4 h
			MEK > Rac1 > PKC = PI3K	24 h
TRIM16	coactivator	Differentiation	MEK	4 h
SMAD3	activator	Differentiation	MEK > PKC	4 h and 24 h
HES1	repressor	Differentiation	MEK, PI3K = PKC	24 h
RELA	TF	Antiproliferation	MEK	4 h
			MEK > PKC	24 h
TWIST2	repressor	Growth	MEK > PI3K > Rac1	4 h
			MEK > Rac1	24 h
ELK3	repressor		MEK > PKC > Rac1	4 h and 24 h
SOX9	TF		MEK > PKC	4h and 24 h
			Rac1	24 h
FOSL1	TF		MEK > PKC > PI3K	4 h and 24 h
FOSB[2]	TF		MEK > PKC	4 h
			Rac1 = PKC	24 h
MAFF[2]	TF/activator/repressor	Proliferation	MEK > PKC	4 h
SKIL[2]	repressor	Proliferation	MEK = PKC	4 h
MXD1[2]	repressor	Proliferation, Differentiation	PKC > MEK	4 h and 24 h
LBH[2,3]	activator		MEK > PKC > Rac1	4 h
			PKC > MEK	24 h
ZNF323	TF		none of the pathway[4]	
THOC4	coactivator		not validated[5]	
JUN	TF	Proliferation	not validated[5]	
TP63	activator/repressor	Proliferation	not validated[5]	
MYC	TF/corepressor?	Proliferation	not validated[5]	

[1]Time point where the induction by EGF was blocked by an inhibitor.
[2]Block PI3K, EGF induced the mRNA of the gene even more.
[3]Block Rac1, EGF induced the mRNA of the gene even more.
[4]none of the pathway: EGF did not signal through any of the inhibited pathways to upregulate the expression of the gene
[5]not validated: qRT-PCR results from the microarray and inhibitor studies did not validate the microarray expression profile.

EGF Increases the Expression of MIRN21 to Promote Keratinocyte Proliferation

MicroRNAs are approximately 22 nucleotides long, non-coding RNA molecules that bind to the 3' untranslated regions (UTR) of target mRNAs to influence the translation or stability of the transcripts. MicroRNAs have been shown to be involved in hair follicle morphogenesis, cutaneous wound healing, psoriasis, autoimmune disorders, and skin carcinogenesis (Bostjancic and Glavac, 2008). In our study, we noticed that microRNA-21 (MIRN21) was significantly upregulated 7.6 and 5.0 fold by EGF at 4 and 8 h, respectively (Figure 28A). This increase was validated by qRT-PCR (Figure 28B). MIRN21 is the only microRNA that is over-expressed in 11 types of solid tumors, including stomach, prostate, head and neck, esophagus, glioblastoma, neuroblastoma, cholangiocarcinoma, breast, lung, colorectal, and pancreatic cancer (Lu et al., 2008; Medina and Slack, 2008). Increased expression of MIRN21 is observed in skin with psoriasis and atopic eczema as compared to healthy skin (Sonkoly et al., 2007). Moreover, activation of EGFR signaling has been shown to increase expression of MIRN21 in lung cancer in never-smokers (Seike et al., 2009). To test the role of MIRN21 in our study, the expression of programmed cell death 4 (PDCD4), a known MIRN21 target, was examined. The RNA level of *PDCD4* was significantly decreased by EGF at 16 and 24 h as observed by both microarray and qRT-PCR results (Figure 28). Inhibition of MIRN21 has been shown to suppress cell proliferation in HeLa cervical carcinoma and hepatocellular cancer cells (Meng et al., 2007; Yao et al., 2009). Since MIRN21 appears to be an EGFR-regulated factor that plays a role in skin diseases and affects cell proliferation, we hypothesized that EGF increases the expression of MIRN21 to promote keratinocyte proliferation by suppressing its target genes. In order to prove

this hypothesis, other experiments need to be performed. First, more validation should be done on other MIRN21 targets such as phosphatase and tensin homologue (PTEN), tropomyosin 1 (TPM1), tissue inhibitor of metalloproteinase 3 (TIMP3), sprout homolog 2 (SPRY2), nuclear factor I/B (NFIB), and AT-rich interactive domain 1A (ARID1A). The interpretation of the results on MIRN21 target genes should be placed in the context of their functions. Keratinocytes should be transfected with either anti-MIRN21 or a control inhibitor, and then treated with or without EGF (10 ng/ml). Cell proliferation assay using bromodeoxyuridine (BrdU) and luciferase reporter gene assays can then be done on these cells. A microarray experiment using RNA from control and anti-MIRN21 cells can be used to identify MIRN21 functionally important targets that contribute to keratinocyte proliferation and differentiation.

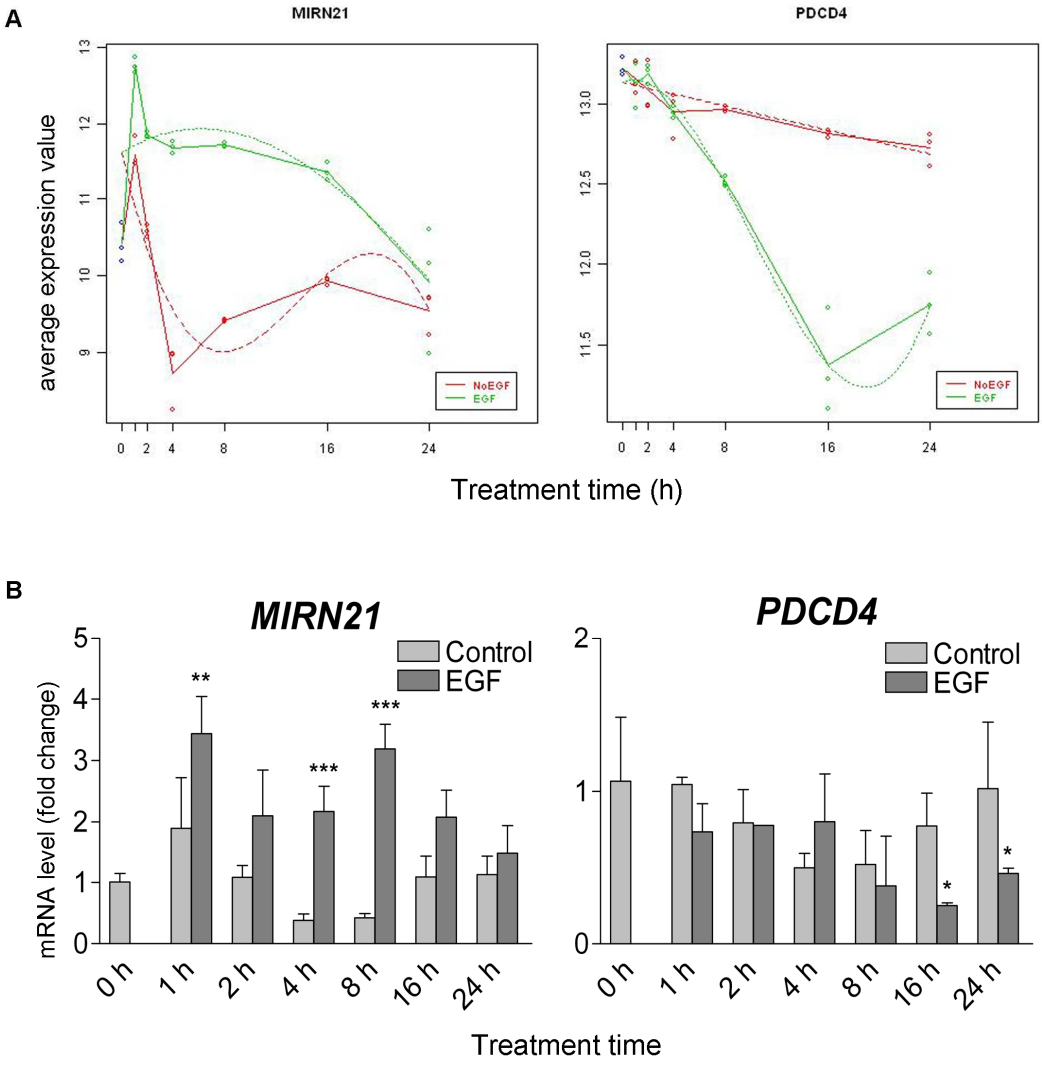

Figure 35. Expression Profiles of MIRN21 and One of Its Target Genes
The effects of EGF on expression of MIRN21 and PDCD4, a target of MIRN21.
(A) Microarray expression profiles of *MIRN21* and one of its target genes, *PDCD4*. NHEKs were grown to 100 % confluent cell density before receiving a last feed. After 48 h of last feed, cells were treated with or without EGF (10 ng/ml) in basal medium for 0, 1, 2, 4, 8, 16, and 24 h. Red dots indicate control samples. Green dots indicate EGF treated samples.
(B) qRT-PCR results of the genes in (A). Results are expressed as relative expression from values obtained in response to t_0 samples. Bars represent means ± SD (n = 3). Two-way ANOVA with Bonferroni post-tests was used to evaluate significant difference. *p < 0.05, **p < 0.001, ***p < 0.0001.

DISCUSSION

Our results provide a vast new understanding of how activation of the EGFR abrogates epidermal permeability barrier function by regulating the expression of enzymes catalyzing lipid biosynthesis, protein precursors of the cornified epithelium, and proteins of intercellular TJs. Establishing an epidermal permeability barrier *in utero* is required for both mice and humans to survive the transition from an aqueous *in utero* to a terrestrial *ex utero* environment. To complete this barrier acquisition, terminally differentiated epidermal cells, lipids, and tight junctions are all required.

The permeability barrier relies on the lipid matrix in the SC, which mainly consists of ceramides, free fatty acids, and cholesterol (Mimeault et al., 2004). Two previous qualitative studies have investigated the effects of EGF on keratinocyte lipid biosynthesis. In the first (Ponec et al., 1997), a slight decrease in Cer content was observed in keratinocytes cultured at the air-liquid interface at 37°C for 14 days in the presence of EGF compared to control. In the second (Gibbs et al., 2000), EGF was reported to decrease the amounts of phospholipids and glucosphingolipids in organotypic cultures of keratinocytes compared to native skin. Neither study identified the molecular mechanism underlying these effects of EGF, nor provided sufficient statistical analysis. A recent study of NHEKs showed that *ACER1* expression is strongly inhibited by EGF and highly induced during epidermal keratinocyte differentiation. This enzyme breaks down ceramide to sphingosine, which can also be derived from sphingosine-1-phosphate (S1P). Sphingosine and S1P are two bioactive lipids that mediate apoptosis, proliferation, and differentiation in keratinocytes (Sun et al., 2008). Here, we provided evidence that EGF decreased several key enzymes affecting free fatty acid and ceramide biosynthesis.

Abnormal levels of these lipid classes have been observed in many skin disorders including psoriasis and atopic dermatitis (Harding et al, 2002; Okamoto et al, 2003; Zheng et al, 2003). It has been reported that the content of the FFA fraction containing 20–26 carbon atoms and the total ceramide amount are significantly reduced in atopic dermatitis and bullous ichthyosiform erythroderma (Paige et al, 1994). Cer1 is of great importance to the organization of the lipid matrix of the SC. It is the only ceramide that can connect the lipid disks in the matrix, allowing the fusion and stabilization of the intercellular lipid lamellae (Bouwstra et al, 1998). A decrease in the Cer1 content of the SC will most likely result in a decrease in the lipids organization. The level of Cer1 is markly decreased in non-erythrodermic lamellar ichthyosis (Paige et al, 1994), and skin psoriasis plaques (Gniadecki et al, 1998). Cer3 and Cer6 make the lipid matrix more coherent through the interaction with other lipid components, and thus enhance the permeability barrier function. Deficiencies of Cer1 and Cer6 are found in Sjögren-Larson syndrome, a genetic disease characterized by ichthyosis, spastic paraplegia and mental retardation (Paige et al, 1994). Collectively, these results strongly support the physiologic connection of the observed effects of EGF on the FFA and ceramide deficiencies which are seen in the SC of patients with psoriasis, AD, and ichthyosis.

The formation of the lipid matrix and CEs takes place concomitantly in the upper granular layer and is essential for maintenance of barrier function (Candi et al., 2005). EGF is known to have an inhibitory effect on the formation of CEs (Sun and Green, 1976). However, the mechanisms underlying the loss of CE after EGF treatment has been restricted to the reduction of few intermediate filaments and their binding proteins (KRT1, KRT10, and FLG), CE precursors (LOR, and IVL), and TGM1 (Gibbs et al., 1998;

Marchese et al., 1990; Monzon et al., 1996; Poumay and Pittelkow, 1995; Reiss and Sartorelli, 1987). Consistent with these studies, we observed a significant reduction in CE competence as well as inhibition of *KRT1*, *KRT10*, *TGM1*, *FLG*, and *LOR* expression. More importantly, we demonstrated that these effects of EGF were through the activation of the EGFR, as inhibition of the EGFR tyrosine kinase blocked these effects. Furthermore, we identified many previously unrecognized EGF-repressed genes, including most of the well characterized proteins that participate in the synthesis of the CE including multiple TGM, SPRR, LCE and S100 proteins (Appendix A) (Candi et al, 2005). Our results greatly advance the current understanding of the molecular mechanisms of EGFR signaling and its inhibition of keratinocyte differentiation.

The regulation of TJ proteins by EGF has been studied in several cell lines including Madin-Darby Canine Kidney cells, TMK-1 gastric cancer cells, and Caco-2 cells (Yoshida et al, 2005; Singh et al, 2004; Samak et al, 2011). However, to our knowledge, there has not been a study of the effects of EGF on TJ barrier function in NHEKs. In NHEK cultures, a continuous network of TJs assemble as the epidermal barrier forms (Yuki et al., 2007). Our experiments demonstrated that EGF reduced levels of CLDN1 and TJP1 by approximately 70%, and this preceded a remarkable 50% reduction in TJ function based on both electrical resistance and permeability assays. TER reflects small-pore water ions permeability, whereas the flux of dextrans measures the permeability of large barrier breaks. These results indicate that EGF disrupts TJ function, making the barrier more susceptable to environmental allergens, one of the characteristics observed in skin of patients with AD.

One of the most important functions of the epidermal barrier is to prevent excessive water loss. TEWL is a technique used to measure the water content property of the skin. High TEWL indicates aberrant barrier function and is associated with various human skin diseases such as atopic dermatitis, psoriasis, contact dermatitis, and ichthyosis (Effendy et al., 1995; Tagami and Yoshikuni, 1985; Tomita et al., 2005; Werner and Lindberg, 1985). To demonstrate the effect of EGF on epidermal barrier function as a whole, we measured TEWL in organotypic cultures, a model to study *in vitro* the skin barrier function (Pasonen-Seppanen et al., 2001; Regnier et al., 1992). This *in vitro* model allows us to compare the effect of EGF on the uniform architecture and metabolism that would otherwise be impossible to obtain in animal models. In addition, there are no limitations in performing laboratory tests on organotypic cultures, whereas making assessment of a product or a treatment on animals is more costly. Here, we showed that EGF increased TEWL in organotypic cultures, indicating that the epidermal barrier integrity is impaired by the treatment. This suggests that EGF plays an active role in making the skin more susceptible to skin diseases.

The regulation of gene expression drives all developmental and differentiation processes. In eukaryotes, TFs define the state of expression and hence the execution of downstream differentiation/morphogenetic events by coordinately integrating signals from upstream developmental/growth factor signaling pathways. Our studies identified several EGF-suppressed TFs including KLF4 and GATA3 that are essential for the initiation and progression of epidermal differentiation. Each of these TFs activates distinct aspects of terminal differentiation. KLF4 is necessary and sufficient for the establishment of a functional epidermal barrier since targeted deletion results in loss of

barrier function and ectopic expression accelerates the formation of the epidermal barrier (Jaubert et al., 2003; Segre et al., 1999). Genes encoding structural components of the CE are misregulated in *Klf4*$^{-/-}$ mutants. Specifically, *Krt1* and *Lor* are downregulated while *Sprr2a*, whose promoter possesses a functional KLF4 binding site, is upregulated in *Klf4*$^{-/-}$ skin (Fischer et al, 1996; Segre et al., 1999). This direction is concordant with the effect of EGF on *KRT1*, *LOR*, and *SPRR2A*, suggesting that the deleterious effect of EGF on KLF4 alters expression of CE genes, leading to an inhibition in CE assembly as demonstrated here and in other studies (Sun and Green, 1976; Monzon et al, 1996). Similarly, epidermal-specific deletion of *Gata3* in mice results in an impaired epidermal barrier and perinatal lethality (de Guzman Strong et al., 2006). However, in contrast to *Klf4*, the *Gata3* deletion strongly affects the expression of genes involved in lipid biosynthesis, with the null allele mice showing significantly lower expression of several critical genes in this pathway including *Alox12b*, *Elovl3* and *4*, and *Acer1* (de Guzman Strong et al., 2006). In the present study, we showed that EGF repressed the expression level of *GATA3* by 62%. This reduction was paralleled by significantly lower levels of expression of multiple lipid metabolism genes including *ALOX12B* (30%), *ACER1* (71%) and *ELOVL3* (76%) and *4* (27%). Of note, mice lacking *Alox12b* or *Elovl4* die perinatally due to defective skin permeability barrier function and severe dehydration. Furthermore, *Alox12b* null mice show significant decreases in certain ω-hydroxyCers that are covalently bound to the CE (Epp et al., 2007), and skin grafts from *Alox12b* null mice exhibit an ichthyosiform phenotype (de Juanes et al., 2009). *Elovl4* null mice show significant decreases in FFA having chain lengths longer than C26, and ceramides with ω-hydroxy VLCFAs (Cameron et al., 2007; Epp et al., 2007; Li et al., 2007b). Free

VLCFAs are required for ceramide synthesis in epidermal barrier and are essential for the highly organized stratum corneum to prevent water penetration. Together, these data suggest that EGF-mediated suppression of *GATA3* could be detrimental to the integrity of the permeability barrier, as major components of the lipid matrix are significantly reduced.

Evidence for additional levels of EGFR-mediated regulation is observed in the example of *TIAM1* (T-lymphoma invasion and metastasis 1), a RAC-specific guanine nucleotide exchange factor, whose level of expression is significantly decreased by EGF. In epithelial cells, RAC1 regulates the formation and function of adheren and TJs in epithelial cells (Lozano et al, 2003). Epidermal keratinocytes from *TIAM1*-deficient mice show impaired junction maturation by lowering levels of TJ proteins such as CLDN1 and TJP1, and TJ barrier function (Mertens et al, 2005). In wild-type keratinocytes, the TIAM1-dependent activated RAC1 binds to PAR3 (protease-activated receptor 3) and threonine phosphorylates PKCζ (protein kinase C, zeta) of the polarity complex (PAR3-PAR6-aPKC) to control the TJ formation (Mertens et al., 2005). Based on these observations, it seems reasonable to hypothesize that EGF diminishes TIAM1-mediated RAC signaling, and thereby inactivates the polarity complex, leading to the reduction of CLDN1 and TJP1 proteins and inhibition of TJ biogenesis. This, in turn, disrupts the permeability barrier function, making the barrier more permissive to many environmental allergens. This increased sensitivity to allergens is one of the characteristics observed in skin of patients with AD (de Benedetto et al., 2011).

In the epidermis, keratinocyte cell fate is tightly regulated by orchestrating large scale changes in gene expression. Here, as previously reported (Okuyama et al., 2004; Wilke et

al., 1988), cell density affected the expression of at least four thousand genes, directing the progression of keratinocytes through a terminal differentiation program. Shown here, EGF counter-regulated more than 50% of these genes, producing the opposite effects on keratinocytes compared to density. Moreover, EGF down-regulated genes are reported to be expressed in suprabasal layers, whereas EGF up-regulated genes are reported to be expressed in the basal layer. EGF is known to suppress keratinocyte differentiation by inhibiting mRNA and protein levels of early and late differentiation markers (Drozdoff and Pledger, 1993; Marchese et al., 1990; Poumay and Pittelkow, 1995). Here, we observed that EGF suppressed the differentiation program by both inhibiting and promoting the expression of genes related to the differentiation or proliferation of keratinocytes, respectively, providing a new view of how EGF is regulating epidermal homeostasis.

Adding to this new view, we found that EGF down-regulated DSG1, an important signaling molecule of epidermal tissue homeostasis. The induction of DSG1 is required to maintain the epidermal barrier integrity and suppress EGFR-mediated ERK1/2 signaling, to promote keratinocyte differentiation (Getsios et al., 2009). The expression of this gene was increased about 12-fold by cell density. However, the induction of DSG1 and its direct transcription factor GRHL1 (Wilanowski et al., 2008) was abolished by EGF, indicating that EGF creates a feedback regulation between EGFR and DSG1. This reciprocal action of DSG1 and EGFR to repress one-another provides a specific example of a mechanism for regulating epidermal homeostasis. Taken together, this raises the possibility that EGF functions as a key regulator that controls cell fate by altering gene

expression through modulating the expression of TFs involved in the terminal differentiation process.

The epidermis is a dynamic tissue in which highly regulated mechanisms exist to balance keratinocyte proliferation and differentiation and maintain epidermal tissue homeostasis. EGF appears to disrupt this balance by down- and up-regulating RNA and protein levels of pro-differentiation and pro-proliferation transcription factors, respectively. An example of a keratinocyte differentiation regulator is FOXN1, a winged helix/forkhead transcription factor, which is reported to regulate the balance between proliferation and differentiation in self-renewing epithelia (Prowse et al., 1999). In mice, rats, and humans, loss-of-function mutations of *FOXN1* leads to nude phenotypes that are characterized by the abnormal development of the epidermis, the lack of visible hair, and the absence of the thymus (Flanagan, 1966; Pantelouris and Macmenamin, 1973). In murine epidermis and hair follicles, loss of *Foxn1* expression impairs terminal differentiation, as the stratum corneum, the inner root sheath, and the hair cuticle and cortex fail to form properly (Kopf-Maier et al., 1990). Ectopic expression of *Foxn1* in the epidermis using the *Ivl* promoter induces hyperproliferation and defects in differentiation (Prowse et al., 1999). In cultures of primary keratinocytes from wild-type mice, *Foxn1* is induced during the early stages of terminal differentiation (Baxter and Brissette, 2002). The RNA levels of FOXN1 were significantly blocked by EGF starting at 4 h and remained repressed through 24 h, leading to a loss of protein at 48 h. Another important regulator of keratinocyte differentiation is POU2F3 (also known as SKN-1A), a TF containing a POU domain. This TF is a candidate for -determining cell fate in skin (Andersen et al., 1993; Goldsborough et al., 1993). POU2F3 is expressed primarily in

the suprabasal layers of the epidermis where it directly regulates expression of *KRT10* (Andersen et al., 1993), *SPRR2A* (Fischer et al., 1996), and *IVL* (Welter et al., 1996) genes. In mice, *in vivo* ablation mutation of *Pou2f3* does not reveal a specific function of the gene (Andersen et al., 1997). However, in *in vitro* raft cultures, POU2F3 plays an important role in maintaining epidermal homeostasis by primarily promoting keratinocyte proliferation, and secondarily by enhancing subsequent keratinocyte differentiation (Hildesheim et al., 2001). RNA levels of *POU2F3* were down-regulated by EGF significantly at 16 and 24 h, while its protein expression was repressed at 48 h by EGF. Another important regulator of keratinocyte differentiation is ELF5, a member of an epithelium-specific subclass of the Ets domain transcription factor family. The *ELF5* transcript is not expressed in undifferentiated keratinocytes, but is induced during keratinocyte differentiation. A potential target for ELF5 is *SPRR2A* gene (Oettgen et al., 1999). RNA and protein levels of ELF5 were down-regulated significantly by EGF starting at 8 h. In our study, EGF also suppressed RNA levels of *NOTCH1* at 4 h. EGFR signaling is reported as negative regulator of the expression of NOTCH1, an important regulator of cell-fate decision, thereby blocking the action of NOTCH1 to promote keratinocyte differentiation in cell culture and skin explants (Kolev et al., 2008). These examples of EGFR signaling as a negative regulator of the expression of FOXN1, POU2F3, ELF5, and NOTCH1, support a model where EGFR signaling influences keratinocyte cell fate by regulating the expression of multiple pro-differentiation transcription factors, each affecting distinct aspects of cell differentiation.

Besides down-regulating pro-differentiation TFs, EGF appeared to up-regulate many transcriptional regulators involved in keratinocyte proliferenation. For example, EGF

significantly increased the transcript levels of *ETS1* at 4 and 8 h. ETS1 is an oncogene that functions as a TF. In mice, Ets1 is mainly expressed in undifferentiated keratinocytes of the skin and its expression is downregulated as cells commit to terminal differentiation program (Nagarajan et al., 2009). Ectopic expression of *ETS1* in differentiated keratinocytes of transgenic mice results in major epidermal defects including shiny, taut, and translucent skin. These mice die shortly after birth due to rapid dehydration (Nagarajan et al., 2010). Induction of *ETS1* leads to an increase in cellular proliferation in the basal layer, absence of the granular layer, and retention of nuclei in the stratum corneum. Overexpression of *ETS1* results in premature expression of the early differentiation marker *KRT10*, whereas the expression of late differentiation markers such as *LOR* and *FLG* are decreased (Nagarajan et al., 2010). Another anti-differentiation regulator that was up-regulated by EGF is SMAD7. SMAD7 has an antagonistic effect on TGFβ signaling, a potent inhibitor of cellular proliferation in skin keratinocytes (Munger et al., 1992). It is suggested that SMAD7 may participate in a negative feedback loop to control TGFβ responses in normal epidermis (He et al., 2001). Mouse Smad7 protein decreases normal differentiation of primary mouse keratinocytes (Liu et al., 2003). EGF also upregulated RNA levels of *MXD1*, a transcriptional repressor belongs to a subfamily of MAX-interacting proteins. The MYC/MAX/MAD network plays a role in cell proliferation, differentiation, and death (Grandori et al., 2000). The MXD1 protein can inhibit cellular growth *in vitro* (Vastrik et al., 1995). MXD1 is highly expressed in differentiating epidermal keratinocytes, whereas its expression is absent in proliferating basal epidermal cells (Lymboussaki et al., 1996). These results

indicate that EGF also controls transcriptional regulators involved in keratinocyte proliferation to affect epidermal homeostasis.

EGFR signaling regulates fundamental aspects in skin biology, including cellular proliferation and differentiation, wound healing, and hair follicle morphogenesis (Schneider et al., 2008). The MAPKs, PI3K, and PLC are the three majors signaling cascades of EGFR signaling. The MAPKs include ERKs, JNKs, and the p38 kinases (Gazel et al., 2008). In cultured keratinocytes, JNK activation inhibits epidermal differentiation (Gazel et al., 2006). The p38 pathway is activated in keratinocytes by oxidative stress, UV light, proinflammatory signals, psoriasis, and wound healing (Kobayashi et al., 2003). It plays an important role in the pathogenesis of pemphigus vulgaris and mediates keratinocyte migration (Li et al., 2004). Activation of ERK produces very similar effects to those of p38 activation (Yano et al., 2004). The PI3K /Akt pathway is activated early during differentiation in cultured keratinocytes and in the intact epidermis. This activation is a key determinant of keratinocyte differentiation and survival (Calautti et al., 2005). EGFR also activates PLC pathway which affects cell migration (Dittmar et al., 2002). Because of the diverse effects of these signaling pathways on keratinocytes, we hope to identify, by inhibiting these signaling pathways, the regulatory networks that control the identified transcription factors and regulate keratinocyte homeostasis. Unfortunately, we found that these pathways significantly overlap in the TF genes that they target even though there are distinct and unique effects among these EGFR signaling pathways. We noticed that EGF affects keratinocyte differentiation and proliferation transcriptional regulators late in time (starting at 8 h after EGF treatment), indicating that these genes may be activated following the synthesis of

immediate early gene products. Our study design did not capture immediate early genes that are transiently and rapidly transcribed in response to the addition of EGF treatment. The transcription of immediate early genes does not require *de novo* protein synthesis. We now believe that immediate early genes determine the genomic response of keratinocyte to the density and EGF treatment, and that EGFR signaling should be mapped to these genes, once identified. As a result, another study design will need to be performed at early times such as 1, 5, 10, and 30 min, in the presence or absence of cycloheximide, an inhibitor of protein synthesis (Godchaux et al., 1967).

Human and nonhuman genetic studies have identified a large number of genes associated with dermatological diseases. Intriguingly, we have shown that among the density-induced genes when compared to the list of EGF-nonresponsive genes, EGF-responsiveness significantly enriches for genes associated with skin disease. For example, EGF resulted in a 67% reduction in the levels of *ABCA12*. Loss of function of *ABCA12* leads to lipid trafficking defects and decreases in the total amount of Cer (Yanagi et al., 2010). Mutations in this gene are causally associated with Harlequin ichthyosis (Akiyama et al., 2005). Similarily, EGF reduced by 29% the level of *SLC27A4*, a gene encoding the long chain fatty acid transporter. Mutations in this gene are associated with ichthyosis premature syndrome (Klar et al., 2009). In contrast to these examples, EGF up-regulates the level of expression of *S100A7*, a transglutaminase substrate/CE precursor that is highly elevated in psoriasis and AD (Glaser et al., 2009; Madsen et al., 1992). While these three genes are exemplary of the effects of EGF on known human skin disease genes, it is important to note that 111 similarly responsive skin disease genes and another 1184 genes not yet associated with skin disease have been identified by this

research. These data provide a new understanding of the molecular mechanisms by which EGF affects epidermal homeostasis and show how imbalance in this signaling pathway may lead to system-wide pathogenesis.

In conclusion, our results provide a systems level understanding of EGFR signaling in repressing keratinocyte differentiation and impairing functional epidermal barrier integrity. Epidermal homeostasis appears to be controlled by the spatial and temporal expression and activity of the EGFR, its ligands, and suppressors thereof. Activation of EGFR preferentially regulates genes known to be associated with skin disease, highlighting the importance of EGFR in skin pathology. Our data provide a valuable resource for further dissecting the molecular events and genetic basis in dermatological diseases.

REFERENCES

Aberer, W., Schuler, G., Stingl, G., Honigsmann, H., and Wolff, K. (1981). Ultraviolet light depletes surface markers of Langerhans cells. J. Invest. Dermatol. *76*, 202-210.

Abramoff, M.D., Magalhaes, P.J., and Ram, S.J. (2004). Image Processing with ImageJ. Biophotonics International *11*, 36-42.

Abts, H.F., Welss, T., Scheuring, S., Scott, F.L., Irving, J.A., Michel, G., Bird, P.I., and Ruzicka, T. (2001). Sequence, organization, chromosomal localization, and alternative splicing of the human serine protease inhibitor gene hurpin (PI13) which is upregulated in psoriasis. DNA Cell Biol *20*, 123-131.

Agero, A.L., Dusza, S.W., Benvenuto-Andrade, C., Busam, K.J., Myskowski, P., and Halpern, A.C. (2006). Dermatologic side effects associated with the epidermal growth factor receptor inhibitors. J Am Acad Dermatol *55*, 657-670.

Ahn, J.Y., Choi, S.E., Jeong, M.S., Park, K.H., Moon, N.J., Joo, S.S., Lee, C.S., Choi, Y.W., Li, K., Lee, M.K., Lee, M.W., and Seo, S.J. (2010). Effect of taxifolin glycoside on atopic dermatitis-like skin lesions in NC/Nga mice. Phytother Res *24*, 1071-1077.

Akhouayri, O., Quelo, I., and St-Arnaud, R. (2005). Sequence-specific DNA binding by the alphaNAC coactivator is required for potentiation of c-Jun-dependent transcription of the osteocalcin gene. Mol Cell Biol *25*, 3452-3460.

Akiyama, M., Sugiyama-Nakagiri, Y., Sakai, K., McMillan, J.R., Goto, M., Arita, K., Tsuji-Abe, Y., Tabata, N., Matsuoka, K., Sasaki, R., Sawamura, D., and Shimizu, H. (2005). Mutations in lipid transporter ABCA12 in harlequin ichthyosis and functional recovery by corrective gene transfer. J Clin Invest *115*, 1777-1784.

Alef, T., Torres, S., Hausser, I., Metze, D., Tursen, U., Lestringant, G.G., and Hennies, H.C. (2009). Ichthyosis, follicular atrophoderma, and hypotrichosis caused by mutations in ST14 is associated with impaired profilaggrin processing. J Invest Dermatol *129*, 862-869.

Allen, M., Ishida-Yamamoto, A., McGrath, J., Davison, S., Iizuka, H., Simon, M., Guerrin, M., Hayday, A., Vaughan, R., Serre, G., Trembath, R., and Barker, J. (2001). Corneodesmosin expression in psoriasis vulgaris differs from normal skin and other inflammatory skin disorders. Lab Invest *81*, 969-976.

An, K.P., Athar, M., Tang, X., Katiyar, S.K., Russo, J., Beech, J., Aszterbaum, M., Kopelovich, L., Epstein, E.H., Jr., Mukhtar, H., and Bickers, D.R. (2002). Cyclooxygenase-2 expression in murine and human nonmelanoma skin cancers: implications for therapeutic approaches. Photochem Photobiol *76*, 73-80.

Andersen, B., Schonemann, M.D., Flynn, S.E., Pearse, R.V., 2nd, Singh, H., and Rosenfeld, M.G. (1993). Skn-1a and Skn-1i: two functionally distinct Oct-2-related factors expressed in epidermis. Science *260*, 78-82.

Andersen, B., Weinberg, W.C., Rennekampff, O., McEvilly, R.J., Bermingham, J.R., Jr., Hooshmand, F., Vasilyev, V., Hansbrough, J.F., Pittelkow, M.R., Yuspa, S.H., and Rosenfeld, M.G. (1997). Functions of the POU domain genes Skn-1a/i and Tst-1/Oct-6/SCIP in epidermal differentiation. Genes Dev *11*, 1873-1884.

