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Thesis

ROLE OF UROCANIC ACID AS AN ENDOGENOUS PHOTOPROTECTANT AND AS A THERAPEUTIC TARGET FOR TREATING UV-INDUCED MELANOMA AND NON-MELANOMA MALIGNANCIES

by

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ABSTRACT

Overexposure to UV (ultraviolet) radiation has been linked to a number of deleterious effects on human health, particularly epidermal malignancies, which consist of both melanoma and non-melanoma skin cancers, basal cell carcinoma and squamous cell carcinoma. In the 1950's, an epidermal compound known as Urocanic Acid (UCA) was discovered whose trans isoform was shown to display photoprotective effects against UV radiation. Not long after, the cis-UCA isomer was found to act as a mediator of immune suppression, causing UCA to be removed from all cosmetic products on the commercial market, most notably sunscreen. Numerous studies conducted after this finding further corroborated cis-UCA's immunosuppressive properties, showing evidence for the ability of cis-UCA to inhibit contact hypersensitivity responses, delayed-type hypersensitivity responses, and allograft rejection. Early evidence for a mechanism of action behind cis-UCA's immunosuppressive properties were widespread, including modulation of antigen-presenting cells, interaction with histamine receptors, and regulation of cytokine expression. The immunosuppressive nature of cis-UCA quickly became associated with an ability to facilitate cancerous progression, particularly regarding epidermal malignancies. Interesting theories were raised about the evolutionary basis for cis-UCA's immunosuppressive nature, including speculation that cis-UCA was

meant to induce immunosuppression following ultraviolet exposure in order to prevent autoimmune responses against sunburned epidermal cells. After the turn of the 20th century, new research continued to facilitate modern day understanding of the role of UCA. Evidence showed that UCA was ultimately derived from filaggrin within the stratum corneum, interacted with key immune effectors including T-lymphocytes and Langerhans cells, and potentially contributed to acidification of the stratum corneum. Despite the negative reputation cis-UCA has received in regards to facilitating skin cancer evasion of the immune system, research has shown that its immunosuppressive effects may allow it to serve as potent anti-inflammatory therapeutic. In regards to skin cancer, targeting of UCA as a therapeutic varies widely. Some have suggested using UCA as a measure of sunscreen efficacy, as an indirect target that when inhibited can reduce tumor growth, and as a biomarker for skin cancer risk. Others have begun developing UCA-based mimics that retain the benefits of UCA, while avoiding any deleterious effects. The role of UCA in non-melanoma and melanoma malignancies is not well understood, making targeting of UCA as a therapeutic challenging. The aim of this paper is to comprehensively review the scientific literature regarding the pre-21st century history of UCA, followed by an in-depth analysis of post-21st century research. The objective is to determine the overall potential of UCA to serve as a therapeutic target for UV-associated health conditions, most notably dermatologic malignancies.