Aoudjehane, L., Pissaia, A., Jr., Scatton, O., Podevin, P., Massault, P.P., Chouzenoux, S., Soubrane, O., Calmus, Y., and Conti, F. (2008). Interleukin-4 induces the activation and collagen production of cultured human intrahepatic fibroblasts via the STAT-6 pathway. Lab Invest *88*, 973-985.

Arakawa, S., Hatano, Y., and Katagiri, K. (2004). Differential expression of mRNA for Th1 and Th2 cytokine-associated transcription factors and suppressors of cytokine signalling in peripheral blood mononuclear cells of patients with atopic dermatitis. Clin Exp Immunol *135*, 505-510.

Arikawa, J., Ishibashi, M., Kawashima, M., Takagi, Y., Ichikawa, Y., and Imokawa, G. (2002). Decreased levels of sphingosine, a natural antimicrobial agent, may be associated with vulnerability of the stratum corneum from patients with atopic dermatitis to colonization by Staphylococcus aureus. J Invest Dermatol *119*, 433-439.

Arnold, I., and Watt, F.M. (2001). c-Myc activation in transgenic mouse epidermis results in mobilization of stem cells and differentiation of their progeny. Curr Biol *11*, 558-568.

Atchley, W.R., and Fitch, W.M. (1995). Myc and Max: molecular evolution of a family of proto-oncogene products and their dimerization partner. Proc. Natl. Acad. Sci. USA. *92*, 10217-10221.

Atit, R., Conlon, R.A., and Niswander, L. (2003). EGF signaling patterns the feather array by promoting the interbud fate. Dev Cell *4*, 231-240.

Atit, R., Sgaier, S.K., Mohamed, O.A., Taketo, M.M., Dufort, D., Joyner, A.L., Niswander, L., and Conlon, R.A. (2006). Beta-catenin activation is necessary and sufficient to specify the dorsal dermal fate in the mouse. Dev Biol *296*, 164-176.

Baden, H.P., Champliaud, M.F., Sundberg, J.P., and Viel, A. (2005). Targeted deletion of the sciellin gene resulted in normal development and maturation. Genesis *42*, 219-228.

Baden, H.P., and Maderson, P.F. (1970). Morphological and biophysical identification of fibrous proteins in the amniote epidermis. J Exp Zool *174*, 225-232.

Bartoletti, A., Cancedda, L., Reid, S.W., Tessarollo, L., Porciatti, V., Pizzorusso, T., and Maffei, L. (2002). Heterozygous knock-out mice for brain-derived neurotrophic factor show a pathway-specific impairment of long-term potentiation but normal critical period for monocular deprivation. J Neurosci *22*, 10072-10077.

Baxter, R.M., and Brissette, J.L. (2002). Role of the nude gene in epithelial terminal differentiation. J. Invest. Dermatol. *118*, 303-309.

Bazzi, H., Fantauzzo, K.A., Richardson, G.D., Jahoda, C.A., and Christiano, A.M. (2007). Transcriptional profiling of developing mouse epidermis reveals novel patterns of coordinated gene expression. Dev Dyn *236*, 961-970.

Bazzi, H., Getz, A., Mahoney, M.G., Ishida-Yamamoto, A., Langbein, L., Wahl, J.K., 3rd, and Christiano, A.M. (2006). Desmoglein 4 is expressed in highly differentiated keratinocytes and trichocytes in human epidermis and hair follicle. Differentiation *74*, 129-140.

Bell, G.I., Fong, N.M., Stempien, M.M., Wormsted, M.A., Caput, D., Ku, L.L., Urdea, M.S., Rall, L.B., and Sanchez-Pescador, R. (1986). Human epidermal growth factor precursor: cDNA sequence, expression in vitro and gene organization. Nucleic Acids Res *14*, 8427-8446.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practival and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society *57*, 11.

Bernard, D., Mehul, B., Thomas-Collignon, A., Delattre, C., Donovan, M., and Schmidt, R. (2005). Identification and characterization of a novel retroviral-like aspartic protease specifically expressed in human epidermis. J Invest Dermatol *125*, 278-287.

Beutler, E., and West, C. (2002). Polymorphisms in glucosylceramide (glucocerebroside) synthase and the Gaucher disease phenotype. Isr Med Assoc J *4*, 986-988.

Bikle, D.D., Ratnam, A., Mauro, T., Harris, J., and Pillai, S. (1996). Changes in calcium responsiveness and handling during keratinocyte differentiation. Potential role of the calcium receptor. J. Clin. Invest. *97*, 1085-1093.

Bilioni, A., Craig, G., Hill, C., and McNeill, H. (2005). Iroquois transcription factors recognize a unique motif to mediate transcriptional repression in vivo. Proc. Natl. Acad. Sci. USA. *102*, 14671-14676.

Birbeck, M.S. (1962). Electron microscopy of melanocytes. Br Med Bull *18*, 220-222.
Blanpain, C., and Fuchs, E. (2006). Epidermal stem cells of the skin. Annu Rev Cell Dev Biol *22*, 339-373.

Blessing, M., Schirmacher, P., and Kaiser, S. (1996). Overexpression of bone morphogenetic protein-6 (BMP-6) in the epidermis of transgenic mice: inhibition or stimulation of proliferation depending on the pattern of transgene expression and formation of psoriatic lesions. J Cell Biol *135*, 227-239.

Bligh, E.G., and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. Can J Biochem Physiol *37*, 911-917.

Bloor, A.J., Kotsopoulou, E., Hayward, P., Champion, B.R., and Green, A.R. (2005). RFP represses transcriptional activation by bHLH transcription factors. Oncogene *24*, 6729-6736.

Boissy, R.E., and Nordlund, J.J. (1996). Biology of melanocytes. (Philadelphia: W.B. Saunders Co.).

Bolling, M.C., Bladergroen, R.S., van Steensel, M.A., Willemsen, M., Jonkman, M.F., and van Geel, M. A novel mutation in the L12 domain of keratin 1 is associated with mild epidermolytic ichthyosis. Br J Dermatol *162*, 875-879.

Boniface, K., Diveu, C., Morel, F., Pedretti, N., Froger, J., Ravon, E., Garcia, M., Venereau, E., Preisser, L., Guignouard, E., Guillet, G., Dagregorio, G., Pene, J., Moles, J.P., Yssel, H., Chevalier, S., Bernard, F.X., Gascan, H., and Lecron, J.C. (2007). Oncostatin M secreted by skin infiltrating T lymphocytes is a potent keratinocyte activator involved in skin inflammation. J Immunol *178*, 4615-4622.

Bonifas, J.M., Rothman, A.L., and Epstein, E.H., Jr. (1991). Epidermolysis bullosa simplex: evidence in two families for keratin gene abnormalities. Science *254*, 1202-1205.
Borgono, C.A., Gavigan, J.A., Alves, J., Bowles, B., Harris, J.L., Sotiropoulou, G., and Diamandis, E.P. (2007). Defining the extended substrate specificity of kallikrein 1-related peptidases. Biol Chem *388*, 1215-1225.

Bostjancic, E., and Glavac, D. (2008). Importance of microRNAs in skin morphogenesis and diseases. Acta Dermatovenerol Alp Panonica Adriat *17*, 95-102.

Bours, V., Franzoso, G., Azarenko, V., Park, S., Kanno, T., Brown, K., and Siebenlist, U. (1993). The oncoprotein Bcl-3 directly transactivates through kappa B motifs via association with DNA-binding p50B homodimers. Cell *72*, 729-739.

Bower, J.M., Camble, R., Gregory, H., Gerring, E.L., and Willshire, I.R. (1975). The inhibition of gastric acid secretion by epidermal growth factor. Experientia *31*, 825-826.
Brattsand, M., and Egelrud, T. (1999). Purification, molecular cloning, and expression of a human stratum corneum trypsin-like serine protease with possible function in desquamation. J Biol Chem *274*, 30033-30040.

Breiden, B., Gallala, H., Doering, T., and Sandhoff, K. (2007). Optimization of submerged keratinocyte cultures for the synthesis of barrier ceramides. Eur J Cell Biol *86*, 657-673.

Briegel, K.J., and Joyner, A.L. (2001). Identification and characterization of Lbh, a novel conserved nuclear protein expressed during early limb and heart development. Dev Biol *233*, 291-304.

Brinkmeier, M.L., Potok, M.A., Cha, K.B., Gridley, T., Stifani, S., Meeldijk, J., Clevers, H., and Camper, S.A. (2003). TCF and Groucho-related genes influence pituitary growth and development. Mol Endocrinol *17*, 2152-2161.

Bruch-Gerharz, D., Schnorr, O., Suschek, C., Beck, K.F., Pfeilschifter, J., Ruzicka, T., and Kolb-Bachofen, V. (2003). Arginase 1 overexpression in psoriasis: limitation of inducible nitric oxide synthase activity as a molecular mechanism for keratinocyte hyperproliferation. Am J Pathol *162*, 203-211.

Brunet, A., Bonni, A., Zigmond, M.J., Lin, M.Z., Juo, P., Hu, L.S., Anderson, M.J., Arden, K.C., Blenis, J., and Greenberg, M.E. (1999). Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell *96*, 857-868.

Burridge, K., Fath, K., Kelly, T., Nuckolls, G., and Turner, C. (1988). Focal adhesions: transmembrane junctions between the extracellular matrix and the cytoskeleton. Annu Rev Cell Biol *4*, 487-525.

Cabral, A., Voskamp, P., Cleton-Jansen, A.M., South, A., Nizetic, D., and Backendorf, C. (2001). Structural organization and regulation of the small proline-rich family of cornified envelope precursors suggest a role in adaptive barrier function. J Biol Chem *276*, 19231-19237.

Calautti, E., Li, J., Saoncella, S., Brissette, J.L., and Goetinck, P.F. (2005). Phosphoinositide 3-kinase signaling to Akt promotes keratinocyte differentiation versus death. J Biol Chem *280*, 32856-32865.

Cameron, D.J., Tong, Z., Yang, Z., Kaminoh, J., Kamiyah, S., Chen, H., Zeng, J., Chen, Y., Luo, L., and Zhang, K. (2007). Essential role of Elovl4 in very long chain fatty acid synthesis, skin permeability barrier function, and neonatal survival. Int J Biol Sci *3*, 111-119.

Candi, E., Oddi, S., Paradisi, A., Terrinoni, A., Ranalli, M., Teofoli, P., Citro, G., Scarpato, S., Puddu, P., and Melino, G. (2002). Expression of transglutaminase 5 in normal and pathologic human epidermis. J Invest Dermatol *119*, 670-677.

Candi, E., Schmidt, R., and Melino, G. (2005). The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol *6*, 328-340.

Capon, F., Boulding, H., Quaranta, M., Mortimer, N.J., Setterfield, J.F., Black, M.M., Trembath, R.C., and Harman, K.E. (2009). Genetic analysis of desmoglein 3 (DSG3) sequence variants in patients with pemphigus vulgaris. Br J Dermatol *161*, 1403-1405.

Carmeliet, P., Ng, Y.S., Nuyens, D., Theilmeier, G., Brusselmans, K., Cornelissen, I., Ehler, E., Kakkar, V.V., Stalmans, I., Mattot, V., Perriard, J.C., Dewerchin, M., Flameng, W., Nagy, A., Lupu, F., Moons, L., Collen, D., D'Amore, P.A., and Shima, D.T. (1999). Impaired myocardial angiogenesis and ischemic cardiomyopathy in mice lacking the vascular endothelial growth factor isoforms VEGF164 and VEGF188. Nat Med *5*, 495-502.

Carpenter, G. (1980a). Epidermal growth factor is a major growth-promoting agent in human milk. Science *210*, 198-199.

Carpenter, G. (1980b). Epidermal growth factor: biology and mechanism of action. Birth Defects Orig Artic Ser *16*, 61-72.

Carpenter, G., and Zendegui, J.G. (1986). Epidermal growth factor, its receptor, and related proteins. Exp Cell Res *164*, 1-10.

Castagnino, P., Biesova, Z., Wong, W.T., Fazioli, F., Gill, G.N., and Di Fiore, P.P. (1995). Direct binding of eps8 to the juxtamembrane domain of EGFR is phosphotyrosine- and SH2-independent. Oncogene *10*, 723-729.

Catterton, W.Z., Escobedo, M.B., Sexson, W.R., Gray, M.E., Sundell, H.W., and Stahlman, M.T. (1979). Effect of epidermal growth factor on lung maturation in fetal rabbits. Pediatr Res *13*, 104-108.

Chang, L.K., Chung, J.Y., Hong, Y.R., Ichimura, T., Nakao, M., and Liu, S.T. (2005). Activation of Sp1-mediated transcription by Rta of Epstein-Barr virus via an interaction with MCAF1. Nucleic Acids Res *33*, 6528-6539.

Chaplin, T., Ayton, P., Bernard, O.A., Saha, V., Della Valle, V., Hillion, J., Gregorini, A., Lillington, D., Berger, R., and Young, B.D. (1995). A novel class of zinc finger/leucine zipper genes identified from the molecular cloning of the t(10;11) translocation in acute leukemia. Blood *85*, 1435-1441.

Charest, J.L., Jennings, J.M., King, W.P., Kowalczyk, A.P., and Garcia, A.J. (2009). Cadherin-mediated cell-cell contact regulates keratinocyte differentiation. J. Invest. Dermatol. *129*, 564-572.

Chariot, A., Castronovo, V., Le, P., Gillet, C., Sobel, M.E., and Gielen, J. (1996). Cloning and expression of a new HOXC6 transcript encoding a repressing protein. Biochem J *319 (Pt 1)*, 91-97.

Chavanas, S., Mechin, M.C., Nachat, R., Adoue, V., Coudane, F., Serre, G., and Simon, M. (2006). Peptidylarginine deiminases and deimination in biology and pathology: relevance to skin homeostasis. J Dermatol Sci *44*, 63-72.

Chen, C.-S.J., Lavker, R.M., Rodeck, U., Risse, B., and Jensen, P.J. (1995a). Use of a Serum-Free Epidermal Culture Model to Show Deleterious Effects of EPidermal Growth Factor on Morphogenesis and Differentiation. J Invest Dermatol *104*, 6.

Chen, C.S., Lavker, R.M., Rodeck, U., Risse, B., and Jensen, P.J. (1995b). Use of a serum-free epidermal culture model to show deleterious effects of epidermal growth factor on morphogenesis and differentiation. J Invest Dermatol *104*, 107-112.

Chen, H., and Sharp, B.M. (2004). Content-rich biological network constructed by mining PubMed abstracts. BMC Bioinformatics *5*, 147.

Chen, H.H., Mullett, S.J., and Stewart, A.F. (2004). Vgl-4, a novel member of the vestigial-like family of transcription cofactors, regulates alpha1-adrenergic activation of gene expression in cardiac myocytes. J. Biol. Chem. *279*, 30800-30806.

Chen, S.H., Arany, I., Apisarnthanarax, N., Rajaraman, S., Tyring, S.K., Horikoshi, T., Brysk, H., and Brysk, M.M. (2000). Response of keratinocytes from normal and psoriatic epidermis to interferon-gamma differs in the expression of zinc-alpha(2)-glycoprotein and cathepsin D. FASEB J *14*, 565-571.

Chen, Y.H., Kim, J.H., and Stallcup, M.R. (2005). GAC63, a GRIP1-dependent nuclear receptor coactivator. Mol Cell Biol *25*, 5965-5972.

Chen, Y.J., Chang, J.T., Lee, L., Wang, H.M., Liao, C.T., Chiu, C.C., Chen, P.J., and Cheng, A.J. (2007). DSG3 is overexpressed in head neck cancer and is a potential molecular target for inhibition of oncogenesis. Oncogene *26*, 467-476.

Cheng, T., Hitomi, K., van Vlijmen-Willems, I.M., de Jongh, G.J., Yamamoto, K., Nishi, K., Watts, C., Reinheckel, T., Schalkwijk, J., and Zeeuwen, P.L. (2006). Cystatin M/E is a high affinity inhibitor of cathepsin V and cathepsin L by a reactive site that is distinct from the legumain-binding site. A novel clue for the role of cystatin M/E in epidermal cornification. J. Biol. Chem. *281*, 15893-15899.

Cheng, T., Tjabringa, G.S., van Vlijmen-Willems, I.M., Hitomi, K., van Erp, P.E., Schalkwijk, J., and Zeeuwen, P.L. (2009). The cystatin M/E-controlled pathway of skin barrier formation: expression of its key components in psoriasis and atopic dermatitis. Br J Dermatol *161*, 253-264.

Chiba, H., Michibata, H., Wakimoto, K., Seishima, M., Kawasaki, S., Okubo, K., Mitsui, H., Torii, H., and Imai, Y. (2004). Cloning of a gene for a novel epithelium-specific cytosolic phospholipase A2, cPLA2delta, induced in psoriatic skin. J Biol Chem *279*, 12890-12897.

Chidgey, M., Brakebusch, C., Gustafsson, E., Cruchley, A., Hail, C., Kirk, S., Merritt, A., North, A., Tselepis, C., Hewitt, J., Byrne, C., Fassler, R., and Garrod, D. (2001). Mice lacking desmocollin 1 show epidermal fragility accompanied by barrier defects and abnormal differentiation. J. Cell Biol. *155*, 821-832.

Choi, Y.S., and Sinha, S. (2006). Determination of the consensus DNA-binding sequence and a transcriptional activation domain for ESE-2. Biochem J *398*, 497-507.

Chokki, M., Mitsuhashi, H., and Kamimura, T. (2006). Metalloprotease-dependent amphiregulin release mediates tumor necrosis factor-alpha-induced IL-8 secretion in the human airway epithelial cell line NCI-H292. Life Sci *78*, 3051-3057.

Cismasiu, V.B., Paskaleva, E., Suman Daya, S., Canki, M., Duus, K., and Avram, D. (2008). BCL11B is a general transcriptional repressor of the HIV-1 long terminal repeat in T lymphocytes through recruitment of the NuRD complex. Virology *380*, 173-181.

Citri, A., and Yarden, Y. (2006). EGF-ERBB signalling: towards the systems level. Nat Rev Mol Cell Biol *7*, 505-516.

Cline, P.R., and Rice, R.H. (1983). Modulation of involucrin and envelope competence in human keratinocytes by hydrocortisone, retinyl acetate, and growth arrest. Cancer Res *43*, 3203-3207.

Coffey, R.J., Jr., Derynck, R., Wilcox, J.N., Bringman, T.S., Goustin, A.S., Moses, H.L., and Pittelkow, M.R. (1987). Production and auto-induction of transforming growth factor-alpha in human keratinocytes. Nature *328*, 817-820.

Cohen, S. (1962). Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the new-born animal. J Biol Chem *237*, 1555-1562.
Cohen, S. (1965). The stimulation of epidermal proliferation by a specific protein (EGF). Dev Biol *12*, 394-407.

Cohen, S., and Carpenter, G. (1975). Human epidermal growth factor: isolation and chemical and biological properties. Proc Natl Acad Sci U S A *72*, 1317-1321.

Cohen, S., and Stastny, M. (1968). Epidermal growth factor. 3. The stimulation of polysome formation in chick embryo epidermis. Biochim Biophys Acta *166*, 427-437.

Coimbra, S., Oliveira, H., Reis, F., Belo, L., Rocha, S., Quintanilha, A., Figueiredo, A., Teixeira, F., Castro, E., Rocha-Pereira, P., and Santos-Silva, A. C-reactive protein and leucocyte activation in psoriasis vulgaris according to severity and therapy. J Eur Acad Dermatol Venereol *24*, 789-796.

Collin, C., Moll, R., Kubicka, S., Ouhayoun, J.P., and Franke, W.W. (1992). Characterization of human cytokeratin 2, an epidermal cytoskeletal protein synthesized late during differentiation. Exp Cell Res *202*, 132-141.

Conesa, A., Nueda, M.J., Ferrer, A., and Talon, M. (2006). maSigPro: a method to identify significantly differential expression profiles in time-course microarray experiments. Bioinformatics *22*, 1096-1102.

Conrad, P.W., Freeman, T.L., Beitner-Johnson, D., and Millhorn, D.E. (1999). EPAS1 trans-activation during hypoxia requires p42/p44 MAPK. J. Biol. Chem. *274*, 33709-33713.

Contzler, R., Favre, B., Huber, M., and Hohl, D. (2005). Cornulin, a new member of the "fused gene" family, is expressed during epidermal differentiation. J. Invest. Dermatol. *124*, 990-997.

Corden, L.D., and McLean, W.H. (1996). Human keratin diseases: hereditary fragility of specific epithelial tissues. Exp Dermatol *5*, 297-307.

Coso, O.A., Chiariello, M., Yu, J.C., Teramoto, H., Crespo, P., Xu, N., Miki, T., and Gutkind, J.S. (1995). The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. Cell *81*, 1137-1146.

Coulombe, P.A., and Fuchs, E. (1990). Elucidating the early stages of keratin filament assembly. J Cell Biol *111*, 153-169.

Coulombe, P.A., Hutton, M.E., Letai, A., Hebert, A., Paller, A.S., and Fuchs, E. (1991). Point mutations in human keratin 14 genes of epidermolysis bullosa simplex patients: genetic and functional analyses. Cell *66*, 1301-1311.

Covaciu, C., Castori, M., De Luca, N., Ghirri, P., Nannipieri, A., Ragone, G., Zambruno, G., and Castiglia, D. (2010). Lethal autosomal recessive epidermolytic ichthyosis due to a novel donor splice-site mutation in KRT10. Br J Dermatol.

Cowger, J.J., Zhao, Q., Isovic, M., and Torchia, J. (2007). Biochemical characterization of the zinc-finger protein 217 transcriptional repressor complex: identification of a ZNF217 consensus recognition sequence. Oncogene *26*, 3378-3386.

Dahlqvist, J., Klar, J., Hausser, I., Anton-Lamprecht, I., Pigg, M.H., Gedde-Dahl, T., Jr., Ganemo, A., Vahlquist, A., and Dahl, N. (2007). Congenital ichthyosis: mutations in ichthyin are associated with specific structural abnormalities in the granular layer of epidermis. J Med Genet *44*, 615-620.

Dale, B.A., Holbrook, K.A., Kimball, J.R., Hoff, M., and Sun, T.T. (1985). Expression of epidermal keratins and filaggrin during human fetal skin development. J Cell Biol *101*, 1257-1269.

Datta, S.R., Brunet, A., and Greenberg, M.E. (1999). Cellular survival: a play in three Akts. Genes Dev *13*, 2905-2927.

Davis, R.J. (2000). Signal transduction by the JNK group of MAP kinases. Cell *103*, 239-252.

De Benedetto, A., Rafaels, N.M., McGirt, L.Y., Ivanov, A.I., Georas, S.N., Cheadle, C., Berger, A.E., Zhang, K., Vidyasagar, S., Yoshida, T., Boguniewicz, M., Hata, T., Schneider, L.C., Hanifin, J.M., Gallo, R.L., Novak, N., Weidinger, S., Beaty, T.H., Leung, D.Y., Barnes, K.C., and Beck, L.A. Tight junction defects in patients with atopic dermatitis. J Allergy Clin Immunol *127*, 773-786 e771-777.

de Guzman Strong, C., Wertz, P.W., Wang, C., Yang, F., Meltzer, P.S., Andl, T., Millar, S.E., Ho, I.C., Pai, S.Y., and Segre, J.A. (2006). Lipid defect underlies selective skin barrier impairment of an epidermal-specific deletion of Gata-3. J Cell Biol *175*, 661-670.

de Juanes, S., Epp, N., Latzko, S., Neumann, M., Furstenberger, G., Hausser, I., Stark, H.J., and Krieg, P. (2009). Development of an ichthyosiform phenotype in Alox12b-deficient mouse skin transplants. J. Invest. Dermatol. *129*, 1429-1436.

de la Serna, I.L., Ohkawa, Y., Higashi, C., Dutta, C., Osias, J., Kommajosyula, N., Tachibana, T., and Imbalzano, A.N. (2006). The microphthalmia-associated transcription factor requires SWI/SNF enzymes to activate melanocyte-specific genes. J. Biol. Chem. *281*, 20233-20241.

de Wit, P.E., Moretti, S., Koenders, P.G., Weterman, M.A., van Muijen, G.N., Gianotti, B., and Ruiter, D.J. (1992). Increasing epidermal growth factor receptor expression in human melanocytic tumor progression. J Invest Dermatol *99*, 168-173.

Deed, R.W., Bianchi, S.M., Atherton, G.T., Johnston, D., Santibanez-Koref, M., Murphy, J.J., and Norton, J.D. (1993). An immediate early human gene encodes an Id-like helix-loop-helix protein and is regulated by protein kinase C activation in diverse cell types. Oncogene *8*, 599-607.

den Hartigh, J.C., van Bergen en Henegouwen, P.M., Verkleij, A.J., and Boonstra, J. (1992). The EGF receptor is an actin-binding protein. J Cell Biol *119*, 349-355.

Derynck, R., Goeddel, D.V., Ullrich, A., Gutterman, J.U., Williams, R.D., Bringman, T.S., and Berger, W.H. (1987). Synthesis of messenger RNAs for transforming growth factors alpha and beta and the epidermal growth factor receptor by human tumors. Cancer Res *47*, 707-712.

Descargues, P., Deraison, C., Bonnart, C., Kreft, M., Kishibe, M., Ishida-Yamamoto, A., Elias, P., Barrandon, Y., Zambruno, G., Sonnenberg, A., and Hovnanian, A. (2005).

Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. Nat Genet *37*, 56-65.

Descargues, P., Deraison, C., Prost, C., Fraitag, S., Mazereeuw-Hautier, J., D'Alessio, M., Ishida-Yamamoto, A., Bodemer, C., Zambruno, G., and Hovnanian, A. (2006). Corneodesmosomal cadherins are preferential targets of stratum corneum trypsin- and chymotrypsin-like hyperactivity in Netherton syndrome. J. Invest. Dermatol. *126*, 1622-1632.

Dhakshinamoorthy, S., and Jaiswal, A.K. (2000). Small maf (MafG and MafK) proteins negatively regulate antioxidant response element-mediated expression and antioxidant induction of the NAD(P)H:Quinone oxidoreductase1 gene. J. Biol. Chem. *275*, 40134-40141.

Diamond, I., Legg, A., Schneider, J.A., and Rozengurt, E. (1978). Glycolysis in quiescent cultures of 3T3 cells. Stimulation by serum, epidermal growth factor, and insulin in intact cells and persistence of the stimulation after cell homogenization. J Biol Chem *253*, 866-871.

DiColandrea, T., Karashima, T., Maatta, A., and Watt, F.M. (2000). Subcellular distribution of envoplakin and periplakin: insights into their role as precursors of the epidermal cornified envelope. J Cell Biol *151*, 573-586.

Ding, H., Benotmane, A.M., Suske, G., Collen, D., and Belayew, A. (1999). Functional interactions between Sp1 or Sp3 and the helicase-like transcription factor mediate basal expression from the human plasminogen activator inhibitor-1 gene. J. Biol. Chem. *274*, 19573-19580.

Dittmar, T., Husemann, A., Schewe, Y., Nofer, J.R., Niggemann, B., Zanker, K.S., and Brandt, B.H. (2002). Induction of cancer cell migration by epidermal growth factor is initiated by specific phosphorylation of tyrosine 1248 of c-erbB-2 receptor via EGFR. FASEB J *16*, 1823-1825.

Dolfi, F., Garcia-Guzman, M., Ojaniemi, M., Nakamura, H., Matsuda, M., and Vuori, K. (1998). The adaptor protein Crk connects multiple cellular stimuli to the JNK signaling pathway. Proc Natl Acad Sci U S A *95*, 15394-15399.

Dong, S., Ying, S., Kojima, T., Shiraiwa, M., Kawada, A., Mechin, M.C., Adoue, V., Chavanas, S., Serre, G., Simon, M., and Takahara, H. (2008). Crucial roles of MZF1 and Sp1 in the transcriptional regulation of the peptidylarginine deiminase type I gene (PADI1) in human keratinocytes. J Invest Dermatol *128*, 549-557.

Dotto, G.P. (1999). Signal transduction pathways controlling the switch between keratinocyte growth and differentiation. Crit Rev Oral Biol Med *10*, 442-457.
Downward, J., Parker, P., and Waterfield, M.D. (1984). Autophosphorylation sites on the epidermal growth factor receptor. Nature *311*, 483-485.

Drozdoff, V., and Pledger, W.J. (1993). Commitment to differentiation and expression of early differentiation markers in murine keratinocytes in vitro are regulated independently of extracellular calcium concentrations. J. Cell Biol. *123*, 909-919.

Dunaeva, M., Michelson, P., Kogerman, P., and Toftgard, R. (2003). Characterization of the physical interaction of Gli proteins with SUFU proteins. J. Biol. Chem. *278*, 5116-5122.

Durchdewald, M., Angel, P., and Hess, J. (2009). The transcription factor Fos: a Janus-type regulator in health and disease. Histol Histopathol *24*, 1451-1461.

Eckert, R.L., and Lee, K.C. (2006). S100A7 (Psoriasin): a story of mice and men. J. Invest. Dermatol. *126*, 1442-1444.

Eckhart, L., Schmidt, M., Mildner, M., Mlitz, V., Abtin, A., Ballaun, C., Fischer, H., Mrass, P., and Tschachler, E. (2008). Histidase expression in human epidermal keratinocytes: regulation by differentiation status and all-trans retinoic acid. J Dermatol Sci *50*, 209-215.

Edwards, M.J. (2008). Therapy directed against thymic stromal lymphopoietin. Drug News Perspect *21*, 312-316.

Effendy, I., Loeffler, H., and Maibach, H.I. (1995). Baseline transepidermal water loss in patients with acute and healed irritant contact dermatitis. Contact Dermatitis *33*, 371-374.

Egberts, F., Heinrich, M., Jensen, J.M., Winoto-Morbach, S., Pfeiffer, S., Wickel, M., Schunck, M., Steude, J., Saftig, P., Proksch, E., and Schutze, S. (2004). Cathepsin D is involved in the regulation of transglutaminase 1 and epidermal differentiation. J Cell Sci *117*, 2295-2307.

Ehlermann, J., Pfisterer, P., and Schorle, H. (2003). Dynamic expression of Kruppel-like factor 4 (Klf4), a target of transcription factor AP-2alpha during murine mid-embryogenesis. Anat Rec A Discov Mol Cell Evol Biol *273*, 677-680.

Eichner, R., Bonitz, P., and Sun, T.T. (1984). Classification of epidermal keratins according to their immunoreactivity, isoelectric point, and mode of expression. J Cell Biol *98*, 1388-1396.

Ekholm, I.E., Brattsand, M., and Egelrud, T. (2000). Stratum corneum tryptic enzyme in normal epidermis: a missing link in the desquamation process? J Invest Dermatol *114*, 56-63.

Elias, P.M., Fartasch, M., Crumrine, D., Behne, M., Uchida, Y., and Holleran, W.M. (2000). Origin of the corneocyte lipid envelope (CLE): observations in harlequin ichthyosis and cultured human keratinocytes. J Invest Dermatol *115*, 765-769.

Elias, P.M., and Menon, G.K. (1991). Structural and lipid biochemical correlates of the epidermal permeability barrier. Adv Lipid Res *24*, 1-26.

Eliyahu, E., Park, J.H., Shtraizent, N., He, X., and Schuchman, E.H. (2007). Acid ceramidase is a novel factor required for early embryo survival. FASEB J *21*, 1403-1409.

Ellis, D.L., Nanney, L.B., and King, L.E., Jr. (1990). Increased epidermal growth factor receptors in seborrheic keratoses and acrochordons of patients with the dysplastic nevus syndrome. J Am Acad Dermatol *23*, 1070-1077.

Endo-Munoz, L., Dahler, A., Teakle, N., Rickwood, D., Hazar-Rethinam, M., Abdul-Jabbar, I., Sommerville, S., Dickinson, I., Kaur, P., Paquet-Fifield, S., and Saunders, N. (2009). E2F7 can regulate proliferation, differentiation, and apoptotic responses in human keratinocytes: implications for cutaneous squamous cell carcinoma formation. Cancer Res *69*, 1800-1808.

Epp, N., Furstenberger, G., Muller, K., de Juanes, S., Leitges, M., Hausser, I., Thieme, F., Liebisch, G., Schmitz, G., and Krieg, P. (2007). 12R-lipoxygenase deficiency disrupts epidermal barrier function. J. Cell Biol. *177*, 173-182.

Essenfelder, G.M., Bruzzone, R., Lamartine, J., Charollais, A., Blanchet-Bardon, C., Barbe, M.T., Meda, P., and Waksman, G. (2004). Connexin30 mutations responsible for hidrotic ectodermal dysplasia cause abnormal hemichannel activity. Hum Mol Genet *13*, 1703-1714.

Farr, C.J., Easty, D.J., Ragoussis, J., Collignon, J., Lovell-Badge, R., and Goodfellow, P.N. (1993). Characterization and mapping of the human SOX4 gene. Mamm Genome *4*, 577-584.

Favre, B., Plantard, L., Aeschbach, L., Brakch, N., Christen-Zaech, S., de Viragh, P.A., Sergeant, A., Huber, M., and Hohl, D. (2007). SLURP1 is a late marker of epidermal differentiation and is absent in Mal de Meleda. J. Invest. Dermatol. *127*, 301-308.

Feng, L., and Prestwich, G.D. (2005). Functional Lipidomic. (Boca Raton: Taylor & Francis Group, LLC).

Ferby, I., Reschke, M., Kudlacek, O., Knyazev, P., Pante, G., Amann, K., Sommergruber, W., Kraut, N., Ullrich, A., Fassler, R., and Klein, R. (2006). Mig6 is a negative regulator of EGF receptor-mediated skin morphogenesis and tumor formation. Nat Med *12*, 568-573.

Filion, G.J., Zhenilo, S., Salozhin, S., Yamada, D., Prokhortchouk, E., and Defossez, P.A. (2006). A family of human zinc finger proteins that bind methylated DNA and repress transcription. Mol Cell Biol *26*, 169-181.

Fischer, D.F., Gibbs, S., van De Putte, P., and Backendorf, C. (1996). Interdependent transcription control elements regulate the expression of the SPRR2A gene during keratinocyte terminal differentiation. Mol Cell Biol *16*, 5365-5374.

Flanagan, S.P. (1966). 'Nude', a new hairless gene with pleiotropic effects in the mouse. Genet Res *8*, 295-309.

Flaxman, B.A. (1972). Cell differentiation and its control in the vertebrate epidermis. American Zoologist *12*, 8.

Flaxman, B.A., and Maderson, P.F. (1976). Growth and differentiation of skin. J. Invest. Dermatol. *67*, 8-14.

Forslind, B., Lindberg, M., Roomans, G.M., Pallon, J., and Werner-Linde, Y. (1997). Aspects on the physiology of human skin: studies using particle probe analysis. Microsc Res Tech *38*, 373-386.

Frey, M.R., Dise, R.S., Edelblum, K.L., and Polk, D.B. (2006). p38 kinase regulates epidermal growth factor receptor downregulation and cellular migration. Embo J *25*, 5683-5692.

Fuchs, E. (2007). Scratching the surface of skin development. Nature *445*, 834-842.
Fuchs, E., and Raghavan, S. (2002). Getting under the skin of epidermal morphogenesis. Nat Rev Genet *3*, 199-209.

Fuller-Pace, F.V., and Ali, S. (2008). The DEAD box RNA helicases p68 (Ddx5) and p72 (Ddx17): novel transcriptional co-regulators. Biochem Soc Trans *36*, 609-612.

Furuse, M., Hata, M., Furuse, K., Yoshida, Y., Haratake, A., Sugitani, Y., Noda, T., Kubo, A., and Tsukita, S. (2002). Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. J. Cell Biol. *156*, 1099-1111.

Gachon, F., Olela, F.F., Schaad, O., Descombes, P., and Schibler, U. (2006). The circadian PAR-domain basic leucine zipper transcription factors DBP, TEF, and HLF modulate basal and inducible xenobiotic detoxification. Cell Metab *4*, 25-36.

Galliano, M.F., Toulza, E., Gallinaro, H., Jonca, N., Ishida-Yamamoto, A., Serre, G., and Guerrin, M. (2006). A novel protease inhibitor of the alpha2-macroglobulin family expressed in the human epidermis. J Biol Chem *281*, 5780-5789.

Gangatirkar, P., Paquet-Fifield, S., Li, A., Rossi, R., and Kaur, P. (2007). Establishment of 3D organotypic cultures using human neonatal epidermal cells. Nat Protoc *2*, 178-186.

Garrett, T.P., McKern, N.M., Lou, M., Elleman, T.C., Adams, T.E., Lovrecz, G.O., Zhu, H.J., Walker, F., Frenkel, M.J., Hoyne, P.A., Jorissen, R.N., Nice, E.C., Burgess, A.W.,

and Ward, C.W. (2002). Crystal structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor alpha. Cell *110*, 763-773.

Garrington, T.P., and Johnson, G.L. (1999). Organization and regulation of mitogen-activated protein kinase signaling pathways. Curr Opin Cell Biol *11*, 211-218.

Garriock, R.J., Vokes, S.A., Small, E.M., Larson, R., and Krieg, P.A. (2001). Developmental expression of the Xenopus Iroquois-family homeobox genes, Irx4 and Irx5. Dev Genes Evol *211*, 257-260.

Gassmann, M., Casagranda, F., Orioli, D., Simon, H., Lai, C., Klein, R., and Lemke, G. (1995). Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. Nature *378*, 390-394.

Gaynor, R.B., Muchardt, C., Diep, A., Mohandas, T.K., Sparkes, R.S., and Lusis, A.J. (1991). Localization of the zinc finger DNA-binding protein HIV-EP1/MBP-1/PRDII-BF1 to human chromosome 6p22.3-p24. Genomics *9*, 758-761.

Gazel, A., Banno, T., Walsh, R., and Blumenberg, M. (2006). Inhibition of JNK promotes differentiation of epidermal keratinocytes. J Biol Chem *281*, 20530-20541.

Gazel, A., Nijhawan, R.I., Walsh, R., and Blumenberg, M. (2008). Transcriptional profiling defines the roles of ERK and p38 kinases in epidermal keratinocytes. J Cell Physiol *215*, 292-308.

Gazel, A., Ramphal, P., Rosdy, M., De Wever, B., Tornier, C., Hosein, N., Lee, B., Tomic-Canic, M., and Blumenberg, M. (2003). Transcriptional profiling of epidermal keratinocytes: comparison of genes expressed in skin, cultured keratinocytes, and reconstituted epidermis, using large DNA microarrays. J. Invest. Dermatol. *121*, 1459-1468.

Getsios, S., Simpson, C.L., Kojima, S., Harmon, R., Sheu, L.J., Dusek, R.L., Cornwell, M., and Green, K.J. (2009). Desmoglein 1-dependent suppression of EGFR signaling promotes epidermal differentiation and morphogenesis. J Cell Biol *185*, 1243-1258.

Geyer, A.S., Ratajczak, P., Pol-Rodriguez, M., Millar, W.S., Garzon, M., and Richard, G. (2005). Netherton syndrome with extensive skin peeling and failure to thrive due to a homozygous frameshift mutation in SPINK5. Dermatology *210*, 308-314.

Ghosh, S., and Karin, M. (2002). Missing pieces in the NF-kappaB puzzle. Cell *109 Suppl*, S81-96.

Giancotti, F.G., Stepp, M.A., Suzuki, S., Engvall, E., and Ruoslahti, E. (1992). Proteolytic processing of endogenous and recombinant beta 4 integrin subunit. J. Cell Biol. *118*, 951-959.

Gibbons, R.J., Wada, T., Fisher, C.A., Malik, N., Mitson, M.J., Steensma, D.P., Fryer, A., Goudie, D.R., Krantz, I.D., and Traeger-Synodinos, J. (2008). Mutations in the chromatin-associated protein ATRX. Hum Mutat *29*, 796-802.

Gibbs, S., Boelsma, E., Kempenaar, J., and Ponec, M. (1998). Temperature-sensitive regulation of epidermal morphogenesis and the expression of cornified envelope precursors by EGF and TGF alpha. Cell Tissue Res *292*, 107-114.

Gibbs, S., Fijneman, R., Wiegant, J., van Kessel, A.G., van De Putte, P., and Backendorf, C. (1993). Molecular characterization and evolution of the SPRR family of keratinocyte differentiation markers encoding small proline-rich proteins. Genomics *16*, 630-637.

Gibbs, S., Silva Pinto, A.N., Murli, S., Huber, M., Hohl, D., and Ponec, M. (2000). Epidermal growth factor and keratinocyte growth factor differentially regulate epidermal migration, growth, and differentiation. Wound Repair Regen *8*, 192-203.

Giudice, G.J., Emery, D.J., Zelickson, B.D., Anhalt, G.J., Liu, Z., and Diaz, L.A. (1993). Bullous pemphigoid and herpes gestationis autoantibodies recognize a common non-collagenous site on the BP180 ectodomain. J Immunol *151*, 5742-5750.

Giudice, G.J., Squiquera, H.L., Elias, P.M., and Diaz, L.A. (1991). Identification of two collagen domains within the bullous pemphigoid autoantigen, BP180. J. Clin. Invest. *87*, 734-738.

Glaser, R., Meyer-Hoffert, U., Harder, J., Cordes, J., Wittersheim, M., Kobliakova, J., Folster-Holst, R., Proksch, E., Schroder, J.M., and Schwarz, T. (2009). The antimicrobial protein psoriasin (S100A7) is upregulated in atopic dermatitis and after experimental skin barrier disruption. J. Invest. Dermatol. *129*, 641-649.

Godchaux, W., 3rd, Adamson, S.D., and Herbert, E. (1967). Effects of cycloheximide on polyribosome function in reticulocytes. J Mol Biol *27*, 57-72.

Goldsborough, A.S., Healy, L.E., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Willison, K.R., and Ashworth, A. (1993). Cloning, chromosomal localization and expression pattern of the POU domain gene Oct-11. Nucleic Acids Res *21*, 127-134.

Gong, X.Q., Shao, Q., Lounsbury, C.S., Bai, D., and Laird, D.W. (2006). Functional characterization of a GJA1 frameshift mutation causing oculodentodigital dysplasia and palmoplantar keratoderma. J. Biol. Chem. *281*, 31801-31811.

Gospodarowicz, D., Ill, C.R., and Birdwell, C.R. (1977). Effects of fibroblast and epidermal growth factors on ovarian cell proliferation in vitro. II. Proliferative response of luteal cells to FGF but not EGF. Endocrinology *100*, 1121-1128.

Grandori, C., Cowley, S.M., James, L.P., and Eisenman, R.N. (2000). The Myc/Max/Mad network and the transcriptional control of cell behavior. Annu Rev Cell Dev Biol *16*, 653-699.

Gray, A., Dull, T.J., and Ullrich, A. (1983). Nucleotide sequence of epidermal growth factor cDNA predicts a 128,000-molecular weight protein precursor. Nature *303*, 722-725.

Green, K.J., Geiger, B., Jones, J.C., Talian, J.C., and Goldman, R.D. (1987). The relationship between intermediate filaments and microfilaments before and during the formation of desmosomes and adherens-type junctions in mouse epidermal keratinocytes. J Cell Biol *104*, 1389-1402.

Green, K.J., and Jones, J.C. (1996). Desmosomes and hemidesmosomes: structure and function of molecular components. FASEB J *10*, 871-881.

Gregory, G.D., Vakoc, C.R., Rozovskaia, T., Zheng, X., Patel, S., Nakamura, T., Canaani, E., and Blobel, G.A. (2007). Mammalian ASH1L is a histone methyltransferase that occupies the transcribed region of active genes. Mol Cell Biol *27*, 8466-8479.

Gregory, H. (1975). Isolation and structure of urogastrone and its relationship to epidermal growth factor. Nature *257*, 325-327.

Groneberg, D.A., Fischer, T.C., Peckenschneider, N., Noga, O., Dinh, Q.T., Welte, T., and Welker, P. (2007). Cell type-specific regulation of brain-derived neurotrophic factor in states of allergic inflammation. Clin Exp Allergy *37*, 1386-1391.

Gruber, R., Wilson, N.J., Smith, F.J., Grabher, D., Steinwender, L., Fritsch, P.O., and Schmuth, M. (2009). Increased pachyonychia congenita severity in patients with concurrent keratin and filaggrin mutations. Br J Dermatol *161*, 1391-1395.

Guo, Y., Yang, M.C., Weissler, J.C., and Yang, Y.S. (2008). Modulation of PLAGL2 transactivation activity by Ubc9 co-activation not SUMOylation. Biochem Biophys Res Commun *374*, 570-575.

Hadj-Rabia, S., Baala, L., Vabres, P., Hamel-Teillac, D., Jacquemin, E., Fabre, M., Lyonnet, S., De Prost, Y., Munnich, A., Hadchouel, M., and Smahi, A. (2004). Claudin-1 gene mutations in neonatal sclerosing cholangitis associated with ichthyosis: a tight junction disease. Gastroenterology *127*, 1386-1390.

Haider, A.S., Peters, S.B., Kaporis, H., Cardinale, I., Fei, J., Ott, J., Blumenberg, M., Bowcock, A.M., Krueger, J.G., and Carucci, J.A. (2006). Genomic analysis defines a cancer-specific gene expression signature for human squamous cell carcinoma and distinguishes malignant hyperproliferation from benign hyperplasia. J. Invest. Dermatol. *126*, 869-881.

Hallberg, B., Rayter, S.I., and Downward, J. (1994). Interaction of Ras and Raf in intact mammalian cells upon extracellular stimulation. J Biol Chem *269*, 3913-3916.

Han, H.J., Russo, J., Kohwi, Y., and Kohwi-Shigematsu, T. (2008). SATB1 reprogrammes gene expression to promote breast tumour growth and metastasis. Nature *452*, 187-193.

Hara-Chikuma, M., Takeda, J., Tarutani, M., Uchida, Y., Holleran, W.M., Endo, Y., Elias, P.M., and Inoue, S. (2004). Epidermal-specific defect of GPI anchor in Pig-a null mice results in Harlequin ichthyosis-like features. J Invest Dermatol *123*, 464-469.

Hashida, R., Ogawa, K., Miyagawa, M., Sugita, Y., Takahashi, E., Nagasu, T., Katsunuma, T., Akasawa, A., Tsujimoto, G., Matsumoto, K., and Saito, H. (2003). Analysis of gene expression in peripheral blood eosinophils from patients with atopic dermatitis by differential display. Int Arch Allergy Immunol *131 Suppl 1*, 26-33.

Hashimoto, K. (2000). Regulation of keratinocyte function by growth factors. J. Dermatol Sci. *24 Suppl 1*, S46-50.

He, C., Hobert, M., Friend, L., and Carlin, C. (2002). The epidermal growth factor receptor juxtamembrane domain has multiple basolateral plasma membrane localization determinants, including a dominant signal with a polyproline core. J Biol Chem *277*, 38284-38293.

He, W., Cao, T., Smith, D.A., Myers, T.E., and Wang, X.J. (2001). Smads mediate signaling of the TGFbeta superfamily in normal keratinocytes but are lost during skin chemical carcinogenesis. Oncogene *20*, 471-483.

Hedge, S.P., Kumar, A., Kurschner, C., and Shapiro, L.H. (1998). c-Maf interacts with c-Myb to regulate transcription of an early myeloid gene during differentiation. Mol Cell Biol *18*, 2729-2737.

Heimberg, M., Weinstein, I., LeQuire, V.S., and Cohen, S. (1965). The induction of fatty liver in neonatal animals by a purified protein (EGF) from mouse submaxillary gland. Life Sci *4*, 1625-1633.

Heinonen, H., Nieminen, A., Saarela, M., Kallioniemi, A., Klefstrom, J., Hautaniemi, S., and Monni, O. (2008). Deciphering downstream gene targets of PI3K/mTOR/p70S6K pathway in breast cancer. BMC Genomics *9*, 348.

Heishi, M., Kagaya, S., Katsunuma, T., Nakajima, T., Yuki, K., Akasawa, A., Maeda, M., Gunji, S., Sugita, Y., Tsujimoto, G., and Saito, H. (2002). High-density oligonucleotide array analysis of mRNA transcripts in peripheral blood cells of severe atopic dermatitis patients. Int Arch Allergy Immunol *129*, 57-66.

Helsby, N.A., Ward, S.A., Parslew, R.A., Friedmann, P.S., and Rhodes, L.E. (1998). Hepatic cytochrome P450 CYP2C activity in psoriasis: studies using proguanil as a probe compound. Acta Derm Venereol *78*, 81-83.

Henderson, Y.C., Frederick, M.J., Wang, M.T., Hollier, L.M., and Clayman, G.L. (2008). LBP-1b, LBP-9, and LBP-32/MGR detected in syncytiotrophoblasts from first-trimester human placental tissue and their transcriptional regulation. DNA Cell Biol *27*, 71-79.

Hennings, H., Kruszewski, F.H., Yuspa, S.H., and Tucker, R.W. (1989). Intracellular calcium alterations in response to increased external calcium in normal and neoplastic keratinocytes. Carcinogenesis *10*, 777-780.

Henry, J., Hsu, C.Y., Haftek, M., Nachat, R., de Koning, H.D., Gardinal-Galera, I., Hitomi, K., Balica, S., Jean-Decoster, C., Schmitt, A.M., Paul, C., Serre, G., and Simon, M. Hornerin is a component of the epidermal cornified cell envelopes. FASEB J *25*, 1567-1576.

Henry, R.W., Mittal, V., Ma, B., Kobayashi, R., and Hernandez, N. (1998). SNAP19 mediates the assembly of a functional core promoter complex (SNAPc) shared by RNA polymerases II and III. Genes Dev *12*, 2664-2672.

Herrmann, T., Grone, H.J., Langbein, L., Kaiser, I., Gosch, I., Bennemann, U., Metzger, D., Chambon, P., Stewart, A.F., and Stremmel, W. (2005). Disturbed epidermal structure in mice with temporally controlled fatp4 deficiency. J. Invest. Dermatol. *125*, 1228-1235.

Herrmann, T., van der Hoeven, F., Grone, H.J., Stewart, A.F., Langbein, L., Kaiser, I., Liebisch, G., Gosch, I., Buchkremer, F., Drobnik, W., Schmitz, G., and Stremmel, W. (2003). Mice with targeted disruption of the fatty acid transport protein 4 (Fatp 4, Slc27a4) gene show features of lethal restrictive dermopathy. J. Cell Biol. *161*, 1105-1115.

Hertl, M., Eming, R., and Veldman, C. (2006). T cell control in autoimmune bullous skin disorders. J. Clin. Invest. *116*, 1159-1166.

Hildesheim, J., Kuhn, U., Yee, C.L., Foster, R.A., Yancey, K.B., and Vogel, J.C. (2001). The hSkn-1a POU transcription factor enhances epidermal stratification by promoting keratinocyte proliferation. J. Cell Sci. *114*, 1913-1923.

Hines, M.D., Jin, H.C., Wheelock, M.J., and Jensen, P.J. (1999). Inhibition of cadherin function differentially affects markers of terminal differentiation in cultured human keratinocytes. J. Cell Sci. *112 (Pt 24)*, 4569-4579.

Ho, S.N., Thomas, D.J., Timmerman, L.A., Li, X., Francke, U., and Crabtree, G.R. (1995). NFATc3, a lymphoid-specific NFATc family member that is calcium-regulated and exhibits distinct DNA binding specificity. J. Biol. Chem. *270*, 19898-19907.

Hoffmann, M.J., Engers, R., Florl, A.R., Otte, A.P., Muller, M., and Schulz, W.A. (2007). Expression changes in EZH2, but not in BMI-1, SIRT1, DNMT1 or DNMT3B are associated with DNA methylation changes in prostate cancer. Cancer Biol Ther *6*, 1403-1412.

Hogenesch, J.B., Gu, Y.Z., Jain, S., and Bradfield, C.A. (1998). The basic-helix-loop-helix-PAS orphan MOP3 forms transcriptionally active complexes with circadian and hypoxia factors. Proc. Natl. Acad. Sci. USA. *95*, 5474-5479.

Hogervorst, F., Kuikman, I., von dem Borne, A.E., and Sonnenberg, A. (1990). Cloning and sequence analysis of beta-4 cDNA: an integrin subunit that contains a unique 118 kd cytoplasmic domain. EMBO J *9*, 765-770.

Hohl, D. (1993). Expression patterns of loricrin in dermatological disorders. Am J Dermatopathol *15*, 20-27.

Hohl, D., de Viragh, P.A., Amiguet-Barras, F., Gibbs, S., Backendorf, C., and Huber, M. (1995). The small proline-rich proteins constitute a multigene family of differentially regulated cornified cell envelope precursor proteins. J Invest Dermatol *104*, 902-909.
Holbrook, K., and Wolff, K. (1987). The structure and development of skin. (New York: McGraw Hill).

Holbrook, K.A. (1989). Biologic structure and function: perspectives on morphologic approaches to the study of the granular layer keratinocyte. J. Invest. Dermatol. *92*, 84S-104S.

Hollenberg, M.D. (1975). Receptors for insulin and epidermal growth factor: relation to synthesis of DNA in cultured rabbit lens epithelium. Arch Biochem Biophys *171*, 371-377.

Holleran, W.M., Takagi, Y., and Uchida, Y. (2006). Epidermal sphingolipids: metabolism, function, and roles in skin disorders. FEBS Lett *580*, 5456-5466.

Holm, S.J., Carlen, L.M., Mallbris, L., Stahle-Backdahl, M., and O'Brien, K.P. (2003). Polymorphisms in the SEEK1 and SPR1 genes on 6p21.3 associate with psoriasis in the Swedish population. Exp Dermatol *12*, 435-444.

Homayouni, R., Heinrich, K., Wei, L., and Berry, M.W. (2005). Gene clustering by latent semantic indexing of MEDLINE abstracts. Bioinformatics *21*, 104-115.

Hoober, J.K., and Cohen, S. (1967). Epidermal growth factor. I. The stimulation of protein and ribonucleic acid synthesis in chick embryo epidermis. Biochim Biophys Acta *138*, 347-356.

Hopkinson, S.B., Riddelle, K.S., and Jones, J.C. (1992). Cytoplasmic domain of the 180-kD bullous pemphigoid antigen, a hemidesmosomal component: molecular and cell biologic characterization. J. Invest. Dermatol. *99*, 264-270.

Hornemann, T., Penno, A., Rutti, M.F., Ernst, D., Kivrak-Pfiffner, F., Rohrer, L., and von Eckardstein, A. (2009). The SPTLC3 subunit of serine palmitoyltransferase generates short chain sphingoid bases. J Biol Chem *284*, 26322-26330.

Horvath, G.C., Kistler, W.S., and Kistler, M.K. (2004). RFX2 is a potential transcriptional regulatory factor for histone H1t and other genes expressed during the meiotic phase of spermatogenesis. Biol Reprod *71*, 1551-1559.

Huang, C.C., Liu, Z., Li, X., Bailey, S.K., Nail, C.D., Foster, K.W., Frost, A.R., Ruppert, J.M., and Lobo-Ruppert, S.M. (2005). KLF4 and PCNA identify stages of tumor initiation in a conditional model of cutaneous squamous epithelial neoplasia. Cancer Biol Ther *4*, 1401-1408.

Huber, M., Rettler, I., Bernasconi, K., Frenk, E., Lavrijsen, S.P., Ponec, M., Bon, A., Lautenschlager, S., Schorderet, D.F., and Hohl, D. (1995). Mutations of keratinocyte transglutaminase in lamellar ichthyosis. Science *267*, 525-528.

Huff, K.R., and Guroff, G. (1978). Epidermal growth factor stimulates the phosphorylation of a specific nuclear protein in chick embryo epidermis. Biochem Biophys Res Commun *85*, 464-472.

Hwang, E.S., Choi, A., and Ho, I.C. (2002). Transcriptional regulation of GATA-3 by an intronic regulatory region and fetal liver zinc finger protein 1. J Immunol *169*, 248-253.

Hwang, J., Kita, R., Kwon, H.S., Choi, E.H., Lee, S.H., Udey, M.C., and Morasso, M.I. Epidermal ablation of Dlx3 is linked to IL-17-associated skin inflammation. Proc Natl Acad Sci U S A *108*, 11566-11571.

Ichimura, T., Watanabe, S., Sakamoto, Y., Aoto, T., Fujita, N., and Nakao, M. (2005). Transcriptional repression and heterochromatin formation by MBD1 and MCAF/AM family proteins. J. Biol. Chem. *280*, 13928-13935.

Ikuta, T., Namiki, T., Fujii-Kuriyama, Y., and Kawajiri, K. (2009). AhR protein trafficking and function in the skin. Biochem Pharmacol *77*, 588-596.

Imataka, H., Nakayama, K., Yasumoto, K., Mizuno, A., Fujii-Kuriyama, Y., and Hayami, M. (1994). Cell-specific translational control of transcription factor BTEB expression. The role of an upstream AUG in the 5'-untranslated region. J. Biol. Chem. *269*, 20668-20673.

Imataka, H., Sogawa, K., Yasumoto, K., Kikuchi, Y., Sasano, K., Kobayashi, A., Hayami, M., and Fujii-Kuriyama, Y. (1992). Two regulatory proteins that bind to the basic

transcription element (BTE), a GC box sequence in the promoter region of the rat P-4501A1 gene. EMBO J *11*, 3663-3671.

Inui, S., Fukuzato, Y., Nakajima, T., Kurata, S., and Itami, S. (2007). Androgen receptor co-activator Hic-5/ARA55 as a molecular regulator of androgen sensitivity in dermal papilla cells of human hair follicles. J. Invest. Dermatol. *127*, 2302-2306.

Inukai, T., Inaba, T., Dang, J., Kuribara, R., Ozawa, K., Miyajima, A., Wu, W., Look, A.T., Arinobu, Y., Iwasaki, H., Akashi, K., Kagami, K., Goi, K., Sugita, K., and Nakazawa, S. (2005). TEF, an antiapoptotic bZIP transcription factor related to the oncogenic E2A-HLF chimera, inhibits cell growth by down-regulating expression of the common beta chain of cytokine receptors. Blood *105*, 4437-4444.

Ishida-Yamamoto, A., Simon, M., Kishibe, M., Miyauchi, Y., Takahashi, H., Yoshida, S., O'Brien, T.J., Serre, G., and Iizuka, H. (2004). Epidermal lamellar granules transport different cargoes as distinct aggregates. J Invest Dermatol *122*, 1137-1144.

Jackson, B., Tilli, C.M., Hardman, M.J., Avilion, A.A., MacLeod, M.C., Ashcroft, G.S., and Byrne, C. (2005). Late cornified envelope family in differentiating epithelia--response to calcium and ultraviolet irradiation. J Invest Dermatol *124*, 1062-1070.

Jaken, S., and Yuspa, S.H. (1988). Early signals for keratinocyte differentiation: role of Ca2+-mediated inositol lipid metabolism in normal and neoplastic epidermal cells. Carcinogenesis *9*, 1033-1038.

Jakobsson, A., Westerberg, R., and Jacobsson, A. (2006). Fatty acid elongases in mammals: their regulation and roles in metabolism. Prog Lipid Res *45*, 237-249.

Jennemann, R., Sandhoff, R., Langbein, L., Kaden, S., Rothermel, U., Gallala, H., Sandhoff, K., Wiegandt, H., and Grone, H.J. (2007). Integrity and barrier function of the epidermis critically depend on glucosylceramide synthesis. J Biol Chem *282*, 3083-3094.

Jerome-Morais, A., Rahn, H.R., Tibudan, S.S., and Denning, M.F. (2009). Role for Protein Kinase C-alpha in Keratinocyte Growth Arrest. J Invest Dermatol.

Jiang, F., Ai, J., Xiao, W., and Wang, Z. (2007). FB1, an E2A fusion partner in childhood leukemia, interacts with U19/EAF2 and inhibits its transcriptional activity. Cancer Lett *253*, 265-272.

Jimbow, K., Quevedo, W.C., Jr., Fitzpatrick, T.B., and Szabo, G. (1976). Some aspects of melanin biology: 1950-1975. J. Invest. Dermatol. *67*, 72-89.

Johansen, C., Kragballe, K., Westergaard, M., Henningsen, J., Kristiansen, K., and Iversen, L. (2005). The mitogen-activated protein kinases p38 and ERK1/2 are increased in lesional psoriatic skin. Br J Dermatol *152*, 37-42.

Johnson, G.L., and Vaillancourt, R.R. (1994). Sequential protein kinase reactions controlling cell growth and differentiation. Curr Opin Cell Biol *6*, 230-238.

Jones, J.C., Asmuth, J., Baker, S.E., Langhofer, M., Roth, S.I., and Hopkinson, S.B. (1994). Hemidesmosomes: extracellular matrix/intermediate filament connectors. Exp Cell Res *213*, 1-11.

Jones, R.J., Dickerson, S., Bhende, P.M., Delecluse, H.J., and Kenney, S.C. (2007). Epstein-Barr virus lytic infection induces retinoic acid-responsive genes through induction of a retinol-metabolizing enzyme, DHRS9. J. Biol. Chem. *282*, 8317-8324.

Jonkman, M.F., de Jong, M.C., Heeres, K., Pas, H.H., van der Meer, J.B., Owaribe, K., Martinez de Velasco, A.M., Niessen, C.M., and Sonnenberg, A. (1995). 180-kD bullous pemphigoid antigen (BP180) is deficient in generalized atrophic benign epidermolysis bullosa. J. Clin. Invest. *95*, 1345-1352.

Jorissen, R.N., Walker, F., Pouliot, N., Garrett, T.P., Ward, C.W., and Burgess, A.W. (2003). Epidermal growth factor receptor: mechanisms of activation and signalling. Exp Cell Res *284*, 31-53.

Jost, M., Kari, C., and Rodeck, U. (2000). The EGF receptor - an essential regulator of multiple epidermal functions. Eur J Dermatol *10*, 505-510.

Joulin, V., Bories, D., Eleouet, J.F., Labastie, M.C., Chretien, S., Mattei, M.G., and Romeo, P.H. (1991). A T-cell specific TCR delta DNA binding protein is a member of the human GATA family. EMBO J *10*, 1809-1816.

Jump, D.B. (2009). Mammalian fatty acid elongases. Methods Mol Biol *579*, 375-389.

Jungersted, J.M., Scheer, H., Mempel, M., Baurecht, H., Cifuentes, L., Hogh, J.K., Hellgren, L.I., Jemec, G.B., Agner, T., and Weidinger, S. (2010). Stratum corneum lipids, skin barrier function and filaggrin mutations in patients with atopic eczema. Allergy *65*, 911-918.

Kalinin, A.E., Kajava, A.V., and Steinert, P.M. (2002). Epithelial barrier function: assembly and structural features of the cornified cell envelope. Bioessays *24*, 789-800.

Kataoka, K., Shioda, S., Ando, K., Sakagami, K., Handa, H., and Yasuda, K. (2004). Differentially expressed Maf family transcription factors, c-Maf and MafA, activate glucagon and insulin gene expression in pancreatic islet alpha- and beta-cells. J Mol Endocrinol *32*, 9-20.

Katou, F., Shirai, N., Kamakura, S., Tagami, H., Nagura, H., and Motegi, K. (2003). Differential expression of cornified cell envelope precursors in normal skin, intraorally transplanted skin and normal oral mucosa. Br J Dermatol *148*, 898-905.

Kelley, C.M., Ikeda, T., Koipally, J., Avitahl, N., Wu, L., Georgopoulos, K., and Morgan, B.A. (1998). Helios, a novel dimerization partner of Ikaros expressed in the earliest hematopoietic progenitors. Curr Biol *8*, 508-515.

Kerschensteiner, D., Liu, H., Cheng, C.W., Demas, J., Cheng, S.H., Hui, C.C., Chow, R.L., and Wong, R.O. (2008). Genetic control of circuit function: Vsx1 and Irx5 transcription factors regulate contrast adaptation in the mouse retina. J Neurosci *28*, 2342-2352.

Kil, S.J., and Carlin, C. (2000). EGF receptor residues leu(679), leu(680) mediate selective sorting of ligand-receptor complexes in early endosomal compartments. J Cell Physiol *185*, 47-60.

Kim, J., Hake, S.B., and Roeder, R.G. (2005). The human homolog of yeast BRE1 functions as a transcriptional coactivator through direct activator interactions. Mol Cell *20*, 759-770.

Kim, K., Choi, J., Heo, K., Kim, H., Levens, D., Kohno, K., Johnson, E.M., Brock, H.W., and An, W. (2008). Isolation and characterization of a novel H1.2 complex that acts as a repressor of p53-mediated transcription. J. Biol. Chem. *283*, 9113-9126.

Kim, M.Y., Jeong, B.C., Lee, J.H., Kee, H.J., Kook, H., Kim, N.S., Kim, Y.H., Kim, J.K., Ahn, K.Y., and Kim, K.K. (2006). A repressor complex, AP4 transcription factor and geminin, negatively regulates expression of target genes in nonneuronal cells. Proc. Natl. Acad. Sci. USA. *103*, 13074-13079.

Kim, S.H., Park, J., Choi, M.C., Kim, H.P., Park, J.H., Jung, Y., Lee, J.H., Oh, D.Y., Im, S.A., Bang, Y.J., and Kim, T.Y. (2007). Zinc-fingers and homeoboxes 1 (ZHX1) binds DNA methyltransferase (DNMT) 3B to enhance DNMT3B-mediated transcriptional repression. Biochem Biophys Res Commun *355*, 318-323.

Kindt, F., Wiegand, S., Loser, C., Nilles, M., Niemeier, V., Hsu, S.Y., Steinhoff, M., Kummer, W., Gieler, U., and Haberberger, R.V. (2007). Intermedin: a skin peptide that is downregulated in atopic dermatitis. J Invest Dermatol *127*, 605-613.

King, I.A., O'Brien, T.J., and Buxton, R.S. (1996). Expression of the "skin-type" desmosomal cadherin DSC1 is closely linked to the keratinization of epithelial tissues during mouse development. J. Invest. Dermatol. *107*, 531-538.

King, L.M., and Francomano, C.A. (2001). Characterization of a human gene encoding nucleosomal binding protein NSBP1. Genomics *71*, 163-173.

Kingo, K., Mossner, R., Ratsep, R., Raud, K., Kruger, U., Silm, H., Vasar, E., Reich, K., and Koks, S. (2008). Association analysis of IL20RA and IL20RB genes in psoriasis. Genes Immun *9*, 445-451.

Kirino, M., Kirino, Y., Takeno, M., Nagashima, Y., Takahashi, K., Kobayashi, M., Murakami, S., Hirasawa, T., Ueda, A., Aihara, M., Ikezawa, Z., and Ishigatsubo, Y. (2008). Heme oxygenase 1 attenuates the development of atopic dermatitis-like lesions in mice: implications for human disease. J Allergy Clin Immunol *122*, 290-297, 297 e291-298.

Kivirikko, S., McGrath, J.A., Baudoin, C., Aberdam, D., Ciatti, S., Dunnill, M.G., McMillan, J.R., Eady, R.A., Ortonne, J.P., Meneguzzi, G., and et al. (1995). A homozygous nonsense mutation in the alpha 3 chain gene of laminin 5 (LAMA3) in lethal (Herlitz) junctional epidermolysis bullosa. Hum Mol Genet *4*, 959-962.