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LIST OF ABBREVIATIONS

5-HT .....5-Hydroxytryptamine Receptors

5-HT2A ..... Serotonin 2A receptor

APC.....Antigen-Presenting Cell

BCC..... Basal Cell Carcinoma

BER..... Base Excision Repair

CD4+..... Cluster of Differentiation 4 Positive

CD8+..... Cluster of Differentiation 8 Positive

CHS.....Contact Hypersensitivity

CPD..... Cyclobutane Pyrimidine Dimer

CSU..... Chronic Spontaneous Urticaria

Cyclic AMP ..... Cyclic Adenosine Monophosphate

DNCB .....Dinitrochlorobenzene

DNFB..... Dinitrofluorobenzene

DTH ..... Delayed-Type Hypersensitivity

FS ..... Basal Cell Carcinoma

GPCR ..... G protein-coupled receptor

HLA-DR ..... Human Leukocyte Antigen – DR

HPLC ..... High Performance Liquid Chromatography

HSV.....Herpes Simplex Virus

IA ..... Immune-associated

IFN- γ .....Interferon Gamma

IL-6 ..... Interleukin-6

IL-8 ..... Interleukin-8

IL-10 ..... Interleukin-10

MHC-2 ..... Major Histocompatibility Complex Class 2

NER ..... Nucleotide Excision Repair

NFKB ..... Basal Cell Carcinoma

NHE1 ..... Sodium-hydrogen Antiporter 1

PDCD4 ..... Programmed Cell Death 4

PGE2 ..... Prostaglandin E2

PUVA ..... Psoralen and Ultraviolet A

ROS ..... Reactive Oxygen Species

SAPK/JNK ..... Stress-activated Protein Kinases/Jun Amino-terminal Kinases

SCC ..... Squamous Cell Carcinoma

SPF ..... Sun Protection Factor

sPLA2 ..... Secretory Phospholipase A2

TAA ..... Tumor-Associated Antigen

TGF-β ..... Transforming growth factor beta

TNCB 2-Chloro-l,3,5-trinitrobenzene

TNF-α ..... Tumor Necrosis Factor Alpha

UAC ..... Urocanic-acid-modified Chitosan

UCA ..... Urocanic Acid

UV ..... Ultraviolet

INTRODUCTION

Health Effects of UV Radiation

Overexposure to ultraviolet (UV) radiation is a critical environmental stressor that has been linked to a plethora of deleterious effects on human health. Skin damage associated with premature aging, ocular impairment such as cataracts, and neoplastic changes are among some of the major sun-related health concerns, summarized in Table 1 (Last, 1993). Additionally, UV radiation has been correlated with immune suppression, further increasing susceptibility to malignant transformation and contraction of infectious disease (Douki, 2016; Last, 1993).

Ecologically important	
DNA damage	Maximum effect on small and single-cell organisms
Impaired growth and photosynthesis	Poor crop yields
Phytoplankton: impaired motility impaired reproductive capacity	Reduced uptake of CO_2
Nitrogen-fixing soil bacteria	Reduced, damaged
Human health effects	
Immunosuppression	Enhanced susceptibility to infection
	Cancer proneness
Dermatological	Sunburn
	Loss of skin elasticity ("Premature aging")
	Photosensitivity
Neoplasia	Melanocytic (malignant melanoma)
	Squamous cell skin cancer
	Basal cell skin cancer
	? Cancer of lip
	? Salivary gland cancer
Ocular	Cataract
	Pterygium

Table 1. Biological effects of ozone depletion/ultraviolet irradiation. The effects of increased ultraviolet irradiation due to ozone depletion impact both global ecological landscapes and human health. On an ecological scale, impaired growth of crops has major consequences, including hunger and starvation, for all living organisms. For human health, increase in risk of skin cancer, both non-melanoma and melanoma forms, are at the severest ends of the spectrum. Taken from Last, 1993.

Skin cancer is one of the most common forms of malignancy in the United States, which includes both melanoma and non-melanoma skin cancers, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) (Last, 1993). It is projected that one in every five individuals in the US will develop skin cancer within their lifetime and nearly 10,000 people are diagnosed daily (Skin Cancer, n.d.). While survival rates for skin cancer are high, particularly for non-melanoma malignancies, current mortality rates of nearly 10,000 people per year nationwide and 65,000 people per year worldwide in addition to the tens of billions of dollars spent per year on treatment serve as a strong basis behind innovative research strategies in advancing prevention, detection, and treatment of skin neoplasms (Skin Cancer, n.d.).

While UV radiation comprises wavelengths between 250 to 400nm, the subtype most associated with development of harmful biological effects is UVB radiation (280 to 320nm) and UVA (320 to 400nm) to a lesser extent (Douki, 2016; De Fabo, 1996). The carcinogenicity of UV radiation is most well-known for its' propensity to induce DNA damage, particularly in keratinocytes, which gives rise to BCC and SCC, and melanocytes, which gives rise to melanoma (Douki, 2016). The two major forms of DNA damage are pyrimidine dimers, most notably cyclobutane pyrimidine dimers (CPDs), and oxidative damage (Douki, 2016). Pyrimidine dimers can be formed through either direct absorption of UVB and UVA irradiation by DNA, a potent endogenous chromophore, or by photosensitizers, endogenous or exogenous chromophores, which induce changes in DNA bases Douki, 2016; Young, 1997). Oxidative damage is produced when absorption of UVA and UVB irradiation by cellular components results in the formation of reactive