Klar, J., Schweiger, M., Zimmerman, R., Zechner, R., Li, H., Torma, H., Vahlquist, A., Bouadjar, B., Dahl, N., and Fischer, J. (2009). Mutations in the fatty acid transport protein 4 gene cause the ichthyosis prematurity syndrome. Am J Hum Genet *85*, 248-253.

Knox, G.E., Reynolds, D.W., Cohen, S., and Alford, C.A. (1978). Alteration of the growth of cytomegalovirus and herpes simplex virus type 1 by epidermal growth factor, a contaminant of crude human chorionic gonadotropin preparations. J Clin Invest *61*, 1635-1644.

Kobayashi, H., Aiba, S., Yoshino, Y., and Tagami, H. (2003). Acute cutaneous barrier disruption activates epidermal p44/42 and p38 mitogen-activated protein kinases in human and hairless guinea pig skin. Exp Dermatol *12*, 734-746.

Kobayashi, T., Hashimoto, K., Okumura, H., Asada, H., and Yoshikawa, K. (1998). Endogenous EGF-family growth factors are necessary for the progression from the G1 to S phase in human keratinocytes. J. Invest. Dermatol. *111*, 616-620.

Koch, P.J., Mahoney, M.G., Ishikawa, H., Pulkkinen, L., Uitto, J., Shultz, L., Murphy, G.F., Whitaker-Menezes, D., and Stanley, J.R. (1997). Targeted disruption of the pemphigus vulgaris antigen (desmoglein 3) gene in mice causes loss of keratinocyte cell adhesion with a phenotype similar to pemphigus vulgaris. J. Cell Biol. *137*, 1091-1102.

Kolev, V., Mandinova, A., Guinea-Viniegra, J., Hu, B., Lefort, K., Lambertini, C., Neel, V., Dummer, R., Wagner, E.F., and Dotto, G.P. (2008). EGFR signalling as a negative regulator of Notch1 gene transcription and function in proliferating keratinocytes and cancer. Nat Cell Biol *10*, 902-911.

Kolly, C., Suter, M.M., and Muller, E.J. (2005). Proliferation, cell cycle exit, and onset of terminal differentiation in cultured keratinocytes: pre-programmed pathways in control of C-Myc and Notch1 prevail over extracellular calcium signals. J. Invest. Dermatol. *124*, 1014-1025.

Komatsu, N., Saijoh, K., Kuk, C., Liu, A.C., Khan, S., Shirasaki, F., Takehara, K., and Diamandis, E.P. (2007). Human tissue kallikrein expression in the stratum corneum and serum of atopic dermatitis patients. Exp Dermatol *16*, 513-519.

Kong, S.E., Banks, C.A., Shilatifard, A., Conaway, J.W., and Conaway, R.C. (2005). ELL-associated factors 1 and 2 are positive regulators of RNA polymerase II elongation factor ELL. Proc. Natl. Acad. Sci. USA. *102*, 10094-10098.

Kong, W., Longaker, M.T., and Lorenz, H.P. (2003). Molecular cloning and expression of keratinocyte proline-rich protein, a novel squamous epithelial marker isolated during skin development. J Biol Chem *278*, 22781-22786.

Kopf-Maier, P., Mboneko, V.F., and Merker, H.J. (1990). Nude mice are not hairless. A morphological study. Acta Anat (Basel) *139*, 178-190.

Krieg, P., Schuppler, M., Koesters, R., Mincheva, A., Lichter, P., and Marks, F. (1997). Repetin (Rptn), a new member of the "fused gene" subgroup within the S100 gene family encoding a murine epidermal differentiation protein. Genomics *43*, 339-348.

Kumar, L., and Matthias, F. (2007). Mfuzz: A software package for soft clustering of microarray data. Bioinformation *2*, 5-7.

Kuramoto, N., Takizawa, T., Matsuki, M., Morioka, H., Robinson, J.M., and Yamanishi, K. (2002). Development of ichthyosiform skin compensates for defective permeability barrier function in mice lacking transglutaminase 1. J. Clin. Invest. *109*, 243-250.

Kyriakis, J.M., and Avruch, J. (2001). Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev *81*, 807-869.

Labastie, M.C., Bories, D., Chabret, C., Gregoire, J.M., Chretien, S., and Romeo, P.H. (1994). Structure and expression of the human GATA3 gene. Genomics *21*, 1-6.

Lacour, J.P., Dubois, D., Pisani, A., and Ortonne, J.P. (1991). Anatomical mapping of Merkel cells in normal human adult epidermis. Br J Dermatol *125*, 535-542.

Lacouture, M.E. (2006). Mechanisms of cutaneous toxicities to EGFR inhibitors. Nat Rev Cancer *6*, 803-812.

Lacz, N.L., Schwartz, R.A., and Kihiczak, G. (2005). Epidermolytic hyperkeratosis: a keratin 1 or 10 mutational event. Int J Dermatol *44*, 1-6.

Lampe, M.A., Williams, M.L., and Elias, P.M. (1983). Human epidermal lipids: characterization and modulations during differentiation. J. Lipid Res. *24*, 131-140.

Lavker, R.M., Miller, S.J., and Sun, T.T. (1993). Epithelial stem cells, hair follicles, and tumor formation. Recent Results Cancer Res *128*, 31-43.

Leclerc, E.A., Huchenq, A., Mattiuzzo, N.R., Metzger, D., Chambon, P., Ghyselinck, N.B., Serre, G., Jonca, N., and Guerrin, M. (2009). Corneodesmosin gene ablation

induces lethal skin-barrier disruption and hair-follicle degeneration related to desmosome dysfunction. J Cell Sci *122*, 2699-2709.

LeClerc, S., Palaniswami, R., Xie, B.X., and Govindan, M.V. (1991). Molecular cloning and characterization of a factor that binds the human glucocorticoid receptor gene and represses its expression. J. Biol. Chem. *266*, 17333-17340.

Lee, H.J., Kim, S., Pelletier, J., and Kim, J. (2004). Stimulation of hTAFII68 (NTD)-mediated transactivation by v-Src. FEBS Lett *564*, 188-198.

Lee, J.H., Kye, K.C., Seo, E.Y., Lee, K., Lee, S.K., Lim, J.S., Seo, Y.J., Kim, C.D., and Park, J.K. (2008). Expression of neutrophil gelatinase-associated lipocalin in calcium-induced keratinocyte differentiation. J Korean Med Sci *23*, 302-306.

Lee, J.Y., Nakane, Y., Koshikawa, N., Nakayama, K., Hayashi, M., and Takenaga, K. (2000). Characterization of a zinc finger protein ZAN75: nuclear localization signal, transcriptional activator activity, and expression during neuronal differentiation of P19 cells. DNA Cell Biol *19*, 227-234.

Lee, K.F., Simon, H., Chen, H., Bates, B., Hung, M.C., and Hauser, C. (1995). Requirement for neuregulin receptor erbB2 in neural and cardiac development. Nature *378*, 394-398.

Lee, Y.S., Yuspa, S.H., and Dlugosz, A.A. (1998). Differentiation of cultured human epidermal keratinocytes at high cell densities is mediated by endogenous activation of the protein kinase C signaling pathway. J. Invest. Dermatol. *111*, 762-766.

Lefevre, C., Bouadjar, B., Ferrand, V., Tadini, G., Megarbane, A., Lathrop, M., Prud'homme, J.F., and Fischer, J. (2006). Mutations in a new cytochrome P450 gene in lamellar ichthyosis type 3. Hum Mol Genet *15*, 767-776.

Lefevre, C., Bouadjar, B., Karaduman, A., Jobard, F., Saker, S., Ozguc, M., Lathrop, M., Prud'homme, J.F., and Fischer, J. (2004). Mutations in ichthyin a new gene on chromosome 5q33 in a new form of autosomal recessive congenital ichthyosis. Hum Mol Genet *13*, 2473-2482.

Leigh, I.M., Lane, E.B., and Watt, F.M., eds. (1994). The Keratinocyte Handbook. (Cambridge).

Lembach, K.J. (1976). Enhanced synthesis and extracellular accumulation of hyaluronic acid during stimulation of quiescent human fibroblasts by mouse epidermal growth factor. J Cell Physiol *89*, 277-288.

Lever, W.F., and Schaumburg-Lever, G. (1990). Histopathology of the skin. (Philadelphia: JB Lippincott).

Lewis, L., Barrandon, Y., Green, H., and Albrecht-Buehler, G. (1987). The reorganization of microtubules and microfilaments in differentiating keratinocytes. Differentiation *36*, 228-233.

Leyvraz, C., Charles, R.P., Rubera, I., Guitard, M., Rotman, S., Breiden, B., Sandhoff, K., and Hummler, E. (2005). The epidermal barrier function is dependent on the serine protease CAP1/Prss8. J Cell Biol *170*, 487-496.

Li, C., and Hung Wong, W. (2001). Model-based analysis of oligonucleotide arrays: model validation, design issues and standard error application. Genome Biol *2*, RESEARCH0032.

Li, C., Lusis, A.J., Sparkes, R., Tran, S.M., and Gaynor, R. (1992). Characterization and chromosomal mapping of the gene encoding the cellular DNA binding protein HTLF. Genomics *13*, 658-664.

Li, H., and Villalobo, A. (2002). Evidence for the direct interaction between calmodulin and the human epidermal growth factor receptor. Biochem J *362*, 499-505.

Li, W., Henry, G., Fan, J., Bandyopadhyay, B., Pang, K., Garner, W., Chen, M., and Woodley, D.T. (2004). Signals that initiate, augment, and provide directionality for human keratinocyte motility. J Invest Dermatol *123*, 622-633.

Li, W., Marshall, C., Mei, L., Gelfand, J., and Seykora, J.T. (2007a). Srcasm corrects Fyn-induced epidermal hyperplasia by kinase down-regulation. J Biol Chem *282*, 1161-1169.

Li, W., Nadelman, C., Henry, G., Fan, J., Muellenhoff, M., Medina, E., Gratch, N.S., Chen, M., Han, J., and Woodley, D. (2001). The p38-MAPK/SAPK pathway is required for human keratinocyte migration on dermal collagen. J Invest Dermatol *117*, 1601-1611.

Li, W., Sandhoff, R., Kono, M., Zerfas, P., Hoffmann, V., Ding, B.C., Proia, R.L., and Deng, C.X. (2007b). Depletion of ceramides with very long chain fatty acids causes defective skin permeability barrier function, and neonatal lethality in ELOVL4 deficient mice. Int J Biol Sci *3*, 120-128.

Li, Y., Du, X., Li, F., Deng, Y., Yang, Z., Wang, Y., Pen, Z., Wang, Z., Yuan, W., Zhu, C., and Wu, X. (2006). A novel zinc-finger protein ZNF436 suppresses transcriptional activities of AP-1 and SRE. Mol Biol Rep *33*, 287-294.

Li, Y., Wheeler, D.L., Alters, W., Chaiswing, L., Verma, A.K., and Oberley, T.D. (2005). Early epidermal destruction with subsequent epidermal hyperplasia is a unique feature of the papilloma-independent squamous cell carcinoma phenotype in PKCepsilon overexpressing transgenic mice. Toxicol Pathol *33*, 684-694.

Liang, G., and Hai, T. (1997). Characterization of human activating transcription factor 4, a transcriptional activator that interacts with multiple domains of cAMP-responsive element-binding protein (CREB)-binding protein. J. Biol. Chem. *272*, 24088-24095.

Lin, D.Y., Fang, H.I., Ma, A.H., Huang, Y.S., Pu, Y.S., Jenster, G., Kung, H.J., and Shih, H.M. (2004). Negative modulation of androgen receptor transcriptional activity by Daxx. Mol Cell Biol *24*, 10529-10541.

Lippens, S., Kockx, M., Knaapen, M., Mortier, L., Polakowska, R., Verheyen, A., Garmyn, M., Zwijsen, A., Formstecher, P., Huylebroeck, D., Vandenabeele, P., and Declercq, W. (2000). Epidermal differentiation does not involve the pro-apoptotic executioner caspases, but is associated with caspase-14 induction and processing. Cell Death Differ *7*, 1218-1224.

Liu, F., Liu, Y., Li, D., Zhu, Y., Ouyang, W., Xie, X., and Jin, B. (2002a). The transcription co-repressor TLE1 interacted with the intracellular region of gpl30 through its Q domain. Mol Cell Biochem *232*, 163-167.

Liu, W., Mei, R., Di, X., Ryder, T., Hubbell, E., Dee, S., Webster, T., Harrington, C., Ho, M., Baid, J., and Smeekens, S. (2002b). Analysis of high density expression microarrays with signed-rank call algorithms. Bioinformatics *18*, 1593-1599.

Liu, X., Lee, J., Cooley, M., Bhogte, E., Hartley, S., and Glick, A. (2003). Smad7 but not Smad6 cooperates with oncogenic ras to cause malignant conversion in a mouse model for squamous cell carcinoma. Cancer Res *63*, 7760-7768.

Liu, Y., Millar, J.S., Cromley, D.A., Graham, M., Crooke, R., Billheimer, J.T., and Rader, D.J. (2008). Knockdown of acyl-CoA:diacylglycerol acyltransferase 2 with antisense oligonucleotide reduces VLDL TG and ApoB secretion in mice. Biochim Biophys Acta *1781*, 97-104.

Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods *25*, 402-408.

Lowenstein, E.J., Daly, R.J., Batzer, A.G., Li, W., Margolis, B., Lammers, R., Ullrich, A., Skolnik, E.Y., Bar-Sagi, D., and Schlessinger, J. (1992). The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. Cell *70*, 431-442.

Lu, Z., Liu, M., Stribinskis, V., Klinge, C.M., Ramos, K.S., Colburn, N.H., and Li, Y. (2008). MicroRNA-21 promotes cell transformation by targeting the programmed cell death 4 gene. Oncogene *27*, 4373-4379.

Lymboussaki, A., Kaipainen, A., Hatva, E., Vastrik, I., Jeskanen, L., Jalkanen, M., Werner, S., Stenback, F., and Alitalo, R. (1996). Expression of Mad, an antagonist of

Myc oncoprotein function, in differentiating keratinocytes during tumorigenesis of the skin. Br J Cancer *73*, 1347-1355.

Ma, J., Zeng, F., Schultz, R.M., and Tseng, H. (2006). Basonuclin: a novel mammalian maternal-effect gene. Development *133*, 2053-2062.

Madison, K.C., Sando, G.N., Howard, E.J., True, C.A., Gilbert, D., Swartzendruber, D.C., and Wertz, P.W. (1998). Lamellar granule biogenesis: a role for ceramide glucosyltransferase, lysosomal enzyme transport, and the Golgi. J Investig Dermatol Symp Proc *3*, 80-86.

Madsen, P., Rasmussen, H.H., Leffers, H., Honore, B., and Celis, J.E. (1992). Molecular cloning and expression of a novel keratinocyte protein (psoriasis-associated fatty acid-binding protein [PA-FABP]) that is highly up-regulated in psoriatic skin and that shares similarity to fatty acid-binding proteins. J Invest Dermatol *99*, 299-305.

Magnusdottir, E., Kalachikov, S., Mizukoshi, K., Savitsky, D., Ishida-Yamamoto, A., Panteleyev, A.A., and Calame, K. (2007). Epidermal terminal differentiation depends on B lymphocyte-induced maturation protein-1. Proc Natl Acad Sci U S A *104*, 14988-14993.

Mainiero, F., Pepe, A., Wary, K.K., Spinardi, L., Mohammadi, M., Schlessinger, J., and Giancotti, F.G. (1995). Signal transduction by the alpha 6 beta 4 integrin: distinct beta 4 subunit sites mediate recruitment of Shc/Grb2 and association with the cytoskeleton of hemidesmosomes. EMBO J *14*, 4470-4481.

Makino, T., Takaishi, M., Morohashi, M., and Huh, N.H. (2001). Hornerin, a novel profilaggrin-like protein and differentiation-specific marker isolated from mouse skin. J. Biol. Chem. *276*, 47445-47452.

Mann, G.B., Fowler, K.J., Gabriel, A., Nice, E.C., Williams, R.L., and Dunn, A.R. (1993). Mice with a null mutation of the TGF alpha gene have abnormal skin architecture, wavy hair, and curly whiskers and often develop corneal inflammation. Cell *73*, 249-261.

Marchese, C., Rubin, J., Ron, D., Faggioni, A., Torrisi, M.R., Messina, A., Frati, L., and Aaronson, S.A. (1990). Human keratinocyte growth factor activity on proliferation and differentiation of human keratinocytes: differentiation response distinguishes KGF from EGF family. J. Cell Physiol. *144*, 326-332.

Marenholz, I., Zirra, M., Fischer, D.F., Backendorf, C., Ziegler, A., and Mischke, D. (2001). Identification of human epidermal differentiation complex (EDC)-encoded genes by subtractive hybridization of entire YACs to a gridded keratinocyte cDNA library. Genome Res *11*, 341-355.

Margolis, B. (1999). The PTB Domain: The Name Doesn't Say It All. Trends Endocrinol Metab *10*, 262-267.

Maricich, S.M., Wellnitz, S.A., Nelson, A.M., Lesniak, D.R., Gerling, G.J., Lumpkin, E.A., and Zoghbi, H.Y. (2009). Merkel cells are essential for light-touch responses. Science *324*, 1580-1582.

Marine, J.C., Bellefroid, E.J., Bourguignon, C., Riviere, M., Lecoq, P.J., Poncelet, D.A., Szpirer, J., Martial, J.A., and Szpirer, C. (1994). Assignment of the human ZNF83 (HPF1) zinc finger gene to chromosome 19q13.3-q13.4. Genomics *21*, 285-286.

Marinkovich, M.P. (2007). Tumour microenvironment: laminin 332 in squamous-cell carcinoma. Nat Rev Cancer *7*, 370-380.

Martin-Nieto, J., and Villalobo, A. (1998). The human epidermal growth factor receptor contains a juxtamembrane calmodulin-binding site. Biochemistry *37*, 227-236.

Martinez, J.A., Williams, C.S., Amann, J.M., Ellis, T.C., Moreno-Miralles, I., Washington, M.K., Gregoli, P., and Hiebert, S.W. (2006). Deletion of Mtgr1 sensitizes the colonic epithelium to dextran sodium sulfate-induced colitis. Gastroenterology *131*, 579-588.

Mascia, F., Mariani, V., Girolomoni, G., and Pastore, S. (2003). Blockade of the EGF receptor induces a deranged chemokine expression in keratinocytes leading to enhanced skin inflammation. Am J Pathol *163*, 303-312.

Masuda, E.S., Naito, Y., Tokumitsu, H., Campbell, D., Saito, F., Hannum, C., Arai, K., and Arai, N. (1995). NFATx, a novel member of the nuclear factor of activated T cells family that is expressed predominantly in the thymus. Mol Cell Biol *15*, 2697-2706.

Matsuki, M., Yamashita, F., Ishida-Yamamoto, A., Yamada, K., Kinoshita, C., Fushiki, S., Ueda, E., Morishima, Y., Tabata, K., Yasuno, H., Hashida, M., Iizuka, H., Ikawa, M., Okabe, M., Kondoh, G., Kinoshita, T., Takeda, J., and Yamanishi, K. (1998). Defective stratum corneum and early neonatal death in mice lacking the gene for transglutaminase 1 (keratinocyte transglutaminase). Proc Natl Acad Sci U S A *95*, 1044-1049.

Matsuo, S., Yamazaki, S., Takeshige, K., and Muta, T. (2007). Crucial roles of binding sites for NF-kappaB and C/EBPs in IkappaB-zeta-mediated transcriptional activation. Biochem J *405*, 605-615.

Matsuzaka, Y., Okamoto, K., Tsuji, H., Mabuchi, T., Ozawa, A., Tamiya, G., and Inoko, H. (2002). Identification of the hRDH-E2 gene, a novel member of the SDR family, and its increased expression in psoriatic lesion. Biochem Biophys Res Commun *297*, 1171-1180.

Matsuzaki, K., Inoue, H., and Kumagai, N. (2004). Re-epithelialisation and the possible involvement of the transcription factor, basonuclin. Int Wound J *1*, 135-140.

Mayer, D.E., Baal, C., Litschauer-Poursadrollah, M., Hemmer, W., and Jarisch, R. (2010). Uncombable hair and atopic dermatitis in a case of trichodento-osseous syndrome. J Dtsch Dermatol Ges *8*, 102-104.

McGrath, J.A., Gatalica, B., Christiano, A.M., Li, K., Owaribe, K., McMillan, J.R., Eady, R.A., and Uitto, J. (1995). Mutations in the 180-kD bullous pemphigoid antigen (BPAG2), a hemidesmosomal transmembrane collagen (COL17A1), in generalized atrophic benign epidermolysis bullosa. Nat Genet *11*, 83-86.

Mechtcheriakova, D., Wlachos, A., Sobanov, J., Kopp, T., Reuschel, R., Bornancin, F., Cai, R., Zemann, B., Urtz, N., Stingl, G., Zlabinger, G., Woisetschlager, M., Baumruker, T., and Billich, A. (2007). Sphingosine 1-phosphate phosphatase 2 is induced during inflammatory responses. Cell Signal *19*, 748-760.

Medina, P.P., and Slack, F.J. (2008). microRNAs and cancer: an overview. Cell Cycle *7*, 2485-2492.

Mehrel, T., Hohl, D., Rothnagel, J.A., Longley, M.A., Bundman, D., Cheng, C., Lichti, U., Bisher, M.E., Steven, A.C., Steinert, P.M., and et al. (1990). Identification of a major keratinocyte cell envelope protein, loricrin. Cell *61*, 1103-1112.

Mehul, B., Bernard, D., Brouard, M., Delattre, C., and Schmidt, R. (2006). Influence of calcium on the proteolytic degradation of the calmodulin-like skin protein (calmodulin-like protein 5) in psoriatic epidermis. Exp Dermatol *15*, 469-477.

Mehul, B., Bernard, D., and Schmidt, R. (2001). Calmodulin-like skin protein: a new marker of keratinocyte differentiation. J Invest Dermatol *116*, 905-909.

Meng, F., Henson, R., Wehbe-Janek, H., Ghoshal, K., Jacob, S.T., and Patel, T. (2007). MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology *133*, 647-658.

Menon, G.K., Elias, P.M., Lee, S.H., and Feingold, K.R. (1992). Localization of calcium in murine epidermis following disruption and repair of the permeability barrier. Cell Tissue Res *270*, 503-512.

Merson, T.D., Dixon, M.P., Collin, C., Rietze, R.L., Bartlett, P.F., Thomas, T., and Voss, A.K. (2006). The transcriptional coactivator Querkopf controls adult neurogenesis. J Neurosci *26*, 11359-11370.

Mertens, A.E., Rygiel, T.P., Olivo, C., van der Kammen, R., and Collard, J.G. (2005). The Rac activator Tiam1 controls tight junction biogenesis in keratinocytes through binding to and activation of the Par polarity complex. J Cell Biol *170*, 1029-1037.

Mesa, K., Gebelein, B., Cook, T., and Urrutia, R. (1996). Identification and characterization of zinc finger encoding genes from the tumoral exocrine pancreatic cell line AR42J. Cancer Lett *103*, 143-149.

Mesko, B., Poliska, S., Szegedi, A., Szekanecz, Z., Palatka, K., Papp, M., and Nagy, L. Peripheral blood gene expression patterns discriminate among chronic inflammatory diseases and healthy controls and identify novel targets. BMC Med Genomics *3*, 15.

Michibata, H., Chiba, H., Wakimoto, K., Seishima, M., Kawasaki, S., Okubo, K., Mitsui, H., Torii, H., and Imai, Y. (2004). Identification and characterization of a novel component of the cornified envelope, cornifelin. Biochem Biophys Res Commun *318*, 803-813.

Miller, T., Williams, K., Johnstone, R.W., and Shilatifard, A. (2000). Identification, cloning, expression, and biochemical characterization of the testis-specific RNA polymerase II elongation factor ELL3. J. Biol. Chem. *275*, 32052-32056.

Mimeault, M., Bonenfant, D., and Batra, S.K. (2004). New advances on the functions of epidermal growth factor receptor and ceramides in skin cell differentiation, disorders and cancers. Skin Pharmacol Physiol *17*, 153-166.

Minden, A., Lin, A., Claret, F.X., Abo, A., and Karin, M. (1995). Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. Cell *81*, 1147-1157.

Ming, M.E., Daryanani, H.A., Roberts, L.P., Baden, H.P., and Kvedar, J.C. (1994). Binding of keratin intermediate filaments (K10) to the cornified envelope in mouse epidermis: implications for barrier function. J Invest Dermatol *103*, 780-784.

Mirancea, N., Hausser, I., Metze, D., Stark, H.J., Boukamp, P., and Breitkreutz, D. (2007). Junctional basement membrane anomalies of skin and mucosa in lipoid proteinosis (hyalinosis cutis et mucosae). J. Dermatol Sci. *45*, 175-185.

Mirmohammadsadegh, A., Tartler, U., Michel, G., Baer, A., Walz, M., Wolf, R., Ruzicka, T., and Hengge, U.R. (2003). HAX-1, identified by differential display reverse transcription polymerase chain reaction, is overexpressed in lesional psoriasis. J Invest Dermatol *120*, 1045-1051.

Missero, C., Di Cunto, F., Kiyokawa, H., Koff, A., and Dotto, G.P. (1996). The absence of p21Cip1/WAF1 alters keratinocyte growth and differentiation and promotes ras-tumor progression. Genes Dev *10*, 3065-3075.

Mitra, P., Xie, R.L., Medina, R., Hovhannisyan, H., Zaidi, S.K., Wei, Y., Harper, J.W., Stein, J.L., van Wijnen, A.J., and Stein, G.S. (2003). Identification of HiNF-P, a key activator of cell cycle-controlled histone H4 genes at the onset of S phase. Mol Cell Biol *23*, 8110-8123.

Mitsuishi, K., Nakamura, T., Sakata, Y., Yuyama, N., Arima, K., Sugita, Y., Suto, H., Izuhara, K., and Ogawa, H. (2005). The squamous cell carcinoma antigens as relevant biomarkers of atopic dermatitis. Clin Exp Allergy *35*, 1327-1333.

Miyoshi, H., Kanekura, T., Aoki, T., and Kanzaki, T. (2005). Beneficial effects of tissue inhibitor of metalloproteinases-2 (TIMP-2) on chronic dermatitis. J Dermatol *32*, 346-353.

Moll, I., Bladt, U., and Jung, E.G. (1990). Presence of Merkel cells in sun-exposed and not sun-exposed skin: a quantitative study. Arch Dermatol Res *282*, 213-216.

Monden, T., Kishi, M., Hosoya, T., Satoh, T., Wondisford, F.E., Hollenberg, A.N., Yamada, M., and Mori, M. (1999). p120 acts as a specific coactivator for 9-cis-retinoic acid receptor (RXR) on peroxisome proliferator-activated receptor-gamma/RXR heterodimers. Mol Endocrinol *13*, 1695-1703.

Monzon, R.I., McWilliams, N., and Hudson, L.G. (1996). Suppression of cornified envelope formation and type 1 transglutaminase by epidermal growth factor in neoplastic keratinocytes. Endocrinology *137*, 1727-1734.

Morasso, M.I., Grinberg, A., Robinson, G., Sargent, T.D., and Mahon, K.A. (1999). Placental failure in mice lacking the homeobox gene Dlx3. Proc Natl Acad Sci U S A *96*, 162-167.

Moriki, T., Maruyama, H., and Maruyama, I.N. (2001). Activation of preformed EGF receptor dimers by ligand-induced rotation of the transmembrane domain. J Mol Biol *311*, 1011-1026.

Morrison, K.M., Miesegaes, G.R., Lumpkin, E.A., and Maricich, S.M. (2009). Mammalian Merkel cells are descended from the epidermal lineage. Dev Biol *336*, 76-83.

Motohashi, H., Katsuoka, F., Engel, J.D., and Yamamoto, M. (2004). Small Maf proteins serve as transcriptional cofactors for keratinocyte differentiation in the Keap1-Nrf2 regulatory pathway. Proc. Natl. Acad. Sci. USA. *101*, 6379-6384.

Muchardt, C., and Yaniv, M. (1993). A human homologue of Saccharomyces cerevisiae SNF2/SWI2 and Drosophila brm genes potentiates transcriptional activation by the glucocorticoid receptor. EMBO J *12*, 4279-4290.

Mulero, J.J., Boyle, B.J., Bradley, S., Bright, J.M., Nelken, S.T., Ho, T.T., Mize, N.K., Childs, J.D., Ballinger, D.G., Ford, J.E., and Rupp, F. (2002). Three new human members of the lipid transfer/lipopolysaccharide binding protein family (LT/LBP). Immunogenetics *54*, 293-300.

Muller, F.B., Huber, M., Kinaciyan, T., Hausser, I., Schaffrath, C., Krieg, T., Hohl, D., Korge, B.P., and Arin, M.J. (2006). A human keratin 10 knockout causes recessive epidermolytic hyperkeratosis. Hum Mol Genet *15*, 1133-1141.

Mumm, J.S., and Kopan, R. (2000). Notch signaling: from the outside in. Dev Biol *228*, 151-165.

Munger, K., Pietenpol, J.A., Pittelkow, M.R., Holt, J.T., and Moses, H.L. (1992). Transforming growth factor beta 1 regulation of c-myc expression, pRB phosphorylation, and cell cycle progression in keratinocytes. Cell Growth Differ *3*, 291-298.

Murphy, D.J., Swigart, L.B., Israel, M.A., and Evan, G.I. (2004). Id2 is dispensable for Myc-induced epidermal neoplasia. Mol Cell Biol *24*, 2083-2090.

Nagarajan, P., Chin, S.S., Wang, D., Liu, S., Sinha, S., and Garrett-Sinha, L.A. (2010). Ets1 blocks terminal differentiation of keratinocytes and induces expression of matrix metalloproteases and innate immune mediators. J. Cell Sci. *123*, 3566-3575.

Nagarajan, P., Parikh, N., Garrett-Sinha, L.A., and Sinha, S. (2009). Ets1 induces dysplastic changes when expressed in terminally-differentiating squamous epidermal cells. PLoS ONE *4*, e4179.

Nair, M., Bilanchone, V., Ortt, K., Sinha, S., and Dai, X. (2007). Ovol1 represses its own transcription by competing with transcription activator c-Myb and by recruiting histone deacetylase activity. Nucleic Acids Res *35*, 1687-1697.

Nanney, L.B., Magid, M., Stoscheck, C.M., and King, L.E., Jr. (1984). Comparison of epidermal growth factor binding and receptor distribution in normal human epidermis and epidermal appendages. J Invest Dermatol *83*, 385-393.

Nanney, L.B., Stoscheck, C.M., and King, L.E. (1988). Characterization of binding and receptors for epidermal growth factor in smooth muscle. Cell Tissue Res *254*, 125-132.

Navarro, P., Chiong, M., Volkwein, K., Moraga, F., Ocaranza, M.P., Jalil, J.E., Lim, S.W., Kim, J.A., Kwon, H.M., and Lavandero, S. (2008). Osmotically-induced genes are controlled by the transcription factor TonEBP in cultured cardiomyocytes. Biochem Biophys Res Commun *372*, 326-330.

Ness, S.L., Edelmann, W., Jenkins, T.D., Liedtke, W., Rustgi, A.K., and Kucherlapati, R. (1998). Mouse keratin 4 is necessary for internal epithelial integrity. J. Biol. Chem. *273*, 23904-23911.

Nguyen, B.C., Lefort, K., Mandinova, A., Antonini, D., Devgan, V., Della Gatta, G., Koster, M.I., Zhang, Z., Wang, J., Tommasi di Vignano, A., Kitajewski, J., Chiorino, G., Roop, D.R., Missero, C., and Dotto, G.P. (2006). Cross-regulation between Notch and p63 in keratinocyte commitment to differentiation. Genes Dev *20*, 1028-1042.

Nguyen, H., Merrill, B.J., Polak, L., Nikolova, M., Rendl, M., Shaver, T.M., Pasolli, H.A., and Fuchs, E. (2009). Tcf3 and Tcf4 are essential for long-term homeostasis of skin epithelia. Nat Genet *41*, 1068-1075.

Nicassio, F., Corrado, N., Vissers, J.H., Areces, L.B., Bergink, S., Marteijn, J.A., Geverts, B., Houtsmuller, A.B., Vermeulen, W., Di Fiore, P.P., and Citterio, E. (2007). Human USP3 is a chromatin modifier required for S phase progression and genome stability. Curr Biol *17*, 1972-1977.

Nie, X.F., Maclean, K.N., Kumar, V., McKay, I.A., and Bustin, S.A. (1995). ERF-2, the human homologue of the murine Tis11d early response gene. Gene *152*, 285-286.

Niebuhr, M., Langnickel, J., Sigel, S., and Werfel, T. (2009). Dysregulation of CD36 upon TLR-2 stimulation in monocytes from patients with atopic dermatitis and the TLR2 R753Q polymorphism. Exp Dermatol.

Nin, M., Katoh, N., Kokura, S., Handa, O., Yoshikawa, T., and Kishimoto, S. (2009). Dichotomous effect of ultraviolet B on the expression of corneodesmosomal enzymes in human epidermal keratinocytes. J. Dermatol Sci. *54*, 17-24.

Ning, J., Zheng, G., and Yang, Y.C. (2008). Tip60 modulates PLAGL2-mediated transactivation by acetylation. J. Cell Biochem. *103*, 730-739.

Nolte, C.J., Oleson, M.A., Bilbo, P.R., and Parenteau, N.L. (1993). Development of a stratum corneum and barrier function in an organotypic skin culture. Arch Dermatol Res *285*, 466-474.

Nomura, I., Gao, B., Boguniewicz, M., Darst, M.A., Travers, J.B., and Leung, D.Y. (2003). Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. J Allergy Clin Immunol *112*, 1195-1202.

Norlen, L. (2001). Skin barrier formation: the membrane folding model. J. Invest. Dermatol. *117*, 823-829.

Norlen, L., Al-Amoudi, A., and Dubochet, J. (2003). A cryotransmission electron microscopy study of skin barrier formation. J Invest Dermatol *120*, 555-560.

Odland, G.F., and Holbrook, K. (1981). The lamellar granules of epidermis. Curr Probl Dermatol *9*, 29-49.

Oettgen, P., Alani, R.M., Barcinski, M.A., Brown, L., Akbarali, Y., Boltax, J., Kunsch, C., Munger, K., and Libermann, T.A. (1997). Isolation and characterization of a novel epithelium-specific transcription factor, ESE-1, a member of the ets family. Mol Cell Biol *17*, 4419-4433.

Oettgen, P., Kas, K., Dube, A., Gu, X., Grall, F., Thamrongsak, U., Akbarali, Y., Finger, E., Boltax, J., Endress, G., Munger, K., Kunsch, C., and Libermann, T.A. (1999). Characterization of ESE-2, a novel ESE-1-related Ets transcription factor that is restricted to glandular epithelium and differentiated keratinocytes. J. Biol. Chem. *274*, 29439-29452.

Ogiso, H., Ishitani, R., Nureki, O., Fukai, S., Yamanaka, M., Kim, J.H., Saito, K., Sakamoto, A., Inoue, M., Shirouzu, M., and Yokoyama, S. (2002). Crystal structure of the complex of human epidermal growth factor and receptor extracellular domains. Cell *110*, 775-787.

Oh, S.W., Kim, M.Y., Lee, J.S., and Kim, S.C. (2006). Keratin 17 mutation in pachyonychia congenita type 2 patient with early onset steatocystoma multiplex and Hutchinson-like tooth deformity. J. Dermatol *33*, 161-164.

Ohno, Y., Suto, S., Yamanaka, M., Mizutani, Y., Mitsutake, S., Igarashi, Y., Sassa, T., and Kihara, A. ELOVL1 production of C24 acyl-CoAs is linked to C24 sphingolipid synthesis. Proc Natl Acad Sci U S A *107*, 18439-18444.

Ohno, Y., Suto, S., Yamanaka, M., Mizutani, Y., Mitsutake, S., Igarashi, Y., Sassa, T., and Kihara, A. (2010). ELOVL1 production of C24 acyl-CoAs is linked to C24 sphingolipid synthesis. Proc. Natl. Acad. Sci. USA. *107*, 18439-18444.

Okada, Y., Matsuura, E., Nagai, R., Sato, T., Watanabe, A., Morita, I., and Doi, T. (2003). PREP1, MEIS1 homolog protein, regulates PF4 gene expression. Biochem Biophys Res Commun *305*, 155-159.

Okuyama, R., LeFort, K., and Dotto, G.P. (2004). A dynamic model of keratinocyte stem cell renewal and differentiation: role of the p21WAF1/Cip1 and Notch1 signaling pathways. J Investig Dermatol Symp Proc *9*, 248-252.

Okuyama, R., LeFort, K., Dotto, G.P. (2004). A Dynamic Model of Keratinocyte Stem Cell Renewal and Differentiation: Role of the $p21^{WAF1/CIP1}$ and Notch1 Signaling Pathways. J Investig Dermatol Symp Proc *9*, 248-252.

Ostendorff, H.P., Bossenz, M., Mincheva, A., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Lichter, P., and Bach, I. (2000). Functional characterization of the gene encoding RLIM, the corepressor of LIM homeodomain factors. Genomics *69*, 120-130.

Oudot, T., Lesueur, F., Guedj, M., de Cid, R., McGinn, S., Heath, S., Foglio, M., Prum, B., Lathrop, M., Prud'homme, J.F., and Fischer, J. (2009). An association study of 22 candidate genes in psoriasis families reveals shared genetic factors with other autoimmune and skin disorders. J Invest Dermatol *129*, 2637-2645.

Owens, D.W., Brunton, V.G., Parkinson, E.K., and Frame, M.C. (2000). E-cadherin at the cell periphery is a determinant of keratinocyte differentiation in vitro. Biochem Biophys Res Commun *269*, 369-376.

Ozcelik, T., Porteus, M.H., Rubenstein, J.L., and Francke, U. (1992). DLX2 (TES1), a homeobox gene of the Distal-less family, assigned to conserved regions on human and mouse chromosomes 2. Genomics *13*, 1157-1161.

Ozden, M.G., Ilter, N., Suer, O.E., Solaktekin, N., and Gurer, M.A. (2008). Increased insulin-like growth factor binding protein-3 expression level in psoriatic tissue before and after systemic treatment with cyclosporine A and methotrexate. Int J Dermatol *47*, 1177-1183.

Pahl, P.M., Hodges, Y.K., Meltesen, L., Perryman, M.B., Horwitz, K.B., and Horwitz, L.D. (1998). ZNF207, a ubiquitously expressed zinc finger gene on chromosome 6p21.3. Genomics *53*, 410-412.

Pandolfi, P.P., Roth, M.E., Karis, A., Leonard, M.W., Dzierzak, E., Grosveld, F.G., Engel, J.D., and Lindenbaum, M.H. (1995). Targeted disruption of the GATA3 gene causes severe abnormalities in the nervous system and in fetal liver haematopoiesis. Nat Genet *11*, 40-44.

Pantelouris, E.M., and Macmenamin, P.N. (1973). Amino acid decarboxylase activity in "nude" mouse liver. Comp Biochem Physiol B *45*, 967-970.

Papait, R., Pistore, C., Negri, D., Pecoraro, D., Cantarini, L., and Bonapace, I.M. (2007). Np95 is implicated in pericentromeric heterochromatin replication and in major satellite silencing. Mol Biol Cell *18*, 1098-1106.

Paragh, G., Ugocsai, P., Vogt, T., Schling, P., Kel, A.E., Tarabin, V., Liebisch, G., Orso, E., Marko, L., Balogh, A., Kobling, T., Remenyik, E., Wikonkal, N.M., Mandl, J., Farwick, M., and Schmitz, G. (2010). Whole genome transcriptional profiling identifies novel differentiation regulated genes in keratinocytes. Exp Dermatol *19*, 297-301.

Park, G.T., Lim, S.E., Jang, S.I., and Morasso, M.I. (2002). Suprabasin, a novel epidermal differentiation marker and potential cornified envelope precursor. J Biol Chem *277*, 45195-45202.

Park, Y.Y., Park, K.C., Shong, M., Lee, S.J., Lee, Y.H., and Choi, H.S. (2007). EID-1 interacts with orphan nuclear receptor SF-1 and represses its transactivation. Mol Cells *24*, 372-377.

Pasonen-Seppanen, S., Suhonen, T.M., Kirjavainen, M., Miettinen, M., Urtti, A., Tammi, M., and Tammi, R. (2001). Formation of permeability barrier in epidermal organotypic culture for studies on drug transport. J. Invest. Dermatol. *117*, 1322-1324.

Pastore, S., Mascia, F., Mariani, V., and Girolomoni, G. (2008). The epidermal growth factor receptor system in skin repair and inflammation. J. Invest. Dermatol. *128*, 1365-1374.

Pastore, S., Mascia, F., Mariotti, F., Dattilo, C., Mariani, V., and Girolomoni, G. (2005). ERK1/2 regulates epidermal chemokine expression and skin inflammation. J Immunol *174*, 5047-5056.

Pati, D., Meistrich, M.L., and Plon, S.E. (1999). Human Cdc34 and Rad6B ubiquitin-conjugating enzymes target repressors of cyclic AMP-induced transcription for proteolysis. Mol Cell Biol *19*, 5001-5013.

Pavez Lorie, E., Ganemo, A., Borgers, M., Wouters, L., Blockhuys, S., van de Plassche, L., Torma, H., and Vahlquist, A. (2009). Expression of retinoid-regulated genes in lamellar ichthyosis vs. healthy control epidermis: changes after oral treatment with liarozole. Acta Derm Venereol *89*, 12-20.

Paznekas, W.A., Boyadjiev, S.A., Shapiro, R.E., Daniels, O., Wollnik, B., Keegan, C.E., Innis, J.W., Dinulos, M.B., Christian, C., Hannibal, M.C., and Jabs, E.W. (2003). Connexin 43 (GJA1) mutations cause the pleiotropic phenotype of oculodentodigital dysplasia. Am J Hum Genet *72*, 408-418.

Pelletier, N., Champagne, N., Stifani, S., and Yang, X.J. (2002). MOZ and MORF histone acetyltransferases interact with the Runt-domain transcription factor Runx2. Oncogene *21*, 2729-2740.

Perez-Soler, R., Delord, J.P., Halpern, A., Kelly, K., Krueger, J., Sureda, B.M., von Pawel, J., Temel, J., Siena, S., Soulieres, D., Saltz, L., and Leyden, J. (2005). HER1/EGFR inhibitor-associated rash: future directions for management and investigation outcomes from the HER1/EGFR inhibitor rash management forum. Oncologist *10*, 345-356.

Perez-Soler, R., and Saltz, L. (2005). Cutaneous adverse effects with HER1/EGFR-targeted agents: is there a silver lining? J Clin Oncol *23*, 5235-5246.

Peus, D., Hamacher, L., and Pittelkow, M.R. (1997). EGF-receptor tyrosine kinase inhibition induces keratinocyte growth arrest and terminal differentiation. J. Invest. Dermatol. *109*, 751-756.

Pi, H., Li, Y., Zhu, C., Zhou, L., Luo, K., Yuan, W., Yi, Z., Wang, Y., Wu, X., and Liu, M. (2002). A novel human SCAN/(Cys)2(His)2 zinc-finger transcription factor ZNF323 in early human embryonic development. Biochem Biophys Res Commun *296*, 206-213.

Pickles, L.M., Roe, S.M., Hemingway, E.J., Stifani, S., and Pearl, L.H. (2002). Crystal structure of the C-terminal WD40 repeat domain of the human Groucho/TLE1 transcriptional corepressor. Structure *10*, 751-761.

Pillai, S., Bikle, D.D., Mancianti, M.L., Cline, P., and Hincenbergs, M. (1990). Calcium regulation of growth and differentiation of normal human keratinocytes: modulation of differentiation competence by stages of growth and extracellular calcium. J Cell Physiol *143*, 294-302.

Plewig, G., and Marples, R.R. (1970). Regional differences of cell sizes in the human stratum corneum. I. J Invest Dermatol *54*, 13-18.

Ponec, M., Gibbs, S., Weerheim, A., Kempenaar, J., Mulder, A., and Mommaas, A.M. (1997). Epidermal growth factor and temperature regulate keratinocyte differentiation. Arch Dermatol Res *289*, 317-326.

Ponec, M., and Weerheim, A. (1990). Retinoids and lipid changes in keratinocytes. Methods Enzymol *190*, 30-41.

Ponec, M., Weerheim, A., Lankhorst, P., and Wertz, P. (2003). New acylceramide in native and reconstructed epidermis. J Invest Dermatol *120*, 581-588.

Porter, R.M., and Lane, E.B. (2003). Phenotypes, genotypes and their contribution to understanding keratin function. Trends Genet *19*, 278-285.

Porter, R.M., Reichelt, J., Lunny, D.P., Magin, T.M., and Lane, E.B. (1998). The relationship between hyperproliferation and epidermal thickening in a mouse model for BCIE. J. Invest. Dermatol. *110*, 951-957.

Potts, R.O., and Francoeur, M.L. (1991). The influence of stratum corneum morphology on water permeability. J Invest Dermatol *96*, 495-499.

Poumay, Y., and Pittelkow, M.R. (1995). Cell density and culture factors regulate keratinocyte commitment to differentiation and expression of suprabasal K1/K10 keratins. J Invest Dermatol *104*, 271-276.

Prenzel, N., Fischer, O.M., Streit, S., Hart, S., and Ullrich, A. (2001). The epidermal growth factor receptor family as a central element for cellular signal transduction and diversification. Endocr Relat Cancer *8*, 11-31.

Prowse, D.M., Lee, D., Weiner, L., Jiang, N., Magro, C.M., Baden, H.P., and Brissette, J.L. (1999). Ectopic expression of the nude gene induces hyperproliferation and defects in differentiation: implications for the self-renewal of cutaneous epithelia. Dev Biol *212*, 54-67.

Pulkkinen, L., Christiano, A.M., Airenne, T., Haakana, H., Tryggvason, K., and Uitto, J. (1994). Mutations in the gamma 2 chain gene (LAMC2) of kalinin/laminin 5 in the junctional forms of epidermolysis bullosa. Nat Genet *6*, 293-297.

Qi, J., Zhang, X., Zhang, H.K., Yang, H.M., Zhou, Y.B., and Han, Z.G. (2006). ZBTB34, a novel human BTB/POZ zinc finger protein, is a potential transcriptional repressor. Mol Cell Biochem *290*, 159-167.

Qian, F., Kruse, U., Lichter, P., and Sippel, A.E. (1995). Chromosomal localization of the four genes (NFIA, B, C, and X) for the human transcription factor nuclear factor I by FISH. Genomics *28*, 66-73.

Radoja, N., Gazel, A., Banno, T., Yano, S., and Blumenberg, M. (2006). Transcriptional profiling of epidermal differentiation. Physiol Genomics *27*, 65-78.

Rall, L.B., Scott, J., Bell, G.I., Crawford, R.J., Penschow, J.D., Niall, H.D., and Coghlan, J.P. (1985). Mouse prepro-epidermal growth factor synthesis by the kidney and other tissues. Nature *313*, 228-231.

Rangarajan, A., Talora, C., Okuyama, R., Nicolas, M., Mammucari, C., Oh, H., Aster, J.C., Krishna, S., Metzger, D., Chambon, P., Miele, L., Aguet, M., Radtke, F., and Dotto, G.P. (2001). Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. EMBO J *20*, 3427-3436.

Rawles, M.E. (1947). Origin of pigment cells from the neural crest in the mouse embryo. Physiol Zool *20*, 248-266.

Ray, A., and Schmitt, D. (1988). [Langerhans cell: CD1 antigens and the Birbeck granule]. Pathol Biol (Paris) *36*, 846-853.

Rebollo, A., and Schmitt, C. (2003). Ikaros, Aiolos and Helios: transcription regulators and lymphoid malignancies. Immunol Cell Biol *81*, 171-175.

Regnier, M., Caron, D., Reichert, U., and Schaefer, H. (1992). Reconstructed human epidermis: a model to study in vitro the barrier function of the skin. Skin Pharmacol *5*, 49-56.

Reich, K., Garbe, C., Blaschke, V., Maurer, C., Middel, P., Westphal, G., Lippert, U., and Neumann, C. (2001). Response of psoriasis to interleukin-10 is associated with suppression of cutaneous type 1 inflammation, downregulation of the epidermal interleukin-8/CXCR2 pathway and normalization of keratinocyte maturation. J Invest Dermatol *116*, 319-329.

Reiss, M., Lipsey, L.R., and Zhou, Z.L. (1991). Extracellular calcium-dependent regulation of transmembrane calcium fluxes in murine keratinocytes. J. Cell Physiol. *147*, 281-291.

Reiss, M., and Sartorelli, A.C. (1987). Regulation of growth and differentiation of human keratinocytes by type beta transforming growth factor and epidermal growth factor. Cancer Res *47*, 6705-6709.

Reynolds, V.H., Boehm, F.H., and Cohen, S. (1965). Enhancement of chemical carcinogenesis by an epidermal growth factor. Surg Forum *16*, 108-109.

Rice, R.H., and Green, H. (1979). Presence in human epidermal cells of a soluble protein precursor of the cross-linked envelope: activation of the cross-linking by calcium ions. Cell *18*, 681-694.

Richardson, R.T., Alekseev, O.M., Grossman, G., Widgren, E.E., Thresher, R., Wagner, E.J., Sullivan, K.D., Marzluff, W.F., and O'Rand, M.G. (2006). Nuclear autoantigenic sperm protein (NASP), a linker histone chaperone that is required for cell proliferation. J. Biol. Chem. *281*, 21526-21534.

Riethmacher, D., Sonnenberg-Riethmacher, E., Brinkmann, V., Yamaai, T., Lewin, G.R., and Birchmeier, C. (1997). Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. Nature *389*, 725-730.

Rigby, A.C., Grant, C.W., and Shaw, G.S. (1998). Solution and solid state conformation of the human EGF receptor transmembrane region. Biochim Biophys Acta *1371*, 241-253.
Ritchie, K., Seah, C., Moulin, J., Isaac, C., Dick, F., and Berube, N.G. (2008). Loss of ATRX leads to chromosome cohesion and congression defects. J. Cell Biol. *180*, 315-324.

Robinson, N.A., Lapic, S., Welter, J.F., and Eckert, R.L. (1997). S100A11, S100A10, annexin I, desmosomal proteins, small proline-rich proteins, plasminogen activator inhibitor-2, and involucrin are components of the cornified envelope of cultured human epidermal keratinocytes. J. Biol. Chem. *272*, 12035-12046.

Rugg, E., Magee, G., Wilson, N., Brandrup, F., Hamburger, J., and Lane, E. (1999). Identification of two novel mutations in keratin 13 as the cause of white sponge naevus. Oral Dis *5*, 321-324.

Samee, N., Geoffroy, V., Marty, C., Schiltz, C., Vieux-Rochas, M., Levi, G., and de Vernejoul, M.C. (2008). Dlx5, a positive regulator of osteoblastogenesis, is essential for osteoblast-osteoclast coupling. Am J Pathol *173*, 773-780.

Sanchez-Diaz, A., Blanco, M.A., Jones, N., and Moreno, S. (2001). HBP2: a new mammalian protein that complements the fission yeast MBF transcription complex. Curr Genet *40*, 110-118.

Sasai, Y., Kageyama, R., Tagawa, Y., Shigemoto, R., and Nakanishi, S. (1992). Two mammalian helix-loop-helix factors structurally related to Drosophila hairy and Enhancer of split. Genes Dev *6*, 2620-2634.

Satijn, D.P., Olson, D.J., van der Vlag, J., Hamer, K.M., Lambrechts, C., Masselink, H., Gunster, M.J., Sewalt, R.G., van Driel, R., and Otte, A.P. (1997). Interference with the

expression of a novel human polycomb protein, hPc2, results in cellular transformation and apoptosis. Mol Cell Biol *17*, 6076-6086.

Sato, H., Taketomi, Y., Isogai, Y., Masuda, S., Kobayashi, T., Yamamoto, K., and Murakami, M. (2009). Group III secreted phospholipase A2 transgenic mice spontaneously develop inflammation. Biochem J *421*, 17-27.

Savage, C.R., Jr., and Cohen, S. (1973). Proliferation of corneal epithelium induced by epidermal growth factor. Exp Eye Res *15*, 361-366.

Savage, C.R., Jr., Hash, J.H., and Cohen, S. (1973). Epidermal growth factor. Location of disulfide bonds. J Biol Chem *248*, 7669-7672.

Savage, C.R., Jr., Inagami, T., and Cohen, S. (1972). The primary structure of epidermal growth factor. J Biol Chem *247*, 7612-7621.

Scarabelli, T.M., Townsend, P.A., Chen Scarabelli, C., Yuan, Z., McCauley, R.B., Di Rezze, J., Patel, D., Putt, J., Allebban, Z., Abboud, J., Chilukuri, K., Gardin, J., Saravolatz, L., Knight, R.A., Latchman, D.S., and Stephanou, A. (2008). Amino acid supplementation differentially modulates STAT1 and STAT3 activation in the myocardium exposed to ischemia/reperfusion injury. Am J Cardiol *101*, 63E-68E.

Scharschmidt, T.C., Man, M.Q., Hatano, Y., Crumrine, D., Gunathilake, R., Sundberg, J.P., Silva, K.A., Mauro, T.M., Hupe, M., Cho, S., Wu, Y., Celli, A., Schmuth, M., Feingold, K.R., and Elias, P.M. (2009). Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory thresholds to irritants and haptens. J Allergy Clin Immunol *124*, 496-506, 506 e491-496.

Schleinitz, N., Fischer, J., Sanchez, A., Veit, V., Harle, J.R., and Pelissier, J.F. (2005). Two new mutations of the ABHD5 gene in a new adult case of Chanarin Dorfman syndrome: an uncommon lipid storage disease. Arch Dermatol *141*, 798-800.

Schneider, J.A., Diamond, I., and Rozengurt, E. (1978). Glycolysis of quiescent cultures of 3T3 cells. Addition of serum, epidermal growth factor, and insulin increases the activity of phosphofructokinase in a protein synthesis-independent manner. J Biol Chem *253*, 872-877.

Schneider, M.R., Werner, S., Paus, R., and Wolf, E. (2008). Beyond wavy hairs: the epidermal growth factor receptor and its ligands in skin biology and pathology. Am J Pathol *173*, 14-24.

Schwartz, D.R., Homanics, G.E., Hoyt, D.G., Klein, E., Abernethy, J., and Lazo, J.S. (1999). The neutral cysteine protease bleomycin hydrolase is essential for epidermal integrity and bleomycin resistance. Proc. Natl. Acad. Sci. USA. *96*, 4680-4685.

Scott, K.L., and Plon, S.E. (2005). CHES1/FOXN3 interacts with Ski-interacting protein and acts as a transcriptional repressor. Gene *359*, 119-126.

Segre, J.A., Bauer, C., and Fuchs, E. (1999). Klf4 is a transcription factor required for establishing the barrier function of the skin. Nat Genet *22*, 356-360.

Seike, M., Goto, A., Okano, T., Bowman, E.D., Schetter, A.J., Horikawa, I., Mathe, E.A., Jen, J., Yang, P., Sugimura, H., Gemma, A., Kudoh, S., Croce, C.M., and Harris, C.C. (2009). MiR-21 is an EGFR-regulated anti-apoptotic factor in lung cancer in never-smokers. Proc. Natl. Acad. Sci. USA. *106*, 12085-12090.

Sekimata, M., and Homma, Y. (2004). Sequence-specific transcriptional repression by an MBD2-interacting zinc finger protein MIZF. Nucleic Acids Res *32*, 590-597.

Senawong, T., Peterson, V.J., and Leid, M. (2005). BCL11A-dependent recruitment of SIRT1 to a promoter template in mammalian cells results in histone deacetylation and transcriptional repression. Arch Biochem Biophys *434*, 316-325.

Sergi, C., Kahl, P., and Otto, H.F. (2000). Immunohistochemical localization of transforming growth factor-alpha and epithelial growth factor receptor in human fetal developing skin, psoriasis and restrictive dermopathy. Pathol Oncol Res *6*, 250-255.

Serre, G., Mils, V., Haftek, M., Vincent, C., Croute, F., Reano, A., Ouhayoun, J.P., Bettinger, S., and Soleilhavoup, J.P. (1991). Identification of late differentiation antigens of human cornified epithelia, expressed in re-organized desmosomes and bound to cross-linked envelope. J Invest Dermatol *97*, 1061-1072.

Shang, Y., Yoshida, T., Amendt, B.A., Martin, J.F., and Owens, G.K. (2008). Pitx2 is functionally important in the early stages of vascular smooth muscle cell differentiation. J. Cell Biol. *181*, 461-473.

Shannon, M., and Stubbs, L. (1998). Analysis of homologous XRCC1-linked zinc-finger gene families in human and mouse: evidence for orthologous genes. Genomics *49*, 112-121.

Shao, H., Zhu, C., Zhao, Z., Guo, M., Qiu, H., Liu, H., Wang, D., Xue, L., Gao, L., Sun, C., and Li, W. (2006). KRAB-containing zinc finger gene ZNF268 encodes multiple alternatively spliced isoforms that contain transcription regulatory domains. Int J Mol Med *18*, 457-463.

Sharov, A.A., Masui, S., Sharova, L.V., Piao, Y., Aiba, K., Matoba, R., Xin, L., Niwa, H., and Ko, M.S. (2008). Identification of Pou5f1, Sox2, and Nanog downstream target genes with statistical confidence by applying a novel algorithm to time course microarray and genome-wide chromatin immunoprecipitation data. BMC Genomics *9*, 269.

Sheng, G., dos Reis, M., and Stern, C.D. (2003). Churchill, a zinc finger transcriptional activator, regulates the transition between gastrulation and neurulation. Cell *115*, 603-613.
Shi, Y., Downes, M., Xie, W., Kao, H.Y., Ordentlich, P., Tsai, C.C., Hon, M., and Evans, R.M. (2001). Sharp, an inducible cofactor that integrates nuclear receptor repression and activation. Genes Dev *15*, 1140-1151.

Shiina, T., Konno, A., Oonuma, T., Kitamura, H., Imaoka, K., Takeda, N., Todokoro, K., and Morimatsu, M. (2004). Targeted disruption of MAIL, a nuclear IkappaB protein, leads to severe atopic dermatitis-like disease. J. Biol. Chem. *279*, 55493-55498.

Shikama, N., Lee, C.W., France, S., Delavaine, L., Lyon, J., Krstic-Demonacos, M., and La Thangue, N.B. (1999). A novel cofactor for p300 that regulates the p53 response. Mol Cell *4*, 365-376.

Shirasawa, S., Sugiyama, S., Baba, I., Inokuchi, J., Sekine, S., Ogino, K., Kawamura, Y., Dohi, T., Fujimoto, M., and Sasazuki, T. (2004). Dermatitis due to epiregulin deficiency and a critical role of epiregulin in immune-related responses of keratinocyte and macrophage. Proc. Natl. Acad. Sci. USA. *101*, 13921-13926.

Sibilia, M., and Wagner, E.F. (1995). Strain-dependent epithelial defects in mice lacking the EGF receptor. Science *269*, 234-238.

Sichtig, N., Korfer, N., and Steger, G. (2007). Papillomavirus binding factor binds to SAP30 and represses transcription via recruitment of the HDAC1 co-repressor complex. Arch Biochem Biophys *467*, 67-75.

Siegenthaler, G., Tomatis, I., Didierjean, L., Jaconi, S., and Saurat, J.H. (1992). Overexpression of cellular retinoic acid-binding protein type II (CRABP-II) and down-regulation of CRABP-I in psoriatic skin. Dermatology *185*, 251-256.

Simon, M., Montezin, M., Guerrin, M., Durieux, J.J., and Serre, G. (1997). Characterization and purification of human corneodesmosin, an epidermal basic glycoprotein associated with corneocyte-specific modified desmosomes. J Biol Chem *272*, 31770-31776.

Smith, E.A., and Fuchs, E. (1998). Defining the interactions between intermediate filaments and desmosomes. J Cell Biol *141*, 1229-1241.

Soltoff, S.P., Carraway, K.L., 3rd, Prigent, S.A., Gullick, W.G., and Cantley, L.C. (1994). ErbB3 is involved in activation of phosphatidylinositol 3-kinase by epidermal growth factor. Mol Cell Biol *14*, 3550-3558.

Song, A., Chen, Y.F., Thamatrakoln, K., Storm, T.A., and Krensky, A.M. (1999). RFLAT-1: a new zinc finger transcription factor that activates RANTES gene expression in T lymphocytes. Immunity *10*, 93-103.

Song, Z., Krishna, S., Thanos, D., Strominger, J.L., and Ono, S.J. (1994). A novel cysteine-rich sequence-specific DNA-binding protein interacts with the conserved X-box motif of the human major histocompatibility complex class II genes via a repeated Cys-His domain and functions as a transcriptional repressor. J Exp Med *180*, 1763-1774.

Sonkoly, E., Wei, T., Janson, P.C., Saaf, A., Lundeberg, L., Tengvall-Linder, M., Norstedt, G., Alenius, H., Homey, B., Scheynius, A., Stahle, M., and Pivarcsi, A. (2007). MicroRNAs: novel regulators involved in the pathogenesis of psoriasis? PLoS One *2*, e610.

Sporn, M.B., Roberts, A.B., Wakefield, L.M., and de Crombrugghe, B. (1987). Some recent advances in the chemistry and biology of transforming growth factor-beta. J Cell Biol *105*, 1039-1045.

Stefansson, K., Brattsand, M., Ny, A., Glas, B., and Egelrud, T. (2006). Kallikrein-related peptidase 14 may be a major contributor to trypsin-like proteolytic activity in human stratum corneum. Biol Chem *387*, 761-768.

Steinberg, M.S., Shida, H., Giudice, G.J., Shida, M., Patel, N.H., and Blaschuk, O.W. (1987). On the molecular organization, diversity and functions of desmosomal proteins. Ciba Found Symp *125*, 3-25.

Stern, C.D. (2005). Neural induction: old problem, new findings, yet more questions. Development *132*, 2007-2021.

Stern, J.L., Cao, J.Z., Xu, J., Mocarski, E.S., and Slobedman, B. (2008). Repression of human cytomegalovirus major immediate early gene expression by the cellular transcription factor CCAAT displacement protein. Virology *378*, 214-225.

Sterneck, E., Zhu, S., Ramirez, A., Jorcano, J.L., and Smart, R.C. (2006). Conditional ablation of C/EBP beta demonstrates its keratinocyte-specific requirement for cell survival and mouse skin tumorigenesis. Oncogene *25*, 1272-1276.

Stevenson, B.R., Siliciano, J.D., Mooseker, M.S., and Goodenough, D.A. (1986). Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. J. Cell Biol. *103*, 755-766.

Stiban, J., Tidhar, R., and Futerman, A.H. Ceramide synthases: roles in cell physiology and signaling. Adv Exp Med Biol *688*, 60-71.

Stiban, J., Tidhar, R., and Futerman, A.H. (2010). Ceramide synthases: roles in cell physiology and signaling. Adv Exp Med Biol *688*, 60-71.

Sticherling, M., Bornscheuer, E., Schroder, J.M., and Christophers, E. (1992). Immunohistochemical studies on NAP-1/IL-8 in contact eczema and atopic dermatitis. Arch Dermatol Res *284*, 82-85.

Stingl, G., Tamaki, K., and Katz, S.I. (1980). Origin and function of epidermal Langerhans cells. Immunol Rev *53*, 149-174.

Stoler, A., Kopan, R., Duvic, M., and Fuchs, E. (1988). Use of monospecific antisera and cRNA probes to localize the major changes in keratin expression during normal and abnormal epidermal differentiation. J Cell Biol *107*, 427-446.

Stone, D.M., Murone, M., Luoh, S., Ye, W., Armanini, M.P., Gurney, A., Phillips, H., Brush, J., Goddard, A., de Sauvage, F.J., and Rosenthal, A. (1999). Characterization of the human suppressor of fused, a negative regulator of the zinc-finger transcription factor Gli. J. Cell Sci. *112 (Pt 23)*, 4437-4448.

Sun, C., Mathur, P., Dupuis, J., Tizard, R., Ticho, B., Crowell, T., Gardner, H., Bowcock, A.M., and Carulli, J. (2006). Peptidoglycan recognition proteins Pglyrp3 and Pglyrp4 are encoded from the epidermal differentiation complex and are candidate genes for the Psors4 locus on chromosome 1q21. Hum Genet *119*, 113-125.

Sun, T.T., and Green, H. (1976). Differentiation of the epidermal keratinocyte in cell culture: formation of the cornified envelope. Cell *9*, 511-521.

Sun, W., Xu, R., Hu, W., Jin, J., Crellin, H.A., Bielawski, J., Szulc, Z.M., Thiers, B.H., Obeid, L.M., and Mao, C. (2008). Upregulation of the human alkaline ceramidase 1 and acid ceramidase mediates calcium-induced differentiation of epidermal keratinocytes. J. Invest. Dermatol. *128*, 389-397.

Sun, Y., Pi, J., Wang, X., Tokar, E.J., Liu, J., and Waalkes, M.P. (2009). Aberrant cytokeratin expression during arsenic-induced acquired malignant phenotype in human HaCaT keratinocytes consistent with epidermal carcinogenesis. Toxicology *262*, 162-170.

Sutter, C.H., Yin, H., Li, Y., Mammen, J.S., Bodreddigari, S., Stevens, G., Cole, J.A., and Sutter, T.R. (2009). EGF receptor signaling blocks aryl hydrocarbon receptor-mediated transcription and cell differentiation in human epidermal keratinocytes. Proc. Natl. Acad. Sci. USA. *106*, 4266-4271.

Suzuki, C., Murakumo, Y., Kawase, Y., Sato, T., Morinaga, T., Fukuda, N., Enomoto, A., Ichihara, M., and Takahashi, M. (2008). A novel GDNF-inducible gene, BMZF3, encodes a transcriptional repressor associated with KAP-1. Biochem Biophys Res Commun *366*, 226-232.

Suzuki, Y., Hamamoto, Y., Ogasawara, Y., Ishikawa, K., Yoshikawa, Y., Sasazuki, T., and Muto, M. (2004). Genetic polymorphisms of killer cell immunoglobulin-like receptors are associated with susceptibility to psoriasis vulgaris. J Invest Dermatol *122*, 1133-1136.

Sybert, V.P., Dale, B.A., and Holbrook, K.A. (1985). Ichthyosis vulgaris: identification of a defect in synthesis of filaggrin correlated with an absence of keratohyaline granules. J Invest Dermatol *84*, 191-194.

Szegedi, A., Payer, E., Czifra, G., Toth, B.I., Schmidt, E., Kovacs, L., Blumberg, P.M., and Biro, T. (2009). Protein kinase C isoenzymes differentially regulate the differentiation-dependent expression of adhesion molecules in human epidermal keratinocytes. Exp Dermatol *18*, 122-129.

Tagami, H., and Yoshikuni, K. (1985). Interrelationship between water-barrier and reservoir functions of pathologic stratum corneum. Arch Dermatol *121*, 642-645.

Takahashi, H., Asano, K., Kinouchi, M., Ishida-Yamamoto, A., Wuepper, K.D., and Iizuka, H. (1998). Structure and transcriptional regulation of the human cystatin A gene. The 12-O-tetradecanoylphorbol-13-acetate (TPA) responsive element-2 site (-272 to -278) on cystatin A gene is critical for TPA-dependent regulation. J. Biol. Chem. *273*, 17375-17380.