oxygen species and the subsequent degradation of key cellular macromolecules including proteins and lipids (Douki, 2016). Inherent DNA repair mechanisms exist to correct damage to DNA and prevent mutations from accumulating during DNA replication; nucleotide excision repair (NER) is responsible for repairing pyrimidine dimers and base excision repair (BER) repairs oxidative DNA modifications. Therefore, the overall persistence of DNA damage and their susceptibility to develop into DNA mutations depends on the balance between damage formation and efficiency of DNA repair mechanisms (Douki, 2016).

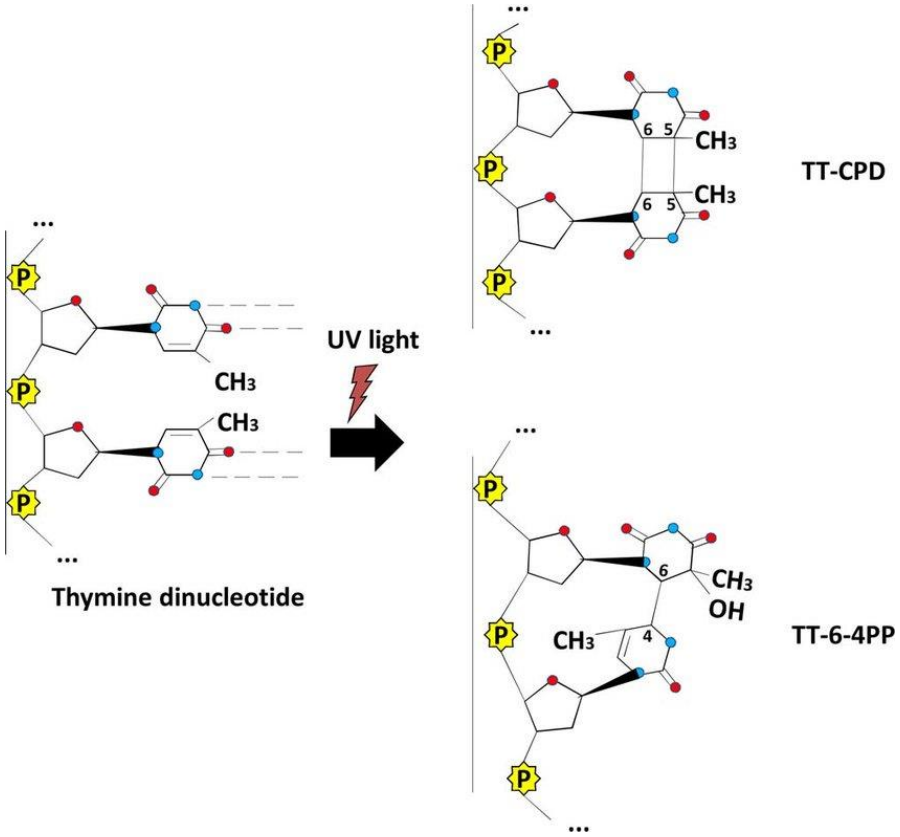

Figure 1. Production of Pyrimidine Dimers. One of the primary damaging effects done to DNA by ultraviolet radiation is the production of pyrimidine dimers. These result in kinks and lesions in the DNA backbone that, if left unfixed by DNA repair mechanisms, can result in deleterious DNA mutations. Taken from Quinet et al., 2018.