Takahashi, H., Honma, M., Ishida-Yamamoto, A., Namikawa, K., Kiyama, H., and Iizuka, H. (2001a). Expression of human cystatin A by keratinocytes is positively regulated via the Ras/MEKK1/MKK7/JNK signal transduction pathway but negatively regulated via the Ras/Raf-1/MEK1/ERK pathway. J. Biol. Chem. *276*, 36632-36638.

Takahashi, H., Ibe, M., Nakamura, S., Ishida-Yamamoto, A., Hashimoto, Y., and Iizuka, H. (2002). Extracellular regulated kinase and c-Jun N-terminal kinase are activated in psoriatic involved epidermis. J Dermatol Sci *30*, 94-99.

Takahashi, K., Nuckolls, G.H., Takahashi, I., Nonaka, K., Nagata, M., Ikura, T., Slavkin, H.C., and Shum, L. (2001b). Msx2 is a repressor of chondrogenic differentiation in migratory cranial neural crest cells. Dev Dyn *222*, 252-262.

Takizawa, Y., Akiyama, M., Nagashima, M., and Shimizu, H. (2000). A novel asparagine-->aspartic acid mutation in the rod 1A domain in keratin 2e in a Japanese family with ichthyosis bullosa of Siemens. J. Invest. Dermatol. *114*, 193-195.

Tanabe, O., McPhee, D., Kobayashi, S., Shen, Y., Brandt, W., Jiang, X., Campbell, A.D., Chen, Y.T., Chang, C., Yamamoto, M., Tanimoto, K., and Engel, J.D. (2007). Embryonic and fetal beta-globin gene repression by the orphan nuclear receptors, TR2 and TR4. EMBO J *26*, 2295-2306.

Tanaka, T., Parry, D.A., Klaus-Kovtun, V., Steinert, P.M., and Stanley, J.R. (1991). Comparison of molecularly cloned bullous pemphigoid antigen to desmoplakin I confirms that they define a new family of cell adhesion junction plaque proteins. J. Biol. Chem. *266*, 12555-12559.

Tanida, S., Joh, T., Itoh, K., Kataoka, H., Sasaki, M., Ohara, H., Nakazawa, T., Nomura, T., Kinugasa, Y., Ohmoto, H., Ishiguro, H., Yoshino, K., Higashiyama, S., and Itoh, M. (2004). The mechanism of cleavage of EGFR ligands induced by inflammatory cytokines in gastric cancer cells. Gastroenterology *127*, 559-569.

Tarutani, M., Itami, S., Okabe, M., Ikawa, M., Tezuka, T., Yoshikawa, K., Kinoshita, T., and Takeda, J. (1997). Tissue-specific knockout of the mouse Pig-a gene reveals important roles for GPI-anchored proteins in skin development. Proc Natl Acad Sci U S A *94*, 7400-7405.

Tatematsu, K., Yoshimoto, N., Koyanagi, T., Tokunaga, C., Tachibana, T., Yoneda, Y., Yoshida, M., Okajima, T., Tanizawa, K., and Kuroda, S. (2005). Nuclear-cytoplasmic shuttling of a RING-IBR protein RBCK1 and its functional interaction with nuclear body proteins. J Biol Chem *280*, 22937-22944.

Tauchi, M., Hida, A., Negishi, T., Katsuoka, F., Noda, S., Mimura, J., Hosoya, T., Yanaka, A., Aburatani, H., Fujii-Kuriyama, Y., Motohashi, H., and Yamamoto, M. (2005). Constitutive expression of aryl hydrocarbon receptor in keratinocytes causes inflammatory skin lesions. Mol Cell Biol *25*, 9360-9368.

Taylor, J.M., Mitchell, W.M., and Cohen, S. (1972). Epidermal growth factor. Physical and chemical properties. J Biol Chem *247*, 5928-5934.

Taylor, J.M., Street, T.L., Hao, L., Copley, R., Taylor, M.S., Hayden, P.J., Stolper, G., Mott, R., Hein, J., Moffatt, M.F., and Cookson, W.O. (2009). Dynamic and physical clustering of gene expression during epidermal barrier formation in differentiating keratinocytes. PLoS One *4*, e7651.

Teige, I., Hvid, H., Svensson, L., Kvist, P.H., and Kemp, K. (2009). Regulatory T cells control VEGF-dependent skin inflammation. J Invest Dermatol *129*, 1437-1445.

Ternes, P., Franke, S., Zahringer, U., Sperling, P., and Heinz, E. (2002). Identification and characterization of a sphingolipid delta 4-desaturase family. J Biol Chem *277*, 25512-25518.

Thompson, P.M., Gotoh, T., Kok, M., White, P.S., and Brodeur, G.M. (2003). CHD5, a new member of the chromodomain gene family, is preferentially expressed in the nervous system. Oncogene *22*, 1002-1011.

Threadgill, D.W., Dlugosz, A.A., Hansen, L.A., Tennenbaum, T., Lichti, U., Yee, D., LaMantia, C., Mourton, T., Herrup, K., Harris, R.C., and et al. (1995). Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. Science *269*, 230-234.

Tian, Q., Kopf, G.S., Brown, R.S., and Tseng, H. (2001). Function of basonuclin in increasing transcription of the ribosomal RNA genes during mouse oogenesis. Development *128*, 407-416.

Tissot, C., and Mechti, N. (1995). Molecular cloning of a new interferon-induced factor that represses human immunodeficiency virus type 1 long terminal repeat expression. J. Biol. Chem. *270*, 14891-14898.

Toker, A., and Newton, A.C. (2000). Akt/protein kinase B is regulated by autophosphorylation at the hypothetical PDK-2 site. J Biol Chem *275*, 8271-8274.

Tomita, Y., Akiyama, M., and Shimizu, H. (2005). Stratum corneum hydration and flexibility are useful parameters to indicate clinical severity of congenital ichthyosis. Exp Dermatol *14*, 619-624.

Tommerup, N., and Vissing, H. (1995). Isolation and fine mapping of 16 novel human zinc finger-encoding cDNAs identify putative candidate genes for developmental and malignant disorders. Genomics *27*, 259-264.

Topley, G.I., Okuyama, R., Gonzales, J.G., Conti, C., and Dotto, G.P. (1999). p21(WAF1/Cip1) functions as a suppressor of malignant skin tumor formation and a determinant of keratinocyte stem-cell potential. Proc Natl Acad Sci U S A *96*, 9089-9094.

Tryggvason, K. (1993). The laminin family. Curr Opin Cell Biol *5*, 877-882.

Tseng, H., Biegel, J.A., and Brown, R.S. (1999). Basonuclin is associated with the ribosomal RNA genes on human keratinocyte mitotic chromosomes. J Cell Sci *112 Pt 18*, 3039-3047.

Tseng, H., and Green, H. (1994). Association of basonuclin with ability of keratinocytes to multiply and with absence of terminal differentiation. J. Cell Biol. *126*, 495-506.

Tsuchida, S., Bonkobara, M., McMillan, J.R., Akiyama, M., Yudate, T., Aragane, Y., Tezuka, T., Shimizu, H., Cruz, P.D., Jr., and Ariizumi, K. (2004). Characterization of Kdap, a protein secreted by keratinocytes. J Invest Dermatol *122*, 1225-1234.

Tsuji, H., Okamoto, K., Matsuzaka, Y., Iizuka, H., Tamiya, G., and Inoko, H. (2003). SLURP-2, a novel member of the human Ly-6 superfamily that is up-regulated in psoriasis vulgaris. Genomics *81*, 26-33.

Uchida, Y., Hama, H., Alderson, N.L., Douangpanya, S., Wang, Y., Crumrine, D.A., Elias, P.M., and Holleran, W.M. (2007). Fatty acid 2-hydroxylase, encoded by FA2H, accounts for differentiation-associated increase in 2-OH ceramides during keratinocyte differentiation. J. Biol. Chem. *282*, 13211-13219.

Uchida, Y., and Holleran, W.M. (2008). Omega-O-acylceramide, a lipid essential for mammalian survival. J. Dermatol Sci. *51*, 77-87.

Ulery, P.G., Rudenko, G., and Nestler, E.J. (2006). Regulation of DeltaFosB stability by phosphorylation. J Neurosci *26*, 5131-5142.

Ullrich, A., Coussens, L., Hayflick, J.S., Dull, T.J., Gray, A., Tam, A.W., Lee, J., Yarden, Y., Libermann, T.A., Schlessinger, J., and et al. (1984). Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature *309*, 418-425.

Van Dijk, M.A., Voorhoeve, P.M., and Murre, C. (1993). Pbx1 is converted into a transcriptional activator upon acquiring the N-terminal region of E2A in pre-B-cell acute lymphoblastoid leukemia. Proc. Natl. Acad. Sci. USA. *90*, 6061-6065.

Vanden Heuvel, G.B., Bodmer, R., McConnell, K.R., Nagami, G.T., and Igarashi, P. (1996). Expression of a cut-related homeobox gene in developing and polycystic mouse kidney. Kidney Int *50*, 453-461.

Varrault, A., Gueydan, C., Delalbre, A., Bellmann, A., Houssami, S., Aknin, C., Severac, D., Chotard, L., Kahli, M., Le Digarcher, A., Pavlidis, P., and Journot, L. (2006). Zac1 regulates an imprinted gene network critically involved in the control of embryonic growth. Dev Cell *11*, 711-722.

Vasireddy, V., Uchida, Y., Salem, N., Jr., Kim, S.Y., Mandal, M.N., Reddy, G.B., Bodepudi, R., Alderson, N.L., Brown, J.C., Hama, H., Dlugosz, A., Elias, P.M., Holleran, W.M., and Ayyagari, R. (2007). Loss of functional ELOVL4 depletes very long-chain fatty acids (> or =C28) and the unique omega-O-acylceramides in skin leading to neonatal death. Hum Mol Genet *16*, 471-482.

Vastrik, I., Kaipainen, A., Penttila, T.L., Lymboussakis, A., Alitalo, R., Parvinen, M., and Alitalo, K. (1995). Expression of the mad gene during cell differentiation in vivo and its inhibition of cell growth in vitro. J Cell Biol *128*, 1197-1208.

Vestergaard, C., Deleuran, M., Gesser, B., and Gronhoj Larsen, C. (2003). Expression of the T-helper 2-specific chemokine receptor CCR4 on CCR10-positive lymphocytes in atopic dermatitis skin but not in psoriasis skin. Br J Dermatol *149*, 457-463.

Vogel, P., Donoviel, M.S., Read, R., Hansen, G.M., Hazlewood, J., Anderson, S.J., Sun, W., Swaffield, J., and Oravecz, T. (2009). Incomplete inhibition of sphingosine 1-phosphate lyase modulates immune system function yet prevents early lethality and non-lymphoid lesions. PLoS One *4*, e4112.

Waikel, R.L., Wang, X.J., and Roop, D.R. (1999). Targeted expression of c-Myc in the epidermis alters normal proliferation, differentiation and UV-B induced apoptosis. Oncogene *18*, 4870-4878.

Wakimoto, K., Chiba, H., Michibata, H., Seishima, M., Kawasaki, S., Okubo, K., Mitsui, H., Torii, H., and Imai, Y. (2003). A novel diacylglycerol acyltransferase (DGAT2) is decreased in human psoriatic skin and increased in diabetic mice. Biochem Biophys Res Commun *310*, 296-302.

Walsh, D.S., Borke, J.L., Singh, B.B., Do, N.N., Hsu, S.D., Balagon, M.V., and Abalos, R.M. (2005). Psoriasis is characterized by altered epidermal expression of caspase 14, a novel regulator of keratinocyte terminal differentiation and barrier formation. J Dermatol Sci *37*, 61-63.

Wang, A., Arantes, S., Conti, C., McArthur, M., Aldaz, C.M., and MacLeod, M.C. (2007). Epidermal hyperplasia and oral carcinoma in mice overexpressing the transcription factor ATF3 in basal epithelial cells. Mol Carcinog *46*, 476-487.

Wang, F., Lee, E., Lowes, M.A., Haider, A.S., Fuentes-Duculan, J., Abello, M.V., Chamian, F., Cardinale, I., and Krueger, J.G. (2006). Prominent production of IL-20 by CD68+/CD11c+ myeloid-derived cells in psoriasis: Gene regulation and cellular effects. J Invest Dermatol *126*, 1590-1599.

Wang, S.S., Devuyst, O., Courtoy, P.J., Wang, X.T., Wang, H., Wang, Y., Thakker, R.V., Guggino, S., and Guggino, W.B. (2000). Mice lacking renal chloride channel, CLC-5, are a model for Dent's disease, a nephrolithiasis disorder associated with defective receptor-mediated endocytosis. Hum Mol Genet *9*, 2937-2945.

Wasmeier, C., Hume, A.N., Bolasco, G., and Seabra, M.C. (2008). Melanosomes at a glance. J. Cell Sci. *121*, 3995-3999.

Watt, F.M., Jordan, P.W., and O'Neill, C.H. (1988). Cell shape controls terminal differentiation of human epidermal keratinocytes. Proc. Natl. Acad. Sci. USA. *85*, 5576-5580.

Watt, F.M., Lo Celso, C., and Silva-Vargas, V. (2006). Epidermal stem cells: an update. Curr Opin Genet Dev *16*, 518-524.

Watt, F.M., Mattey, D.L., and Garrod, D.R. (1984). Calcium-induced reorganization of desmosomal components in cultured human keratinocytes. J. Cell Biol. *99*, 2211-2215.

Weemaes, C.M., Stoop, J.W., and Berrens, L. (1977). Inactivation of heamolytic complement by house dust allergen in the serum of children with atopic diseases. Clin Allergy *7*, 75-82.

Wei, H., Gan, B., Wu, X., and Guan, J.L. (2009). Inactivation of FIP200 leads to inflammatory skin disorder, but not tumorigenesis, in conditional knock-out mouse models. J Biol Chem *284*, 6004-6013.

Wei, J., Gorman, T.E., Liu, X., Ith, B., Tseng, A., Chen, Z., Simon, D.I., Layne, M.D., and Yet, S.F. (2005). Increased neointima formation in cysteine-rich protein 2-deficient mice in response to vascular injury. Circ Res *97*, 1323-1331.

Wells, J., Lee, B., Cai, A.Q., Karapetyan, A., Lee, W.J., Rugg, E., Sinha, S., Nie, Q., and Dai, X. (2009). Ovol2 suppresses cell cycling and terminal differentiation of keratinocytes by directly repressing c-Myc and Notch1. J. Biol. Chem. *284*, 29125-29135.

Welter, J.F., Gali, H., Crish, J.F., and Eckert, R.L. (1996). Regulation of human involucrin promoter activity by POU domain proteins. J. Biol. Chem. *271*, 14727-14733.

Werner, Y., and Lindberg, M. (1985). Transepidermal water loss in dry and clinically normal skin in patients with atopic dermatitis. Acta Derm Venereol *65*, 102-105.

Wheelock, M.J., and Jensen, P.J. (1992). Regulation of keratinocyte intercellular junction organization and epidermal morphogenesis by E-cadherin. J. Cell Biol. *117*, 415-425.

Whittock, N.V., and Bower, C. (2003). Genetic evidence for a novel human desmosomal cadherin, desmoglein 4. J. Invest. Dermatol. *120*, 523-530.

Wilanowski, T., Caddy, J., Ting, S.B., Hislop, N.R., Cerruti, L., Auden, A., Zhao, L.L., Asquith, S., Ellis, S., Sinclair, R., Cunningham, J.M., and Jane, S.M. (2008). Perturbed desmosomal cadherin expression in grainy head-like 1-null mice. EMBO J *27*, 886-897.

Wildey, G.M., and Howe, P.H. (2009). Runx1 is a co-activator with FOXO3 to mediate transforming growth factor beta (TGFbeta)-induced Bim transcription in hepatic cells. J. Biol. Chem. *284*, 20227-20239.

Wilke, M.S., Hsu, B.M., Wille, J.J., Jr., Pittelkow, M.R., and Scott, R.E. (1988). Biologic mechanisms for the regulation of normal human keratinocyte proliferation and differentiation. Am J Pathol *131*, 171-181.

Wille, J.J., Jr., Pittelkow, M.R., Shipley, G.D., and Scott, R.E. (1984). Integrated control of growth and differentiation of normal human prokeratinocytes cultured in serum-free medium: clonal analyses, growth kinetics, and cell cycle studies. J Cell Physiol *121*, 31-44.

Wolf, S.S., Patchev, V.K., and Obendorf, M. (2007). A novel variant of the putative demethylase gene, s-JMJD1C, is a coactivator of the AR. Arch Biochem Biophys *460*, 56-66.

Wood, S.H., Clements, D.N., Ollier, W.E., Nuttall, T., McEwan, N.A., and Carter, S.D. (2009). Gene expression in canine atopic dermatitis and correlation with clinical severity scores. J Dermatol Sci *55*, 27-33.

Wu, Z., Hansmann, B., Meyer-Hoffert, U., Glaser, R., and Schroder, J.M. (2009a). Molecular identification and expression analysis of filaggrin-2, a member of the S100 fused-type protein family. PLoS One *4*, e5227.

Wu, Z., Irizarry, R., Gentleman, R., Murilla, F., and Spencer, F. (2004). Model-Based Background Adjustment for Oligonucleotide Expression Arrays. J. Am. Stat. Assoc. *99*, 909-917.

Wu, Z., Meyer-Hoffert, U., Reithmayer, K., Paus, R., Hansmann, B., He, Y., Bartels, J., Glaser, R., Harder, J., and Schroder, J.M. (2009b). Highly complex peptide aggregates of the S100 fused-type protein hornerin are present in human skin. J Invest Dermatol *129*, 1446-1458.

Xia, X., Qian, S., Soriano, S., Wu, Y., Fletcher, A.M., Wang, X.J., Koo, E.H., Wu, X., and Zheng, H. (2001). Loss of presenilin 1 is associated with enhanced beta-catenin signaling and skin tumorigenesis. Proc. Natl. Acad. Sci. USA. *98*, 10863-10868.

Xia, Y.P., Li, B., Hylton, D., Detmar, M., Yancopoulos, G.D., and Rudge, J.S. (2003). Transgenic delivery of VEGF to mouse skin leads to an inflammatory condition resembling human psoriasis. Blood *102*, 161-168.

Xiao, W., Jiang, F., and Wang, Z. (2006). ELL binding regulates U19/Eaf2 intracellular localization, stability, and transactivation. Prostate *66*, 1-12.

Xie, Z., Singleton, P.A., Bourguignon, L.Y., and Bikle, D.D. (2005). Calcium-induced human keratinocyte differentiation requires src- and fyn-mediated phosphatidylinositol 3-kinase-dependent activation of phospholipase C-gamma1. Mol Biol Cell *16*, 3236-3246.

Yamada, K., Kawata, H., Shou, Z., Hirano, S., Mizutani, T., Yazawa, T., Sekiguchi, T., Yoshino, M., Kajitani, T., and Miyamoto, K. (2003). Analysis of zinc-fingers and homeoboxes (ZHX)-1-interacting proteins: molecular cloning and characterization of a member of the ZHX family, ZHX3. Biochem J *373*, 167-178.

Yamagami, S., Ebihara, N., and Amano, S.Y. (2005). Chemokine receptor gene expression in giant papillae of atopic keratoconjunctivitis. Mol Vis *11*, 192-200.

Yamane, Y., Moriyama, K., Yasuda, C., Miyata, S., Aihara, M., Ikezawa, Z., and Miyazaki, K. (2009). New horny layer marker proteins for evaluating skin condition in atopic dermatitis. Int Arch Allergy Immunol *150*, 89-101.

Yanagi, T., Akiyama, M., Nishihara, H., Ishikawa, J., Sakai, K., Miyamura, Y., Naoe, A., Kitahara, T., Tanaka, S., and Shimizu, H. (2010). Self-improvement of keratinocyte differentiation defects during skin maturation in ABCA12-deficient harlequin ichthyosis model mice. Am J Pathol *177*, 106-118.

Yang, C.F., Hwu, W.L., Yang, L.C., Chung, W.H., Chien, Y.H., Hung, C.F., Chen, H.C., Tsai, P.J., Fann, C.S., Liao, F., and Chen, Y.T. (2008). A promoter sequence variant of ZNF750 is linked with familial psoriasis. J. Invest. Dermatol. *128*, 1662-1668.

Yano, S., Komine, M., Fujimoto, M., Okochi, H., and Tamaki, K. (2004). Mechanical stretching in vitro regulates signal transduction pathways and cellular proliferation in human epidermal keratinocytes. J Invest Dermatol *122*, 783-790.

Yao, Q., Xu, H., Zhang, Q.Q., Zhou, H., and Qu, L.H. (2009). MicroRNA-21 promotes cell proliferation and down-regulates the expression of programmed cell death 4 (PDCD4) in HeLa cervical carcinoma cells. Biochem Biophys Res Commun *388*, 539-542.

Yoon, S.W., Kim, T.Y., Sung, M.H., Kim, C.J., and Poo, H. (2005). Comparative proteomic analysis of peripheral blood eosinophils from healthy donors and atopic dermatitis patients with eosinophilia. Proteomics *5*, 1987-1995.

Yoshida, T., Fukuda, T., Okabe, S., Hatano, M., Miki, T., Hirosawa, S., Miyasaka, N., Isono, K., and Tokuhisa, T. (1996). The BCL6 gene is predominantly expressed in keratinocytes at their terminal differentiation stage. Biochem Biophys Res Commun *228*, 216-220.

Yoshida, Y., Morita, K., Mizoguchi, A., Ide, C., and Miyachi, Y. (2001). Altered expression of occludin and tight junction formation in psoriasis. Arch Dermatol Res *293*, 239-244.

Yoshikawa, N., Shimizu, N., Sano, M., Ohnuma, K., Iwata, S., Hosono, O., Fukuda, K., Morimoto, C., and Tanaka, H. (2008). Role of the hinge region of glucocorticoid receptor for HEXIM1-mediated transcriptional repression. Biochem Biophys Res Commun *371*, 44-49.

Young, C., Allen, M.H., Cuthbert, A., Ameen, M., Veal, C., Leman, J., Burden, A.D., Kirby, B., Griffiths, C.E., Trembath, R.C., Mathew, C.G., and Barker, J.N. (2003a). A Crohn's disease-associated insertion polymorphism (3020insC) in the NOD2 gene is not associated with psoriasis vulgaris, palmo-plantar pustular psoriasis or guttate psoriasis. Exp Dermatol *12*, 506-509.

Young, P., Boussadia, O., Halfter, H., Grose, R., Berger, P., Leone, D.P., Robenek, H., Charnay, P., Kemler, R., and Suter, U. (2003b). E-cadherin controls adherens junctions in the epidermis and the renewal of hair follicles. EMBO J *22*, 5723-5733.

Yu, X., Sharma, K.D., Takahashi, T., Iwamoto, R., and Mekada, E. (2002). Ligand-independent dimer formation of epidermal growth factor receptor (EGFR) is a step separable from ligand-induced EGFR signaling. Mol Biol Cell *13*, 2547-2557.

Yuan, Y., and Xu, J. (2007). Loss-of-function deletion of the steroid receptor coactivator-1 gene in mice reduces estrogen effect on the vascular injury response. Arterioscler Thromb Vasc Biol *27*, 1521-1527.

Yuki, T., Haratake, A., Koishikawa, H., Morita, K., Miyachi, Y., and Inoue, S. (2007). Tight junction proteins in keratinocytes: localization and contribution to barrier function. Exp Dermatol *16*, 324-330.

Yuspa, S.H., Kilkenny, A.E., Steinert, P.M., and Roop, D.R. (1989). Expression of murine epidermal differentiation markers is tightly regulated by restricted extracellular calcium concentrations in vitro. J. Cell Biol. *109*, 1207-1217.

Zamansky, G.B., Nguyen, U., and Chou, I.N. (1991). An immunofluorescence study of the calcium-induced coordinated reorganization of microfilaments, keratin intermediate filaments, and microtubules in cultured human epidermal keratinocytes. J Invest Dermatol *97*, 985-994.

Zeeuwen, P.L., van Vlijmen-Willems, I.M., Hendriks, W., Merkx, G.F., and Schalkwijk, J. (2002). A null mutation in the cystatin M/E gene of ichq mice causes juvenile lethality and defects in epidermal cornification. Hum Mol Genet *11*, 2867-2875.

Zhang, D., Yoon, H.G., and Wong, J. (2005a). JMJD2A is a novel N-CoR-interacting protein and is involved in repression of the human transcription factor achaete scute-like homologue 2 (ASCL2/Hash2). Mol Cell Biol *25*, 6404-6414.

Zhang, J., Zhi, H.Y., Ding, F., Luo, A.P., and Liu, Z.H. (2005b). Transglutaminase 3 expression in C57BL/6J mouse embryo epidermis and the correlation with its differentiation. Cell Res *15*, 105-110.

Zhang, J.Y., Green, C.L., Tao, S., and Khavari, P.A. (2004). NF-kappaB RelA opposes epidermal proliferation driven by TNFR1 and JNK. Genes Dev *18*, 17-22.

Zhang, X.J. (2009). [Enlightenment from genome-wide association study to genetics of psoriasis]. Zhejiang Da Xue Xue Bao Yi Xue Ban *38*, 333-337.

Zhang, Y., and Chellappan, S.P. (1995). Cloning and characterization of human DP2, a novel dimerization partner of E2F. Oncogene *10*, 2085-2093.

Zheng, Y., Peng, Z., Wang, Y., Tan, S., Xi, Y., and Wang, G. (2003). Alteration and significance of heparin-binding epidermal-growth-factor-like growth factor in psoriatic epidermis. Dermatology *207*, 22-27.

Zhou, B., Zhong, Q., Minoo, P., Li, C., Ann, D.K., Frenkel, B., Morrisey, E.E., Crandall, E.D., and Borok, Z. (2008). Foxp2 inhibits Nkx2.1-mediated transcription of SP-C via interactions with the Nkx2.1 homeodomain. Am J Respir Cell Mol Biol *38*, 750-758.

Zhou, X., Liao, J., Meyerdierks, A., Feng, L., Naumovski, L., Bottger, E.C., and Omary, M.B. (2000). Interferon-alpha induces nmi-IFP35 heterodimeric complex formation that is affected by the phosphorylation of IFP35. J. Biol. Chem. *275*, 21364-21371.

Zhu, A.J., and Watt, F.M. (1999). beta-catenin signalling modulates proliferative potential of human epidermal keratinocytes independently of intercellular adhesion. Development *126*, 2285-2298.

Zhu, S., Oh, H.S., Shim, M., Sterneck, E., Johnson, P.F., and Smart, R.C. (1999). C/EBPbeta modulates the early events of keratinocyte differentiation involving growth arrest and keratin 1 and keratin 10 expression. Mol Cell Biol *19*, 7181-7190.

Zuo, Y., Zhuang, D.Z., Han, R., Isaac, G., Tobin, J.J., McKee, M., Welti, R., Brissette, J.L., Fitzgerald, M.L., and Freeman, M.W. (2008). ABCA12 maintains the epidermal lipid permeability barrier by facilitating formation of ceramide linoleic esters. J. Biol. Chem. *283*, 36624-36635.

Zwang, Y., and Yarden, Y. (2006). p38 MAP kinase mediates stress-induced internalization of EGFR: implications for cancer chemotherapy. EMBO J *25*, 4195-4206.

APPENDICES

Appendix A - Literature Support of Genes Involved in Cornified Envelope Formation (Refer to Chapter 3, Figure 16)

Transcript cluster ID	Gene Symbol	Gene Description	Density FC[1]	EGF FC[2]	Significant Index for EGF effect[3]	Function in skin	References
7931859	CALML5	calmodulin-like 5	10.11	-3.04	1	Enzyme	(Mehul et al., 2001)
8026398	CASP14	caspase 14	9.19	-1.99	1	Enzyme	(Lippens et al., 2000)
8179716	CDSN	corneodesmosin	31.89	1.34	1	CE	(Simon et al., 1997)
7964834	CPM	carboxypeptidase M	11.77	-1.93	1	CE	(Taylor et al., 2009)
7920178	CRNN	cornulin	4.03	-2.17	1	CE	(Contzler et al., 2005)
7945663	CTSD	cathepsin D	2.70	-1.44	1	Enzyme	(Egberts et al., 2004)
8162652	CTSL2	cathepsin L2	3.03	1.71	1	Enzyme	(Cheng et al., 2006)
8026424	CYP4F22	cytochrome P450, family 4, subfamily F, polypeptide 22	18.64	-2.35	1	Enzyme?	(Lefevre et al., 2006)
8036079	DMKN	dermokine	8.63	-1.34	1	CE	(Bazzi et al., 2007)
8022728	DSC1	desmocollin 1	16.56	-2.81	1	Cell adhesion	(King et al., 1996; Taylor et al., 2009)
8020724	DSG1	desmoglein 1	11.67	-1.60	1	Cell adhesion	(Getsios et al., 2009)
8020762	DSG3	desmoglein 3 (pemphigus vulgaris antigen)	1.62	1.19	1	Cell adhesion	(Koch et al., 1997)
8127767	ELOVL4	elongation of very long chain fatty acids-like 4	11.12	-1.92	1	Fatty acid elongase	(Cameron et al., 2007)
7920165	FLG	filaggrin	55.43	-1.54	1	CE, EDC	(Dale et al., 1985; Kalinin et al., 2002)
7920175	FLG2	filaggrin family member 2	65.91	-1.82	1	CE	(Wu et al., 2009a)
7965606	HAL	histidine ammonia-lyase	44.86	-3.76	1	Enzyme	(Eckhart et al., 2008)
8103769	HPGD	hydroxyprostaglandin dehydrogenase 15-(NAD)	25.24	-1.77	1	CE	(Taylor et al., 2009)

[1]Density FC: fold change between the 50% and 100% confluent cell density untreated samples.
[2]EGF FC: fold change between control and EGF at 100% confluent cell density.
[3]Indicates whether the effect of EGF on the gene is statistically significant; 1: significant, 0: non-significant
CE: cornified envelope; EDC: epidermal differentiation complex on human chromosome 1q21

Appendix A - Literature Support of Genes Involved in Cornified Envelope Formation (Refer to Chapter 3, Figure 16) (cont.)

Transcript cluster ID	Gene Symbol	Gene Description	Density FC[1]	EGF FC[2]	Significant Index for EGF effect[3]	Function in skin	References
7920155	HRNR	hornerin	48.67	-2.20	1	CE	(Henry et al.; Makino et al., 2001)
8038670	KLK5	kallikrein-related peptidase 5	2.33	-1.14	1	Tryptic enzyme, desquamation	(Borgono et al., 2007)
7905515	KPRP	keratinocyte proline-rich protein	12.23	2.31	1	marker of keratinocyte late differentiation	(Kong et al., 2003)
7963491	KRT1	keratin 1	42.74	-3.35	1	Structural proteins	(Stoler et al., 1988)
8015104	KRT10	keratin 10	22.67	-2.50	1	Structural proteins	(Ming et al., 1994)
7963479	KRT2	keratin 2	3.75	-1.95	1	Structural proteins	(Collin et al., 1992)
7963534	KRT4	keratin 4	12.95	-6.29	1	Structural proteins	(Bazzi et al., 2007)
7963502	KRT77	keratin 77	11.77	-3.04	1	Structural proteins	(Bazzi et al., 2007)
8036072	KRTDAP	keratinocyte differentiation-associated protein	21.19	-2.07	1	Regulator of keratinocyte differentiation	(Tsuchida et al., 2004)
7905525	LCE1B	late cornified envelope 1B	4.48	3.22	1	CE, EDC	(Jackson et al., 2005)
7920193	LCE1C	late cornified envelope 1C	1.54	1.25	1	CE, EDC	(Jackson et al., 2005)
7920191	LCE3A	late cornified envelope 3A	2.01	3.00	1	CE, EDC	(Jackson et al., 2005)
7920185	LCE3D	late cornified envelope 3D	52.44	1.81	1	CE, EDC	(Jackson et al., 2005)
7920182	LCE3E	late cornified envelope 3E	9.82	2.57	1	CE, EDC	(Jackson et al., 2005)
7905563	LOR	loricrin	44.32	-19.49	1	CE, EDC	(Kalinin et al., 2002; Mehrel et al., 1990)
7898413	PADI1	peptidyl arginine deiminase, type I	2.97	1.70	1	Deiminase filaggrin and KRT1	(Dong et al., 2008)
8001007	PRSS8	protease, serine, 8	2.23	1.34	1	serine protease	(Leyvraz et al., 2005)
7920146	RPTN	repetin	35.51	-2.27	1	CE, EDC	(Krieg et al., 1997) (Kalinin et al., 2002)

[1]Density FC: fold change between the 50% and 100% confluent cell density untreated samples.
[2]EGF FC: fold change between control and EGF at 100% confluent cell density.
[3]Indicates whether the effect of EGF on the gene is statistically significant; 1: significant, 0: non-significant
CE: cornified envelope; EDC: epidermal differentiation complex on human chromosome 1q21

Appendix A - Literature Support of Genes Involved in Cornified Envelope Formation (Refer to Chapter 3, Figure 16) (cont.)

Transcript cluster ID	Gene Symbol	Gene Description	Density FC[1]	EGF FC[2]	Significant Index for EGF effect[3]	Function in skin	References
7920238	S100A12	S100 calcium binding protein A12	16.11	-2.64	1	CE, EDC	(Kalinin et al., 2002)
7920252	S100A7	S100 calcium binding protein A7	27.47	1.98	1	CE, EDC	(Eckert and Lee, 2006)
7920244	S100A8	S100 calcium binding protein A8	4.68	-1.31	1	CE, EDC	(Taylor et al., 2009)
8109001	SPINK5	serine peptidase inhibitor, Kazal type 5	21.52	-1.38	1	Serine Protease Inhibitor	(Descargues et al., 2005)
7920205	SPRR2A	small proline-rich protein 2A	1.52	1.80	1	CE, EDC	(Gibbs et al., 1993)
7920196	SPRR2D	small proline-rich protein 2D	9.40	1.35	1	CE, EDC	(Katou et al., 2003)
7905548	SPRR3	small proline-rich protein 3	36.63	-2.34	1	CE, EDC	(Hohl et al., 1995)
7978222	TGM1	transglutaminase 1	5.36	-1.38	1	Enzyme	(Kalinin et al., 2002; Matsuki et al., 1998)
8060432	TGM3	transglutaminase 3	3.13	-1.86	1	Enzyme	(Kalinin et al., 2002; Zhang et al., 2005b)
7988050	TGM5	transglutaminase 5	5.01	-1.85	1	Enzyme	(Candi et al., 2002)
7953775	A2ML1	alpha-2-macroglobulin-like 1	4.23	1.09	0	protease inhibitor	(Galliano et al., 2006)
8052828	ASPRV1	aspartic peptidase, retroviral-like 1	11.24	-1.01	0	Protease, desquamation ?	(Bernard et al., 2005)
8037179	CNFN	cornifelin	14.77	1.05	0	CE	(Michibata et al., 2004)
7905486	CRCT1	cysteine-rich C-terminal 1	22.59	1.13	0	EDC	(Marenholz et al., 2001)
7941505	CST6	cystatin E/M	12.42	1.14	0	CE	(Zeeuwen et al., 2002)
8082058	CSTA	cystatin A (stefin A)	3.90	-1.12	0	protease inhibitor	(Kalinin et al., 2002; Takahashi et al., 1998; Takahashi et al., 2001a)

[1]Density FC: fold change between the 50% and 100% confluent cell density untreated samples.
[2]EGF FC: fold change between control and EGF at 100% confluent cell density.
[3]Indicates whether the effect of EGF on the gene is statistically significant; 1: significant, 0: non-significant
CE: cornified envelope; EDC: epidermal differentiation complex on human chromosome 1q21

Appendix A - Literature Support of Genes Involved in Cornified Envelope Formation (Refer to Chapter 3, Figure 16) (cont.)

Transcript cluster ID	Gene Symbol	Gene Description	Density FC[1]	EGF FC[2]	Significant Index for EGF effect[3]	Function in skin	References
8020740	DSG4	desmoglein 4	1.50	1.20	0	Cell adhesion	(Bazzi et al., 2006; Whittock and Bower, 2003)
8116780	DSP	desmoplakin	1.57	-1.06	0	Cell adhesion	(Kalinin et al., 2002)
8018579	EVPL	envoplakin	1.50	1.08	0	Cell adhesion	(DiColandrea et al., 2000; Kalinin et al., 2002)
7905533	IVL	involucrin	7.79	-1.15	0	CE, EDC	(Kalinin et al., 2002; Robinson et al., 1997; Taylor et al., 2009)
8038770	KLK14	kallikrein-related peptidase 14	1.50	1.08	0	serine-type endopeptidase, desquamation	(Stefansson et al., 2006)
8038695	KLK7	kallikrein-related peptidase 7	11.31	-1.07	0	Chymotryptic enzyme, desquamation	(Nin et al., 2009; Taylor et al., 2009)
8015133	KRT23	keratin 23 (histone deacetylase inducible)	54.47	-1.13	0	Structural proteins	(Taylor et al., 2009)
7905528	LCE1A	late cornified envelope 1A	2.13	1.17	0	CE, EDC	(Jackson et al., 2005)
7905523	LCE1D	late cornified envelope 1D	1.76	-1.11	0	CE, EDC	(Jackson et al., 2005)
7905507	LCE2A	late cornified envelope 2A	2.77	1.03	0	CE, EDC	(Jackson et al., 2005)
7905505	LCE2B	late cornified envelope 2B	1.56	-1.03	0	CE, EDC	(Kalinin et al., 2002)
7905503	LCE2C	late cornified envelope 2C	4.74	1.39	0	CE, EDC	(Jackson et al., 2005)
7905500	LCE2D	late cornified envelope 2D	3.23	1.38	0	CE, EDC	(Jackson et al., 2005)
7908672	PKP1	plakophilin 1	1.89	-1.04	0	Cell adhesion	(Smith and Fuchs, 1998)
7999253	PPL	periplakin	3.51	1.10	0	Cell adhesion	(Kalinin et al., 2002)
7905571	S100A9	S100 calcium binding protein A9	4.19	1.29	0	CE, EDC	(Kalinin et al., 2002; Taylor et al., 2009)

[1]Density FC: fold change between the 50% and 100% confluent cell density untreated samples.
[2]EGF FC: fold change between control and EGF at 100% confluent cell density.
[3]Indicates whether the effect of EGF on the gene is statistically significant; 1: significant, 0: non-significant
CE: cornified envelope; EDC: epidermal differentiation complex on human chromosome 1q21

Appendix A - Literature Support of Genes Involved in Cornified Envelope Formation (Refer to Chapter 3, Figure 16) (cont.)

Transcript cluster ID	Gene Symbol	Gene Description	Density FC[1]	EGF FC[2]	Significant Index for EGF effect[3]	Function in skin	References
8093950	S100P	S100 calcium binding protein P	6.69	-1.17	0	CE, EDC	(Taylor et al., 2009)
8036103	SBSN	suprabasin	25.63	-1.07	0	CE, EDC	(Park et al., 2002)
7969493	SCEL	sciellin	11.33	-1.08	0	CE, EDC	(Baden et al., 2005)
7905544	SPRR1A	small proline-rich protein 1A	3.94	-1.16	0	CE, EDC	(Robinson et al., 1997)
7905553	SPRR1B	small proline-rich protein 1B (cornifin)	1.88	-1.03	0	CE, EDC	(Robinson et al., 1997)
7920201	SPRR2B	small proline-rich protein 2B	46.13	-1.08	0	CE, EDC	(Taylor et al., 2009)
7920214	SPRR2E	small proline-rich protein 2E	31.50	-1.19	0	CE, EDC	(Cabral et al., 2001; Katou et al., 2003)
7920217	SPRR2G	small proline-rich protein 2G	18.90	-1.01	0	CE, EDC	(Cabral et al., 2001; Katou et al., 2003)
7905536	SPRR4	small proline-rich protein 4	8.31	1.29	0	CE, EDC	(Cabral et al., 2001)

[1]Density FC: fold change between the 50% and 100% confluent cell density untreated samples.
[2]EGF FC: fold change between control and EGF at 100% confluent cell density.
[3]Indicates whether the effect of EGF on the gene is statistically significant; 1: significant, 0: non-significant
CE: cornified envelope; EDC: epidermal differentiation complex on human chromosome 1q21

Appendix B - List of 114 Genes Related to Skin Diseases Based on Literature (Refer to Chapter 3, Figure 19)

Transcript Cluster ID	Gene Symbol	Gene Description	Density FC[1]	EGF FC[2]	Skin Diseases	Level expressed in skin disease	References
8058708	**ABCA12**	**ATP-binding cassette, sub-family A (ABC1), member 12**	**8.07**	**-1.25**	**ichthyosis**	**Knockout mice die shortly after birth as water rapidly evaporates from their skin; glucosylceramides transport (Jiang et al, 2009), Harlequin ichthyosis (Yanagi et al, 2010)**	**(Akiyama et al., 2005; Zuo et al., 2008)**
8079153	ABHD5	abhydrolase domain containing 5	4.76	-1.97	Chanarin Dorfman syndrome	Mutation	(Schleinitz et al., 2005)
7932616	ABI1	abl-interactor 1	1.94	-1.35	psoriasis		(Sticherling et al., 1992)
7938390	ADM	adrenomedullin	2.38	-1.79	atopic dermatitis	Decreased	(Kindt et al., 2007)
8131614	AHR	aryl hydrocarbon receptor	2.26	-2.17	skin carcinogenesis	Consitutive activation	(Ikuta et al., 2009; Tauchi et al., 2005)
8012309	**ALOX12B**	**arachidonate 12-lipoxygenase, 12R type**	**19.23**	**-1.34**	**ichthyosis**	**Knockout mice died within 3-5 hours after birth**	**(Epp et al., 2007)**
7983910	AQP9	aquaporin 9	26.04	-1.79	psoriasis	Elevated	(Mesko et al.)
8122058	ARG1	arginase, liver	16.45	-8.41	psoriasis	Elevated	(Bruch-Gerharz et al., 2003)
8149534	**ASAH1**	**N-acylsphingosine amidohydrolase (acid ceramidase) 1**	**2.18**	**-1.71**	**atopic dermatitis**	**Knockout mice is lethal (embryonic lethal); Decreased**	**(Arikawa et al., 2002; Eliyahu et al., 2007)**
7947230	BDNF	brain-derived neurotrophic factor	1.78	-1.52	Decreased in atopic dermatitis	knockout mice died	(Bartoletti et al., 2002; Groneberg et al., 2007)
8014008	BLMH	bleomycin hydrolase	2.07	-1.66	dermatitis	Decrease	(Schwartz et al., 1999)
8116818	BMP6	bone morphogenetic protein 6	1.88	-1.43	psoriasis	Overexpressed	(Blessing et al., 1996)
8075600	BPIL2	bactericidal/permeability-increasing protein-like 2	28.94	-1.50	psoriasis	Elevated	(Mulero et al., 2002)
7931859	CALML5	calmodulin-like 5	10.11	-3.04	psoriasis	Elevated	(Mehul et al., 2006)
8026398	CASP14	caspase 14, apoptosis-related cysteine peptidase	9.19	-1.99	Psoriasis	Decrease	(Walsh et al., 2005)
8079117	CCBP2	chemokine binding protein 2	1.93	-1.87	atopic dermatitis	Elevated	(Vestergaard et al., 2003)

[1]Density FC: fold change between the 50% and 100% confluent cell density untreated samples.
[2]EGF FC: fold change between control and EGF at 100% confluent cell density.
Bolded entries are genes essential for the development of epidermal barrier in mice.

Appendix B - List of 114 Genes Related to Skin Diseases Based on Literature (Refer to Chapter 3, Figure 19) (cont.)

Transcript Cluster ID	Gene Symbol	Gene Description	Density FC[1]	EGF FC[2]	Skin Diseases	Level expressed in skin disease	References
8097461	CCRN4L	CCR4 carbon catabolite repression 4-like (S. cerevisiae)	3.65	-2.44	atopic dermatitis	Elevated	(Yamagami et al., 2005)
8133876	CD36	CD36 molecule (thrombospondin receptor)	17.85	-8.66	atopic dermatitis	Dysregulated	(Niebuhr et al., 2009)
8179716	**CDSN**	**corneodesmosin**	**31.89**	**1.34**	**psoriasis**	**Conditional knockout mice results in neonatal death due to epidermal tearing upon mechanical stress**	**(Allen et al., 2001; Leclerc et al., 2009)**
8063386	CEBPB	CCAAT/enhancer binding protein (C/EBP), beta	2.43	-1.75	mild epidermal hyperplasia; resistant to carcinogen-induced skin tumorigenesis	Knockout mice developed a mild epidermal hyperplasia	(Sterneck et al., 2006; Zhu et al., 1999)
8179351	CFB	complement factor B	1.57	-1.38	atopic dermatitis	Decreased	(Weemaes et al., 1977)
8167603	CLCN5	chloride channel 5	1.93	-1.28	Dent's disease	Mutation	(Wang et al., 2000)
7921099	CRABP2	cellular retinoic acid binding protein 2	2.24	-1.69	psoriasis	Elevated	(Siegenthaler et al., 1992)
7945663	CTSD	cathepsin D	2.70	-1.44	psoriasis	Decreased	(Chen et al., 2000)
8162652	CTSL2	cathepsin L2	3.03	1.71	atopic dermatitis	Decreased	(Cheng et al., 2009)
7995552	CYLD	cylindromatosis (turban tumor syndrome)	1.81	-1.65	psoriasis	SNPs	(Oudot et al., 2009)
7929466	CYP2C18	cytochrome P450, family 2, subfamily C, polypeptide 18	8.15	-5.00	psoriasis	Decreased	(Helsby et al., 1998)
8026424	CYP4F22	cytochrome P450, family 4, subfamily F, polypeptide 22	18.64	-2.35	lamellar ichthyosis type 3	Mutations	(Lefevre et al., 2006)
7942613	DGAT2	diacylglycerol O-acyltransferase homolog 2 (mouse)	16.71	-2.30	psoriasis	Decreased; knockout mice are lipopenic and die shortly after birth	(Liu et al., 2008; Wakimoto et al., 2003)
8046124	DHRS9	dehydrogenase/reductase (SDR family) member 9	14.50	-2.05	Epstein-Barr virus lytic infection		(Jones et al., 2007)
8016609	DLX3	distal-less homeobox 3	2.10	-1.36	atopic dermatitis, psoriasis	Knockout mice died midgestation	(Hwang et al.; Mayer et al., 2010; Morasso et al., 1999)
8022728	DSC1	desmocollin 1	16.56	-2.81	dermatitis	Deficient mice developed dermatitis	(Chidgey et al., 2001)

[1]Density FC: fold change between the 50% and 100% confluent cell density untreated samples.
[2]EGF FC: fold change between control and EGF at 100% confluent cell density.
Bolded entries are genes essential for the development of epidermal barrier in mice.

Appendix B - List of 114 Genes Related to Skin Diseases Based on Literature (Refer to Chapter 3, Figure 19) (cont.)

Transcript Cluster ID	Gene Symbol	Gene Description	Density FC[1]	EGF FC[2]	Skin Diseases	Level expressed in skin disease	References
8020724	DSG1	desmoglein 1	11.67	-1.60	Netherton syndrome	Decrease	(Descargues et al., 2006)
8020762	DSG3	desmoglein 3 (pemphigus vulgaris antigen)	1.62	1.19	pemphigus vulgaris; overexpressed in head and neck cancer		(Capon et al., 2009; Chen et al., 2007)
7905220	ECM1	extracellular matrix protein 1	2.89	1.43	lipid proteinosis		(Mirancea et al., 2007)
8127767	**ELOVL4**	**elongation of very long chain fatty acids like 4**	**11.12**	**-1.92**	**Stargardt's disease**	**Knockout mice lead to neonatal death**	**(Li et al., 2007b; Vasireddy et al., 2007)**
8095728	EREG	epiregulin	1.78	-1.51	dermatitis	Deficient mice developed chronic dermatitis	(Shirasawa et al., 2004)
8147049	FABP5	fatty acid binding protein 5 (psoriasis-associated)	6.04	-1.88	atopic dermatitis	Elevated	(Yamane et al., 2009)
7920165	FLG	filaggrin	55.43	-1.54	atopic dermatitis	Mutation	(Scharschmidt et al., 2009)
8128956	FYN	FYN oncogene related to SRC, FGR, YES	1.59	-1.37	epidermal hyperplasia	Increase	(Li et al., 2007a)
7926105	**GATA3**	**GATA binding protein 3**	**2.05**	**-1.62**	**atopic dermatitis**	**Knockout mice died at E11; lipid defect; increase in atopic dermatitis**	**(Arakawa et al., 2004; de Guzman Strong et al., 2006; Pandolfi et al., 1995)**
8121749	GJA1	gap junction protein, alpha 1, 43kDa	2.02	-1.55	knockout mice die after birth or survive up to 5 hours after birth; oculodentodigital dysplasia	Mutation	(Gong et al., 2006; Paznekas et al., 2003)
7970448	GJB6	gap junction protein, beta 6, 30kDa	6.25	-2.22	hidrotic ectodermal dysplasia	Mutation	(Essenfelder et al., 2004)
8006940	GRB7	growth factor receptor-bound protein 7	2.73	1.49	atopic dermatitis	Decreased	(Yoon et al., 2005)
7905733	HAX1	HCLS1 associated protein X-1	1.64	-1.62	psoriasis	Elevated	(Mirmohammadsadegh et al., 2003)
8114572	HBEGF	heparin-binding EGF-like growth factor	2.86	-3.12	psoriasis	Elevated	(Zheng et al., 2003)
8072678	HMOX1	heme oxygenase (decycling) 1	3.94	1.93	atopic dermatitis	Elevated	(Kirino et al., 2008)
7920155	HRNR	hornerin	48.67	-2.20	atopic dermatitis	Elevated	(Wu et al., 2009b)
8139488	IGFBP3	insulin-like growth factor binding protein 3	7.15	-6.11	psoriasis	Elevated	(Ozden et al., 2008)

[1]Density FC: fold change between the 50% and 100% confluent cell density untreated samples.
[2]EGF FC: fold change between control and EGF at 100% confluent cell density.
Bolded entries are genes essential for the development of epidermal barrier in mice.

Appendix B - List of 114 Genes Related to Skin Diseases Based on Literature (Refer to Chapter 3, Figure 19) (cont.)

Transcript Cluster ID	Gene Symbol	Gene Description	Density FC[1]	EGF FC[2]	Skin Diseases	Level expressed in skin disease	References
8044574	IL1RN	interleukin 1 receptor antagonist	2.77	1.27	dermatitis, psoriasis	SNPs	(Oudot et al., 2009)
7909261	IL20	interleukin 20	1.70	-1.54	psoriasis	Elevated	(Wang et al., 2006)
8129837	IL20RA	interleukin 20 receptor, alpha	6.61	-2.79	psoriasis	Haplotype	(Kingo et al., 2008)
8048227	IL8RB	interleukin 8 receptor, beta	7.82	-1.75	psoriasis	Elevated	(Nomura et al., 2003)
8050719	ITSN2	intersectin 2	2.42	-1.22	atopic dermatitis	Elevated	(Hashida et al., 2003)
8039884	KIR2DL5A	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 5A	1.57	-1.29	psoriasis	Elevated	(Suzuki et al., 2004)
8163002	**KLF4**	**Kruppel-like factor 4 (gut)**	**6.29**	**-1.37**	**Skin squamous cell carcinoma, hyperplasia, dysplasia**	**Knockout mice die postnatally due to dehydration**	**(Ehlermann et al., 2003; Huang et al., 2005; Segre et al., 1999)**
8038633	KLK1	kallikrein 1	2.32	-1.66	atopic dermatitis	Elevated	(Komatsu et al., 2007)
8038747	KLK12	kallikrein-related peptidase 12	11.22	-1.41	atopic dermatitis	Elevated	(Komatsu et al., 2007)
8038670	KLK5	kallikrein-related peptidase 5	2.33	-1.14	atopic dermatitis	Elevated	(Komatsu et al., 2007)
8038683	KLK6	kallikrein-related peptidase 6	18.60	1.41	atopic dermatitis	Elevated	(Komatsu et al., 2007)
8038707	KLK8	kallikrein-related peptidase 8	5.21	-1.17	atopic dermatitis	Elevated	(Komatsu et al., 2007)
7963491	KRT1	keratin 1	42.74	-3.35	hyperkeratosis ; ichthyosis	Mutations	(Bolling et al.; Lacz et al., 2005; Muller et al., 2006)
8015104	KRT10	keratin 10	22.67	-2.50	epidermolytic ichtyosis; epidermolytic hyperkeratosis ; acanthosis	Mutations	(Covaciu et al., 2010; Muller et al., 2006; Porter et al., 1998)
8015323	KRT13	keratin 13	6.48	-3.18	White Sponge Nevus (acanthosis);	Mutations	(Rugg et al., 1999; Sun et al., 2009)
8015376	KRT16	keratin 16	2.91	-1.17	benign hyperplasia; pachyonychia congenita		(Gruber et al., 2009; Haider et al., 2006)
7963479	KRT2	keratin 2	3.75	-1.95	ichthyosis bullosa of Siemens	Mutations	(Takizawa et al., 2000)
7963534	KRT4	keratin 4	12.95	-6.29	hyperkeratosis ; ichthyosis	Mutations	(Ness et al., 1998; Pavez Lorie et al., 2009)
7963406	KRT6B	keratin 6B	1.61	-1.10	pachyonychia congenita		(Oh et al., 2006)

[1]Density FC: fold change between the 50% and 100% confluent cell density untreated samples.
[2]EGF FC: fold change between control and EGF at 100% confluent cell density.
Bolded entries are genes essential for the development of epidermal barrier in mice.

Appendix B - List of 114 Genes Related to Skin Diseases Based on Literature (Refer to Chapter 3, Figure 19) (cont.)

Transcript Cluster ID	Gene Symbol	Gene Description	Density FC[1]	EGF FC[2]	Skin Diseases	Level expressed in skin disease	References
8015357	KRT9	keratin 9	4.24	-3.39	epidermolytic palmoplantar keratoderma	Mutations	(Corden and McLean, 1996)
8036072	KRTDAP	keratinocyte differentiation-associated protein	21.19	-2.07	psoriasis	Elevated	(Tsuchida et al., 2004)
7920191	LCE3A	late cornified envelope 3A	2.01	3.00	psoriasis	SNPs	(Zhang, 2009)
7920185	LCE3D	late cornified envelope 3D	52.44	1.81	psoriasis	SNPs	(Zhang, 2009)
8158167	LCN2	lipocalin 2	7.69	2.61	psoriasis	Elevated	(Lee et al., 2008)
7905563	LOR	loricrin	44.32	-19.49	psoriasis, dermatitis	Decrease	(Hohl, 1993)
8153346	LYNX1	Ly6/neurotoxin 1	9.06	-1.32	psoriasis	Elevated	(Tsuji et al., 2003)
8119016	MAPK13	mitogen-activated protein kinase 13	2.00	-1.14	psoriasis	Elevated	(Johansen et al., 2005)
8000811	MAPK3	mitogen-activated protein kinase 3	1.53	1.57	psoriasis	Elevated	(Johansen et al., 2005)
8081386	NFKBIZ	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	1.51	1.23	atopic dermatitis	Deficient mice developed atopic dermatitis	(Shiina et al., 2004)
7986675	NIPA1	non imprinted in Prader-Willi/Angelman syndrome 1	1.71	-1.16	Ichthyosis	Mutation	(Lefevre et al., 2004)
8109563	NIPAL4	NIPA-like domain containing 4	5.16	-1.21	congenital ichtyosis	Mutation	(Dahlqvist et al., 2007)
7995539	NOD2	nucleotide-binding oligomerization domain containing 2	3.05	-1.67	psoriasis	Mutation	(Young et al., 2003a)
8105908	OCLN	occludin	9.02	-2.30	psoriasis	Widely expressed	(Yoshida et al., 2001)
8075316	OSM	oncostatin M	1.55	-1.19	psoriasis, atopic dermatitis	Elevated	(Boniface et al., 2007)
7898413	PADI1	peptidyl arginine deiminase, type I	2.97	1.70	psoriasis	Decreased	(Chavanas et al., 2006)
7920228	PGLYRP 4	peptidoglycan recognition protein 4	15.32	-2.08	psoriasis	SNPs	(Sun et al., 2006)
8171418	**PIGA**	**phosphatidylinositol glycan anchor biosynthesis, class A**	**1.56**	**-1.83**	**ichthyosis**	**Knockout mice died within a few days after birth, Harlequin ichthyosis-like features; mutation**	**(Hara-Chikuma et al., 2004; Tarutani et al., 1997)**

[1]Density FC: fold change between the 50% and 100% confluent cell density untreated samples.
[2]EGF FC: fold change between control and EGF at 100% confluent cell density.
Bolded entries are genes essential for the development of epidermal barrier in mice.

Appendix B - List of 114 Genes Related to Skin Diseases Based on Literature (Refer to Chapter 3, Figure 19) (cont.)

Transcript Cluster ID	Gene Symbol	Gene Description	Density FC[1]	EGF FC[2]	Skin Diseases	Level expressed in skin disease	References
8075468	PLA2G3	phospholipase A2, group III	1.90	-1.45	hyperkeratosis, acanthosis	Overexpressed	(Sato et al., 2009)
7987792	PLA2G4D	phospholipase A2, group IVD (cytosolic)	6.93	-1.38	psoriasis, atopic dermatitis	Elevated	(Chiba et al., 2004)
8041763	PRKCE	protein kinase C, epsilon	19.56	-8.40	hyperplasia	Overexpressed	(Li et al., 2005)
8001007	PRSS8	protease, serine, 8	2.23	1.34	ichthyosis	Decreased	(Alef et al., 2009)
7975545	PSEN1	presenilin 1	1.58	-1.44	skin tumorigenesis		(Xia et al., 2001)
8124865	PSORS1C2	psoriasis susceptibility 1 candidate 2	1.81	1.14	psoriasis	SNPs	(Holm et al., 2003)
7922976	PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	3.70	-2.37	dermatitis, human nonmelanoma	Elevated	(Ahn et al., 2010; An et al., 2002)
8150757	RB1CC1	RB1-inducible coiled-coil 1	2.60	-1.24	acanthosis, hyperkeratosis	Double knockout with p53	(Wei et al., 2009)
8025278	RETN	resistin	1.68	-1.54	psoriasis	Elevated	(Coimbra et al.)
7920252	S100A7	S100 calcium binding protein A7	27.47	1.98	atopic dermatitis, psoriasis	Elevated	(Glaser et al., 2009)
8150889	SDR16C5	short chain dehydrogenase/reductase family 16C, member 5	12.84	-4.22	psoriasis	Increased	(Matsuzaka et al., 2002)
8021603	SERPINB13	serpin peptidase inhibitor, clade B (ovalbumin), member 13	4.13	-1.36	psoriasis	Elevated	(Abts et al., 2001)
8023696	SERPINB3	serpin peptidase inhibitor, clade B (ovalbumin), member 3	15.24	-1.51	atopic dermatitis	Elevated	(Mitsuishi et al., 2005)
8023688	SERPINB4	serpin peptidase inhibitor, clade B (ovalbumin), member 4	14.47	-1.38	atopic dermatitis	Elevated	(Yamane et al., 2009)
7928171	SGPL1	sphingosine-1-phosphate lyase 1	1.62	-1.46	atopic dermatitis in dog	Elevated; knockout mice died 15 weeks after birth due to non-lymphoid lesions	(Vogel et al., 2009; Wood et al., 2009)
8048717	SGPP2	sphingosine-1-phosphate phosphotase 2	10.21	-2.79	psoriasis	Elevated	(Mechtcheriakova et al., 2007)
8158224	**SLC27A4**	**solute carrier family 27 (fatty acid transporter), member 4**	**1.72**	**-1.19**	**ichthyosis**	**Knockout mice died shortly after birth; hyperkeratosis; mutations**	**(Herrmann et al., 2005; Herrmann et al., 2003; Klar et al., 2009)**

[1]Density FC: fold change between the 50% and 100% confluent cell density untreated samples.
[2]EGF FC: fold change between control and EGF at 100% confluent cell density.
Bolded entries are genes essential for the development of epidermal barrier in mice.

Appendix B - List of 114 Genes Related to Skin Diseases Based on Literature (Refer to Chapter 3, Figure 19) (cont.)

Transcript Cluster ID	Gene Symbol	Gene Description	Density FC[1]	EGF FC[2]	Skin Diseases	Level expressed in skin disease	References
8153336	SLURP1	secreted LY6/PLAUR domain containing 1	1.86	-1.36	Mal de Meleda	Mutation	(Favre et al., 2007)
8109001	SPINK5	serine peptidase inhibitor, Kazal type 5	21.52	-1.38	Netherton syndrome	Mutation	(Geyer et al., 2005)
7978222	**TGM1**	**null mice died because of impaired barrier skin functiontransglutaminase 1 (K polypeptide epidermal type I, protein-glutamine-gamma-glutamyltransferase)**	**5.36**	**-1.38**	**lamellar ichthyosis**	**Knockout mice died 5 hrs after birth due to impaired skin barrier function**	**(Huber et al., 1995; Kuramoto et al., 2002; Matsuki et al., 1998)**
8060432	TGM3	transglutaminase 3 (E polypeptide, protein-glutamine-gamma-glutamyltransferase)	3.13	-1.86	Darier's disease	Misregulated	(Candi et al., 2002)
7988050	TGM5	transglutaminase 5	5.01	-1.85	ichthyosis	Misregulated	(Candi et al., 2002)
8018966	TIMP2	TIMP metallopeptidase inhibitor 2	2.75	1.27	eczema	Decreased	(Miyoshi et al., 2005)
8092169	TNFSF10	tumor necrosis factor (ligand) superfamily, member 10	2.75	-2.40	atopic dermatitis	Elevated	(Heishi et al., 2002)
8107270	TSLP	thymic stromal lymphopoietin	2.01	-2.24	atopic dermatitis	Elevated	(Edwards, 2008)
8157216	**UGCG**	**UDP-glucose ceramide glucosyltransferase**	**2.72**	**-1.27**	**Gaucher disease**	**Conditional knockout mice had extreme TEWL leading to death 4 days after birth.**	**(Beutler and West, 2002; Jennemann et al., 2007)**
8119898	VEGFA	vascular endothelial growth factor A	1.97	-2.04	acanthosis	Knockout mice died in utero due to defect in blood island formation, elevated	(Carmeliet et al., 1999; Teige et al., 2009; Xia et al., 2003)
8019541	ZNF750	zinc finger protein 750	6.15	-1.42	psoriasis	Mutation	(Yang et al., 2008)

[1]Density FC: fold change between the 50% and 100% confluent cell density untreated samples.
[2]EGF FC: fold change between control and EGF at 100% confluent cell density.
Bolded entries are genes essential for the development of epidermal barrier in mice.

Appendix C – Literature Support of the 245 Known Transcriptional Regulators (Refer to Chapter 4, Tables 7 and 8)

Probe Set	Gene Symbol	Gene title	MAX FC[1]	MIN FC[2]	Type of transcriptional regulators	Reference
221011_s_at	LBH	limb bud and heart development homolog (mouse)	159.74	1.00	activator	(Briegel and Joyner, 2001)
204420_at	FOSL1	FOS-like antigen 1	77.30	2.19	TF	(Finzer et al., 2000)
228964_at	PRDM1	PR domain containing 1, with ZNF domain	34.29	2.16	repressor	(Magnusdottir et al., 2007)
202768_at	FOSB	FBJ murine osteosarcoma viral oncogene homolog B	33.03	-1.00	TF	(Ulery et al., 2006)
228033_at	E2F7	E2F transcription factor 7	30.66	1.14	repressor	(Endo-Munoz et al., 2009)
206877_at	MXD1	MAX dimerization protein 1	29.95	1.00	repressor	(Grandori et al., 2000)
202935_s_at	SOX9	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	27.98	3.00	TF	(Pan et al., 2008)
206127_at	ELK3	ELK3, ETS-domain protein (SRF accessory protein 2)	24.38	1.02	repressor or activator	(Chen et al., 2003; Wasylyk et al., 2005)
209189_at	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	24.33	-2.46	TF	(Durchdewald et al., 2009)
209878_s_at	RELA	v-rel reticuloendotheliosis viral oncogene homolog A, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3, p65 (avian)	17.64	-4.63	TF/activator	(Ghosh and Karin, 2002)
36711_at	MAFF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	15.58	1.70	TF	(Motohashi et al., 2004)
229404_at	TWIST2	twist homolog 2 (Drosophila)	13.90	-1.18	repressor	(Lee et al., 2003)
226319_s_at	LOC644811 /// THOC4	THO complex 4 /// similar to THO complex subunit 4 (Tho4) (Ally of AML-1 and LEF-1) (Transcriptional coactivator Aly/REF) (bZIP-enhancing factor BEF)	12.26	-1.21	coactivator	(Mertz et al., 2007)
211834_s_at	TP63	tumor protein p63	11.58	-1.15	activator	(Senoo et al., 2007; Yang et al., 1998; Zhu et al., 2007)
224833_at	ETS1	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	11.13	1.40	TF	(Jung et al., 2005)
1555832_s_at	KLF6	Kruppel-like factor 6	9.60	1.45	activator	Rubinstein et al, 2004
1552477_a_at	IRF6	interferon regulatory factor 6	9.09	-1.14	TF	(Ben et al., 2005)
238482_at	KLF7	Kruppel-like factor 7 (ubiquitous)	9.08	1.27	TF/coactivator	(Matsumoto et al., 1998)
1560224_at	AHCTF1	AT hook containing transcription factor 1	9.04	1.00	TF	(Okita et al., 2004; Rasala et al., 2006)

[1]EGF Max FC: maximum fold change observed by EGF over the whole treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the whole treatment time.

Appendix C - Literature Support of the 245 Known Transcriptional Regulators (Refer to Chapter 4, Tables 7 and 8)

Probe Set	Gene Symbol	Gene title	MAX FC[1]	MIN FC[2]	Type of transcriptional regulators	Reference
201169_s_at	BHLHB2	basic helix-loop-helix domain containing, class B, 2	8.18	1.09	repressor	(Iizuka and Horikawa, 2008; Nakashima et al., 2008; Rossner et al., 2008)
226711_at	FOXN2	forkhead box N2	7.86	1.72	TF	(Li et al., 1992)Li et al, 1992
1554311_a_at	SUPT6H	suppressor of Ty 6 homolog (S. cerevisiae)	7.82	2.62	chromatin structure	(Chiang et al., 1996; Yoh et al., 2007)
206788_s_at	CBFB	core-binding factor, beta subunit	7.74	-1.05	coactivator	(Sakakura et al., 2005)
205659_at	HDAC9	histone deacetylase 9	7.42	-1.01	corepressor	(Zhang et al., 2001)
1554411_at	CTNNB1	catenin (cadherin-associated protein), beta 1, 88kDa	7.20	1.28	coactivator	(Zhu and Watt, 1999)
44783_s_at	HEY1	hairy/enhancer-of-split related with YRPW motif 1	6.98	-1.07	repressor	(Fischer et al., 2005)
40446_at	PHF1	PHD finger protein 1	6.64	-1.04	repressor	(Sarma et al., 2008)
227261_at	KLF12	Kruppel-like factor 12	6.60	1.03	corepressor	(Roth et al., 2000)
209651_at	TGFB1I1	transforming growth factor beta 1 induced transcript 1	6.47	-1.10	coactivator	(Inui et al., 2007)
228634_s_at	CSDA	Cold shock domain protein A	6.15	-1.59	repressor	(Coles et al., 1996)
210541_s_at	TRIM27	tripartite motif-containing 27	6.00	1.01	repressor	(Bloor et al., 2005)
204790_at	SMAD7	SMAD family member 7	6.00	1.11	TF/activator	(Liu et al., 2003)
208003_s_at	NFAT5	nuclear factor of activated T-cells 5, tonicity-responsive	5.38	-1.15	TF	(Navarro et al., 2008)
228625_at	CITED4	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 4	5.35	-1.32	coactivator	(Labalette et al., 2004)
213032_at	NFIB	nuclear factor I/B	5.06	-1.08	TF/activator	(Mukhopadhyay et al., 2007)
200879_s_at	EPAS1	endothelial PAS domain protein 1	4.97	-2.57	activator	(Conrad et al., 1999)
225289_at	STAT3	signal transducer and activator of transcription 3 (acute-phase response factor)	4.93	-2.02	TF	(Brantley et al., 2008; Snyder et al., 2008)
1552487_a_at	BNC1	basonuclin 1	4.84	1.40	TF	(Matsuzaki et al., 2004)
236429_at	ZNF83	zinc finger protein 83	4.67	1.40	TF	(Marine et al., 1994)
1555611_s_at	MBD1	methyl-CpG binding domain protein 1	4.65	-1.52	corepressor	(Fujita et al., 2000)
216997_x_at	TLE4	transducin-like enhancer of split 4 (E(sp1) homolog, Drosophila)	4.47	-1.77	corepressor	(Brantjes et al., 2001; Eberhard et al., 2000)
202875_s_at	PBX2	pre-B-cell leukemia homeobox 2	4.39	1.75	activator	(Okada et al., 2003)

[1]EGF Max FC: maximum fold change observed by EGF over the whole treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the whole treatment time.

Appendix C - Literature Support of the 245 Known Transcriptional Regulators (Refer to Chapter 4, Tables 7 and 8)

Probe Set	Gene Symbol	Gene title	MAX FC[1]	MIN FC[2]	Type of transcriptional regulators	Reference
206307_s_at	FOXD1	forkhead box D1	4.28	1.97	TF	(Ernstsson et al., 1996)
215111_s_at	TSC22D1	TSC22 domain family, member 1	4.23	1.32	repressor	(Choi et al., 2005; Iida et al., 2007; Sommer et al., 2006)
214600_at	TEAD1	TEA domain family member 1 (SV40 transcriptional enhancer factor)	4.12	-1.06	activator	(Xiao et al., 1991)
211603_s_at	ETV4	ets variant gene 4 (E1A enhancer binding protein, E1AF)	4.10	-1.05	activator	Zhu et al, 2005
203258_at	DRAP1	DR1-associated protein 1 (negative cofactor 2 alpha)	4.06	-1.42	repressor/corepressor	(Castano et al., 2000; Mermelstein et al., 1996)
201417_at	SOX4	SRY (sex determining region Y)-box 4	3.99	1.10	TF	(Farr et al., 1993)
206675_s_at	SKIL	SKI-like oncogene	3.93	1.21	repressor	(Fitsialos et al., 2007)
228785_at	ZNF281	Zinc finger protein 281	3.89	1.96	repressor	(Law et al., 1999)
221763_at	JMJD1C	jumonji domain containing 1C	3.70	1.37	coactivator	(Wolf et al., 2007)
210971_s_at	ARNTL	aryl hydrocarbon receptor nuclear translocator-like	3.66	-3.87	TF/coactivator?	(Hogenesch et al., 1998)
1555639_a_at	RBM14	RNA binding motif protein 14	3.66	-1.01	coactivator	Yang et al, 2007
208735_s_at	CTDSP2	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 2	3.62	-1.34	activator	(Thompson et al., 2006)
200919_at	PHC2	polyhomeotic homolog 2 (Drosophila)	3.58	1.09	repressor	(Gunster et al., 1997)
219729_at	PRRX2	paired related homeobox 2	3.56	-1.18	TF	(Mitchell et al., 2006)
203313_s_at	TGIF1	TGFB-induced factor homeobox 1	3.51	1.42	corepressor of SMAD2	(Hamid et al., 2008; Wotton et al., 2001; Wotton et al., 1999)
224760_at	SP1	Sp1 transcription factor	3.42	-1.06	TF	(Mora-Lopez et al., 2008)
205375_at	MDFI	MyoD family inhibitor	3.42	-1.62	repressor	(Chen et al., 1996; Ma et al., 2003)
201368_at	ZFP36L2	zinc finger protein 36, C3H type-like 2	3.41	1.17	TF	(Nie et al., 1995)
207109_at	POU2F3	POU class 2 homeobox 3	3.40	-4.89	repressor	(Shiina et al., 2004)
226206_at	MAFK	v-maf musculoaponeurotic fibrosarcoma oncogene homolog K (avian)	3.39	1.71	repressor	(Dhakshinamoorthy and Jaiswal, 2000)
225798_at	JAZF1	JAZF zinc finger 1	3.35	-1.02	repressor	(Nakajima et al., 2004)
206604_at	OVOL1	ovo-like 1(Drosophila)	3.31	-1.34	repressor	(Nair et al., 2007)

[1]EGF Max FC: maximum fold change observed by EGF over the whole treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the whole treatment time.

Appendix C - Literature Support of the 245 Known Transcriptional Regulators (Refer to Chapter 4, Tables 7 and 8)

Probe Set	Gene Symbol	Gene title	MAX FC[1]	MIN FC[2]	Type of transcriptional regulators	Reference
203874_s_at	SMARCA 1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1	3.27	1.05	chromatin structure	(Banting et al., 2005)
1554980_a_at	ATF3	activating transcription factor 3	3.20	1.00	corepressor, repressor of Nrf2	(Wang et al., 2007)
201465_s_at	JUN	jun oncogene	3.14	1.14	TF	(Bohmann et al., 1987; Chen and Chang, 2000)
210086_at	HR	hairless homolog (mouse)	3.05	-1.96	TF	(Ahmad et al., 1998)
225265_at	RBMS1	RNA binding motif, single stranded interacting protein 1	3.04	1.23	activator/repressor	Normura et al, 2005
1565254_s_at	ELL	elongation factor RNA polymerase II	3.02	1.04	TF	(Kong et al., 2005)
232231_at	RUNX2	runt-related transcription factor 2	2.98	1.02	activator	(Pratap et al., 2008)
206173_x_at	GABPB2	GA binding protein transcription factor, beta subunit 2	2.98	1.03	TF	(Watanabe et al., 1993)
219199_at	AFF4	AF4/FMR2 family, member 4	2.93	1.23	repressor	(Niedzielski et al., 2007)
1558560_s_at	BLZF1	basic leucine zipper nuclear factor 1 (JEM-1)	2.90	-1.12	coactivator	(Duprez et al., 1997; Tong et al., 1999)
235791_x_at	CHD1	chromodomain helicase DNA binding protein 1	2.90	1.36	chromatin structure	(Woodage et al., 1997)
226952_at	EAF1	ELL associated factor 1	2.86	-1.22	activator	(Simone et al., 2001)
210655_s_at	FOXO3	forkhead box O3	2.85	-1.55	activator	(Brunet et al., 1999)
219657_s_at	KLF3	Kruppel-like factor 3 (basic)	2.83	-1.68	repressor	(Perdomo et al., 2005; Sue et al., 2008)
202171_at	VEZF1	vascular endothelial zinc finger 1	2.82	1.22	TF	(Aitsebaomo et al., 2001; Miyashita et al., 2004)
210365_at	RUNX1	runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene)	2.82	-1.02	co-activator	(Wildey and Howe, 2009)
201862_s_at	LRRFIP1	leucine rich repeat (in FLII) interacting protein 1	2.81	1.21	repressor	(Rikiyama et al., 2003; Suriano et al., 2005)
210554_s_at	CTBP2	C-terminal binding protein 2	2.80	1.06	corepressor	(Zhao et al., 2006)
202599_s_at	NRIP1	nuclear receptor interacting protein 1	2.73	1.17	coactivator/repressor	(Rytinki and Palvimo, 2008; Zschiedrich et al., 2008)
201332_s_at	STAT6	signal transducer and activator of transcription 6, interleukin-4 induced	2.72	-1.91	activator	(Aoudjehane et al., 2008)
201845_s_at	RYBP	RING1 and YY1 binding protein	2.71	1.13	corepressor/repressor	(Garcia et al., 1999)
223780_s_at	MED13	mediator complex subunit 13	2.71	1.11	coactivator	(Rachez et al., 1999)

[1]EGF Max FC: maximum fold change observed by EGF over the whole treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the whole treatment time.

Appendix C - Literature Support of the 245 Known Transcriptional Regulators (Refer to Chapter 4, Tables 7 and 8)

Probe Set	Gene Symbol	Gene title	MAX FC[1]	MIN FC[2]	Type of transcriptional regulators	Reference
227111_at	ZBTB34	zinc finger and BTB domain containing 34	2.70	1.13	repressor	(Qi et al., 2006)
204341_at	TRIM16 /// TRIM16L	tripartite motif-containing 16 /// tripartite motif-containing 16-like	2.68	1.00	TF	(Beer et al., 2002)
219850_s_at	EHF	ets homologous factor	2.66	1.05	activator/repressor	(Kas et al., 2000; Silverman et al., 2002)
204512_at	HIVEP1	human immunodeficiency virus type I enhancer binding protein 1	2.64	1.12	corepressor	(Gaynor et al., 1991)
235473_at	MED6	Mediator complex subunit 6	2.63	-1.39	coactivator	(Lee et al., 1997)
243683_at	MORF4L2	Mortality factor 4 like 2	2.62	-2.18	activator/repressor	(Tominaga et al., 2003)
218284_at	SMAD3	SMAD family member 3	2.62	-1.12	activator	(Ashcroft et al., 1999; Descargues et al., 2008; Flanders et al., 2002)
227718_at	PURB	purine-rich element binding protein B	2.60	-1.06	repressor	(Knapp et al., 2006; Knapp et al., 2007; Zhang et al., 2008)
203275_at	IRF2	interferon regulatory factor 2	2.56	-1.30	repressor	(Chae et al., 2008; Han et al., 2008)
203394_s_at	HES1	hairy and enhancer of split 1, (Drosophila)	2.49	-1.53	repressor	(Sasai et al., 1992)
203739_at	ZNF217	zinc finger protein 217	2.35	1.29	repressor	(Cowger et al., 2007)
202147_s_at	IFRD1	interferon-related developmental regulator 1	2.33	-1.53	corepressor/coactivator	(Batta and Kundu, 2007; Dieplinger et al., 2007)
223586_at	ARNTL2	aryl hydrocarbon receptor nuclear translocator-like 2	2.30	1.20	TF, activator	(Ikeda et al., 2000; Schoenhard et al., 2002)
224013_s_at	SOX7	SRY (sex determining region Y)-box 7	2.24	-3.24	activator	(Niimi et al., 2004)
217729_s_at	AES	amino-terminal enhancer of split	2.20	-2.11	repressor/corepressor	(Liu et al., 2002a)
200049_at	MYST2	MYST histone acetyltransferase 2	2.18	-2.68	chromatin structure	(Georgiakaki et al., 2006; Iizuka and Stillman, 1999)
225539_at	ZNF295	zinc finger protein 295	2.16	-2.93	repressor	(Wang et al., 2005)
1559449_a_at	ZNF254	Zinc finger protein 254	1.98	-6.17	TF	(Han et al., 1999)
223714_at	ZNF256	zinc finger protein 256	1.95	-3.16	repressor	(Suzuki et al., 2008)
202431_s_at	MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	1.95	-1.03	TF	(Murphy et al., 2004)
202191_s_at	GAS7	growth arrest-specific 7	1.93	-1.12	TF	(Ju et al., 1998)

[1]EGF Max FC: maximum fold change observed by EGF over the whole treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the whole treatment time.

Appendix C - Literature Support of the 245 Known Transcriptional Regulators (Refer to Chapter 4, Tables 7 and 8)

Probe Set	Gene Symbol	Gene title	MAX FC[1]	MIN FC[2]	Type of transcriptional regulators	Reference
209292_at	ID4	Inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	1.93	-4.84	corepressor	(Pagliuca et al., 1995)
1553613_s_at	FOXC1	forkhead box C1	1.92	-1.70	TF	(Berry et al., 2006; Berry et al., 2005)
204908_s_at	BCL3	B-cell CLL/lymphoma 3	1.91	-1.08	activator	(Bours et al., 1993)
236128_at	ZNF91	zinc finger protein 91	1.88	-4.27	repressor	(Nishimura et al., 2001)
218486_at	KLF11	Kruppel-like factor 11	1.87	1.08	repressor	(Ellenrieder et al., 2002)
228483_s_at	TAF9B	TAF9B RNA polymerase II, TATA box binding protein (TBP)-associated factor, 31kDa	1.76	-2.40	coactivator	(Lu and Levine, 1995)
238520_at	TRERF1	Transcriptional regulating factor 1	1.75	-5.95	coactivator	(Gizard et al., 2006)
208763_s_at	TSC22D3	TSC22 domain family, member 3	1.73	-4.91	TF	(Vogel et al., 1996)
212436_at	TRIM33	tripartite motif-containing 33	1.73	1.14	repressor	(He et al., 2006; Venturini et al., 1999)
1554415_at	TAF5L	TAF5-like RNA polymerase II, p300/CBP-associated factor (PCAF)-associated factor, 65kDa	1.70	-1.07	coactivator	(Kuninger et al., 2006; Okumura et al., 2006)
219854_at	ZNF14	zinc finger protein 14	1.70	-2.86	coactivator	(Kouzu-Fujita et al., 2009)
226574_at	PSPC1	paraspeckle component 1	1.67	-4.35	coactivator	(Kuwahara et al., 2006)
219388_at	GRHL2	grainyhead-like 2 (Drosophila)	1.64	-5.31	TF	(Peters et al., 2002)
207980_s_at	CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	1.61	-2.67	coactivator	(Qu et al., 2007)
229500_at	SLC30A9	solute carrier family 30 (zinc transporter), member 9	1.61	-1.27	coactivator	(Chen et al., 2005)
226066_at	MITF	microphthalmia-associated transcription factor	1.60	-4.61	TF/activator	(de la Serna et al., 2006)
213006_at	CEBPD	CCAAT/enhancer binding protein (C/EBP), delta	1.59	-3.36	activator/TF	(Clarkson et al., 1995)
213140_s_at	SS18L1	synovial sarcoma translocation gene on chromosome 18-like 1	1.58	-2.91	activator	(Aizawa et al., 2004)
225543_at	GTF3C4	MRNA full length insert cDNA clone EUROIMAGE 1674211	1.55	-2.61	TF	(Dumay-Odelot et al., 2007; Hsieh et al., 1999)
226652_at	USP3	ubiquitin specific peptidase 3	1.53	-3.45	chromatin structure	(Nicassio et al., 2007)
221827_at	RBCK1	RanBP-type and C3HC4-type zinc finger containing 1	1.50	-1.21	TF	(Tatematsu et al., 2005)
1559881_s_at	ZNF12	zinc finger protein 12	1.49	-4.58	repressor	(Zhao et al., 2006b)

[1]EGF Max FC: maximum fold change observed by EGF over the whole treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the whole treatment time.

Appendix C - Literature Support of the 245 Known Transcriptional Regulators (Refer to Chapter 4, Tables 7 and 8)

Probe Set	Gene Symbol	Gene title	MAX FC[1]	MIN FC[2]	Type of transcriptional regulators	Reference
204069_at	MEIS1	Meis homeobox 1	1.49	-15.96	activator	(Okada et al., 2003)
218149_s_at	ZNF395	zinc finger protein 395	1.49	-5.23	repressor	(Sichtig et al., 2007)
225760_at	MYSM1	myb-like, SWIRM and MPN domains 1	1.47	-1.98	coactivator	(Zhu et al., 2007)
217547_x_at	ZNF675	zinc finger protein 675	1.46	-4.30	repressor	(Shin et al., 2002)
204702_s_at	NFE2L3	nuclear factor (erythroid-derived 2)-like 3	1.43	-2.34	TF	(Nouhi et al., 2007; Sankaranarayanan and Jaiswal, 2004)
227642_at	TFCP2L1	Transcription factor CP2-like 1	1.42	-1.56	repressor	(Rodda et al., 2001)
218859_s_at	ESF1	ESF1, nucleolar pre-rRNA processing protein, homolog (S. cerevisiae)	1.40	-3.39	repressor	(Oda et al., 2004)
225655_at	UHRF1	ubiquitin-like, containing PHD and RING finger domains, 1	1.40	-1.45	chromatin structure	(Papait et al., 2007)
208670_s_at	EID1	EP300 interacting inhibitor of differentiation 1	1.40	-1.08	repressor	(Park et al., 2007)
223424_s_at	ZSCAN21	zinc finger and SCAN domain containing 21	1.39	-4.67	activator	(Chowdhury et al., 1992)
227680_at	ZNF326	zinc finger protein 326	1.39	-2.73	activator	(Lee et al., 2000)
235201_at	FOXP2	forkhead box P2	1.37	-4.88	TF/repressor	(Zhou et al., 2008)
209105_at	NCOA1	nuclear receptor coactivator 1	1.37	-2.48	coactivator	(Yuan and Xu, 2007)
225026_at	CHD6	chromodomain helicase DNA binding protein 6	1.37	-2.79	chromatin structure	(Thompson et al., 2003)
200779_at	ATF4	activating transcription factor 4 (tax-responsive enhancer element B67)	1.36	-1.14	activator	(Liang and Hai, 1997)
203204_s_at	JMJD2A	jumonji domain containing 2A	1.36	-2.85	repressor	(Zhang et al., 2005a)
212420_at	ELF1	E74-like factor 1 (ets domain transcription factor)	1.36	-2.75	activator	(Oettgen et al., 1997)
219551_at	EAF2	ELL associated factor 2	1.35	-11.40	activator	(Jiang et al., 2007; Xiao et al., 2006)
244743_x_at	LOC730295 /// LOC731265 /// ZNF138	zinc finger protein 138 /// similar to Zinc finger protein 431	1.34	-3.39	repressor	(Tommerup and Vissing, 1995)
222028_at	ZNF45	zinc finger protein 45	1.33	-2.68	TF	(Shannon and Stubbs, 1998)
211778_s_at	OVOL2	ovo-like 2 (Drosophila)	1.31	-6.56	repressor	(Wells et al., 2009)
223506_at	ZC3H8	zinc finger CCCH-type containing 8	1.31	-2.72	repressor	(Hwang et al., 2002)
231929_at	IKZF2	IKAROS family zinc finger 2 (Helios)	1.29	-2.77	TF/activator	(Kelley et al., 1998; Rebollo and Schmitt, 2003)

[1]EGF Max FC: maximum fold change observed by EGF over the whole treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the whole treatment time.

Appendix C - Literature Support of the 245 Known Transcriptional Regulators (Refer to Chapter 4, Tables 7 and 8)

Probe Set	Gene Symbol	Gene title	MAX FC[1]	MIN FC[2]	Type of transcriptional regulators	Reference
207147_at	DLX2	distal-less homeobox 2	1.29	-6.52	TF?	(Ozcelik et al., 1992)
1553348_a_at	NFX1	nuclear transcription factor, X-box binding 1	1.28	-3.19	TF/repressor	(Song et al., 1994)
227884_at	TAF15	TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 68kDa	1.27	-4.55	coactivator	(Lee et al., 2004)
212462_at	MYST4	MYST histone acetyltransferase (monocytic leukemia) 4	1.27	-3.08	coactivator	(Merson et al., 2006; Pelletier et al., 2002)
208859_s_at	ATRX /// LOC728849	alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae) /// similar to transcriptional regulator ATRX isoform 1	1.27	-2.97	chromatin structure	(Gibbons et al., 2008; Ritchie et al., 2008)
225992_at	MLLT10	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 10	1.25	-3.30	TF	(Chaplin et al., 1995)
202925_s_at	PLAGL2	pleiomorphic adenoma gene-like 2	1.25	-2.87	activator	(Guo et al., 2008; Ning et al., 2008)
229394_s_at	GRLF1	glucocorticoid receptor DNA binding factor 1	1.23	-2.81	repressor	(LeClerc et al., 1991)
213203_at	SNAPC5	small nuclear RNA activating complex, polypeptide 5, 19kDa	1.23	-3.15	TF	(Henry et al., 1998)
225840_at	TEF	thyrotrophic embryonic factor	1.23	-6.39	TF	(Gachon et al., 2006; Inukai et al., 2005)
238549_at	CBFA2T2	core-binding factor, runt domain, alpha subunit 2; translocated to, 2	1.23	-2.11	corepressor	(Martinez et al., 2006)
223218_s_at	NFKBIZ	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	1.22	-3.01	activator	(Matsuo et al., 2007)
210253_at	HTATIP2	HIV-1 Tat interactive protein 2, 30kDa	1.22	-3.23	coactivator	(Wolf et al., 2007)
226352_at	JMY	junction-mediating and regulatory protein	1.21	-5.68	coactivator	(Shikama et al., 1999)
222683_at	RNF20	ring finger protein 20	1.21	-2.61	coactivator	(Kim et al., 2005)
202983_at	HLTF	helicase-like transcription factor	1.21	-3.83	activator	(Ding et al., 1999)
223135_s_at	BBX	bobby sox homolog (Drosophila)	1.21	-2.95	activator	(Sanchez-Diaz et al., 2001)
222895_s_at	BCL11B	B-cell CLL/lymphoma 11B (zinc finger protein)	1.21	-2.66	repressor	(Cismasiu et al., 2008)
212148_at	PBX1	Pre-B-cell leukemia homeobox 1	1.21	-50.88	activator	(Van Dijk et al., 1993)
217862_at	PIAS1	protein inhibitor of activated STAT, 1	1.20	-3.03	coactivator/corepressor	(Lin et al., 2004)
203964_at	NMI	N-myc (and STAT) interactor	1.19	-7.72	transcription cofactor	(Zhou et al., 2000)

[1]EGF Max FC: maximum fold change observed by EGF over the whole treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the whole treatment time.

Appendix C - Literature Support of the 245 Known Transcriptional Regulators (Refer to Chapter 4, Tables 7 and 8)

Probe Set	Gene Symbol	Gene title	MAX FC[1]	MIN FC[2]	Type of transcriptional regulators	Reference
206495_s_at	MIZF	MBD2-interacting zinc finger	1.19	-2.05	repressor/activator	(Mitra et al., 2003; Sekimata and Homma, 2004)
210555_s_at	NFATC3	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3	1.19	-8.83	coactivator	(Ho et al., 1995; Masuda et al., 1995)
200887_s_at	STAT1	signal transducer and activator of transcription 1, 91kDa	1.19	-2.86	TF	(Scarabelli et al., 2008)
201970_s_at	NASP	nuclear autoantigenic sperm protein (histone-binding)	1.18	-7.93	chromatin structure	(Richardson et al., 2006)
242121_at	RNF12	Ring finger protein 12	1.18	-1.80	corepressor	(Ostendorff et al., 2000)
218031_s_at	FOXN3	forkhead box N3	1.17	-4.09	repressor	(Scott and Plon, 2005)
226872_at	RFX2	regulatory factor X, 2 (influences HLA class II expression)	1.17	-15.30	TF	(Horvath et al., 2004)
212769_at	TLE3	transducin-like enhancer of split 3 (E(sp1) homolog, Drosophila)	1.16	-1.97	corepressor	(Brinkmeier et al., 2003)
206724_at	CBX4	chromobox homolog 4 (Pc class homolog, Drosophila)	1.16	-4.66	corepressor	(Satijn et al., 1997)
213293_s_at	TRIM22	tripartite motif-containing 22	1.16	-5.21	corepressor	(Tissot and Mechti, 1995)
221606_s_at	NSBP1	nucleosomal binding protein 1	1.16	-2.78	activator	(King and Francomano, 2001)
203358_s_at	EZH2	enhancer of zeste homolog 2 (Drosophila)	1.16	-3.57	chromatin structure/repressor?	(Hoffmann et al., 2007)
206542_s_at	SMARCA2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2	1.15	-2.83	coactvator	(Muchardt and Yaniv, 1993)
208718_at	DDX17	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	1.15	-2.71	coactivator/corepressor	(Fuller-Pace and Ali, 2008)
218902_at	NOTCH1	Notch homolog 1, translocation-associated (Drosophila)	1.14	-2.74	TF	(Nguyen et al., 2006)
1552685_a_at	GRHL1	grainyhead-like 1 (Drosophila)	1.14	-1.89	TF	(Henderson et al., 2008)
226157_at	TFDP2	Transcription factor Dp-2 (E2F dimerization partner 2)	1.13	-4.60	TF	(Zhang and Chellappan, 1995)
225935_at	CUX1 // ---	CDNA clone IMAGE:4816860	1.13	-3.35	repressor	(Stern et al., 2008; Vanden Heuvel et al., 1996)
201996_s_at	SPEN	spen homolog, transcriptional regulator (Drosophila)	1.13	-3.08	repressor	(Shi et al., 2001)
222667_s_at	ASH1L	ash1 (absent, small, or homeotic)-like (Drosophila)	1.13	-2.75	chromatin structure	(Gregory et al., 2007)
206858_s_at	HOXC6	homeobox C6	1.13	-3.44	corepressor	(Chariot et al., 1996)

[1]EGF Max FC: maximum fold change observed by EGF over the whole treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the whole treatment time.

Appendix C - Literature Support of the 245 Known Transcriptional Regulators (Refer to Chapter 4, Tables 7 and 8)

Probe Set	Gene Symbol	Gene title	MAX FC[1]	MIN FC[2]	Type of transcriptional regulators	Reference
204791_at	NR2C1	nuclear receptor subfamily 2, group C, member 1	1.12	-2.63	repressor	(Tanabe et al., 2007)
202227_s_at	BRD8	bromodomain containing 8	1.12	-2.71	coactivator	(Monden et al., 1999)
209911_x_at	HIST1H2BD	histone cluster 1, H2bd	1.12	-1.72	chromatin structure	(Zhu et al., 2005)
207002_s_at	PLAGL1	pleiomorphic adenoma gene-like 1	1.12	-6.93	TF	(Varrault et al., 2006)
219878_s_at	KLF13	Kruppel-like factor 13	1.12	-1.91	activator	(Song et al., 1999)
228038_at	SOX2	SRY (sex determining region Y)-box 2	1.10	-11.43	activator	(Sharov et al., 2008)
202963_at	RFX5	regulatory factor X, 5 (influences HLA class II expression)	1.10	-3.54	activator	Villard et al, 2000
222016_s_at	ZNF323	zinc finger protein 323	1.10	-26.86	TF	(Pi et al., 2002)
203140_at	BCL6	B-cell CLL/lymphoma 6 (zinc finger protein 51)	1.10	-5.00	corepressor	(Yoshida et al., 1996)
201566_x_at	ID2 /// ID2B	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein /// inhibitor of DNA binding 2B, dominant negative helix-loop-helix protein	1.09	-11.54	repressor	(Murphy et al., 2004)
209538_at	ZNF32	zinc finger protein 32	1.07	-2.75	TF	(Mesa et al., 1996)
222749_at	SUFU	suppressor of fused homolog (Drosophila)	1.07	-3.10	corepressor	(Dunaeva et al., 2003; Stone et al., 1999)
214004_s_at	VGLL4	vestigial like 4 (Drosophila)	1.05	-2.95	transcription cofactor	(Chen et al., 2004)
213707_s_at	DLX5	distal-less homeobox 5	1.05	-5.10	activator	(Samee et al., 2008)
207558_s_at	PITX2	paired-like homeodomain 2	1.04	-3.54	TF	(Shang et al., 2008)
209989_at	ZNF268	zinc finger protein 268	1.04	-2.64	activator	(Shao et al., 2006)
226113_at	ZNF436	zinc finger protein 436	1.04	-2.88	repressor	(Li et al., 2006)
239937_at	ZNF207	Zinc finger protein 207	1.03	-2.19	TF	(Pahl et al., 1998)
203542_s_at	KLF9	Kruppel-like factor 9	1.02	-3.88	TF	(Imataka et al., 1994; Imataka et al., 1992)
223210_at	CHURC1	churchill domain containing 1	1.02	-2.63	activator	(Sheng et al., 2003)
1559078_at	BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein)	1.00	-2.82	repressor	(Senawong et al., 2005)
209604_s_at	GATA3	GATA binding protein 3	-1.00	-3.89	TF	(Joulin et al., 1991; Labastie et al., 1994)
1566324_a_at	MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)	-1.00	-4.05	activator/repressor	(Hedge et al., 1998; Kataoka et al., 2004)
207826_s_at	ID3	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	-1.01	-13.90	corepressor	(Deed et al., 1993)

[1]EGF Max FC: maximum fold change observed by EGF over the whole treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the whole treatment time.

Appendix C - Literature Support of the 245 Known Transcriptional Regulators (Refer to Chapter 4, Tables 7 and 8)

Probe Set	Gene Symbol	Gene title	MAX FC[1]	MIN FC[2]	Type of transcriptional regulators	Reference
202815_s_at	HEXIM1	hexamethylene bis-acetamide inducible 1	-1.02	-2.76	repressor	(Yoshikawa et al., 2008) Yik et al, 2003
203221_at	TLE1	transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	-1.02	-2.68	corepressor	(Pickles et al., 2002)
220625_s_at	ELF5	E74-like factor 5 (ets domain transcription factor)	-1.03	-29.70	activator	(Choi and Sinha, 2006)
214058_at	MYCL1	v-myc myelocytomatosis viral oncogene homolog 1, lung carcinoma derived (avian)	-1.03	-4.90	TF	(Atchley and Fitch, 1995)
211126_s_at	CSRP2	cysteine and glycine-rich protein 2	-1.04	-2.94	coactivator	(Wei et al., 2005)
219041_s_at	REPIN1	replication initiator 1	-1.05	-4.53	repressor	(Kim et al., 2006)
210993_s_at	SMAD1	SMAD family member 1	-1.05	-3.74	activator	(He et al., 2001)
203408_s_at	SATB1	SATB homeobox 1	-1.05	-3.42	chromatin structure	(Han et al., 2008)
209398_at	HIST1H1C	histone cluster 1, H1c	-1.06	-1.93	repressor	(Kim et al., 2008)
204999_s_at	ATF5	activating transcription factor 5	-1.06	-2.65	TF/corepressor	(Pati et al., 1999)
229638_at	IRX3	iroquois homeobox 3	-1.07	-10.58	repressor	(Bilioni et al., 2005)
210319_x_at	MSX2	msh homeobox 2	-1.08	-6.69	repressor	(Takahashi et al., 2001b)
221531_at	WDR61	WD repeat domain 61	-1.08	-3.31	coactivator	Zhu et al, 2005 (Genes Dev)
216197_at	ATF7IP	activating transcription factor 7 interacting protein	-1.09	-5.25	activator or repressor	(Chang et al., 2005; Ichimura et al., 2005)
223213_s_at	ZHX1	zinc fingers and homeoboxes 1	-1.10	-3.78	repressor	(Kim et al., 2007; Yamada et al., 2003)
222146_s_at	TCF4	transcription factor 4	-1.10	-16.36	activator/repressor	(Nguyen et al., 2009)
207469_s_at	PIR	pirin (iron-binding nuclear protein)	-1.10	-11.73	transcription cofactor	(Pang et al, 2004)
224976_at	NFIA	nuclear factor I/A	-1.11	-4.52	TF/activator	(Qian et al., 1995)
219517_at	ELL3	elongation factor RNA polymerase II-like 3	-1.15	-1.43	regulator	(Miller et al., 2000)
210239_at	IRX5	iroquois homeobox 5	-1.15	-15.05	TF	(Kerschensteiner et al., 2008)
222018_at	NACA /// NACA3P /// NACAP1	nascent polypeptide-associated complex alpha subunit /// nascent-polypeptide-associated complex alpha polypeptide pseudogene 1 /// NACA family member 3 pseudogene	-1.16	-2.76	coactivator	(Akhouayri et al., 2005)
227047_x_at	ZBTB4	zinc finger and BTB domain containing 4	-1.18	-2.62	repressor	(Filion et al., 2006)
223275_at	PRMT6	protein arginine methyltransferase 6	-1.18	-15.03	transcriptional regulator/chromatin structure	(Hyllus et al., 2007; Miranda et al., 2005)

[1]EGF Max FC: maximum fold change observed by EGF over the whole treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the whole treatment time.

Appendix C - Literature Support of the 245 Known Transcriptional Regulators (Refer to Chapter 4, Tables 7 and 8)

Probe Set	Gene Symbol	Gene title	MAX FC[1]	MIN FC[2]	Type of transcriptional regulators	Reference
212346_s_at	MXD4	MAX dimerization protein 4	-1.19	-3.12	repressor	(Hurlin et al., 1995; Marcotte et al., 2005)
226895_at	NFIC	Nuclear factor I/C (CCAAT-binding transcription factor)	-1.23	-3.91	TF/activator	(Qian et al., 1995; Wenzelides et al., 1996)
220225_at	IRX4	iroquois homeobox 4	-1.27	-3.37	TF	(Garriock et al., 2001)
217991_x_at	SSBP3	single stranded DNA binding protein 3	-1.30	-2.72	TF	(Wu, 2006)

[1]EGF Max FC: maximum fold change observed by EGF over the whole treatment time.
[2]EGF Min FC: minimum fold change observed by EGF over the whole treatment time.