Rise of Urocanic Acid

Because the cell types most vulnerable to neoplastic malformations, keratinocytes and melanocytes, reside in the stratum basale, the deepest layer of the epithelium, they receive protection from the barrier created by the remaining four layers above. These layers' guard against UV damage by direct reflection of radiation as well as absorption by endogenous chromophores (Barresi et al. 2011). In the 1950's, a novel link was found between UV radiation and a compound known as Urocanic acid (UCA) (Eckhart, 2016). This molecule was found to reside in the most superficial layer of the epidermis, the stratum corneum, and it was established the *trans* isoform served as an efficient absorber of UV radiation (Young, 1997). The novel ability of *trans*-UCA to act as a natural protectant against solar irradiation quickly resulted in it being marketed in various cosmetic and sunscreen products (Young, 1997). It was later taken off the market in the 1990's, when it was discovered that the *cis* isoform of UCA displays immunosuppressive properties; this finding was supported by a well-known study conducted by De Fabo and Noonan in 1983 (De Fabo, 1996). Although further research into both the protective effects and immunosuppressive effects of various isoforms of UCA dwindled in the following years, there still existed great debate and controversy regarding the beneficial versus harmful effects of UCA. Recent research has revitalized the possibility of UCA in serving a valuable role in protection against UV radiation and as a potential therapeutic target against both non-melanoma and melanoma skin cancers (Barresi et al., 2011).

SPECIFIC AIMS

The controversial role of Urocanic Acid as both a photoprotectant and immunosuppresor is currently still under debate. The following comprehensive review of scientific literature serves to accomplish several objectives. First, a condensed description of the history of Urocanic Acid research is provided, emphasizing the factors that have contributed to the modern day debate regarding this chromophore. Second, this thesis aims to conduct an in-depth analysis of the current research regarding the revitalized support of UCA in serving as a potent photoprotectant against UV radiation. Both *in vitro* and *in vivo* studies will be analyzed, including current Phase 1 clinical trials. In addition, both benefits and risks will be extensively researched. Lastly, the final objective of this review is to conclude on the overall potential of UCA to serve as a therapeutic target for UV-associated health conditions, most notably dermatologic malignancies.

HISTORY OF UROCANIC ACID

Discovery and Popularity

One of the main functions of the epidermal layer is photoprotection, which is carried out by light-absorbing compounds, including lipids, keratins, melanin, and porphyrins (Baden & Pathak, 1967). In the early 1950's, a compound known as Urocanic acid (UCA) was identified as an additional photoprotective constituent of the epidermis. The very first description of UCA dates back to an 1874 study by Jaffe, where the compound now known to be Urocanic acid was found in the urine of canines (Norval, Simpson, & Ross, 1989). Numerous studies beginning in the early 1950's identified the presence of epidermal UCA using techniques including chromatography, spectrophotometry, and staining (Norval et al., 1989). Zenisek et al. found UCA in human sweat, Tabachnick found UCA in guinea pig epidermis, and Baden and Pathak found UCA in the epidermis of a murine model (Baden & Pathak, 1967; Tabachnick, 1957; Zenisek, Kral, & Hais, 1955). Finally, in 1961, Everett et al. found UCA present in human epidermal layers (Everett, Anglin, & Bever, 1961).

Accounting for roughly 0.7% of the dry weight of the human epidermis, UCA is a major absorber of light in the UV range (Baden & Pathak, 1967). UCA exhibited absorption profiles that were either stronger than or equal to current sunscreens available on the commercial market, prompting researchers to speculate on its' pharmacological potential (Zenisek et al., 1955). Following the discovery of UCA, this pushed many researchers during the 1950's to test the potential of UCA to act as a natural sunscreen against erythema-producing radiation. Erythema is a dark reddening of the skin caused by

increased blood flow to dermal capillaries; one cause of erythema is solar radiation, which results in the commonly known sunburn. A 1957 study by Tabachnick showed that UCA was responsible for 80% of all UV-absorption that occurred in guinea pig epidermal samples (Tabachnick, 1957). Additionally, a later 1961 study by Everett et al. showed that a formulation containing 5% UCA was able to confer erythema protection to human skin (Everett et al., 1961). To test the hypothesis that UCA could be utilized as an effective sunscreen, Baden & Pathak treated subjects with oral, intradermal, and topical applications of UCA followed by solar radiation (Baden & Pathak, 1967). They found that subjects treated with topical UCA received sunburns that were half as severe as controls who did not receive UCA (Baden & Pathak, 1967). They also found that oral and intradermal routes of administration were ineffective in protecting against UV irradiation (Baden & Pathak, 1967).

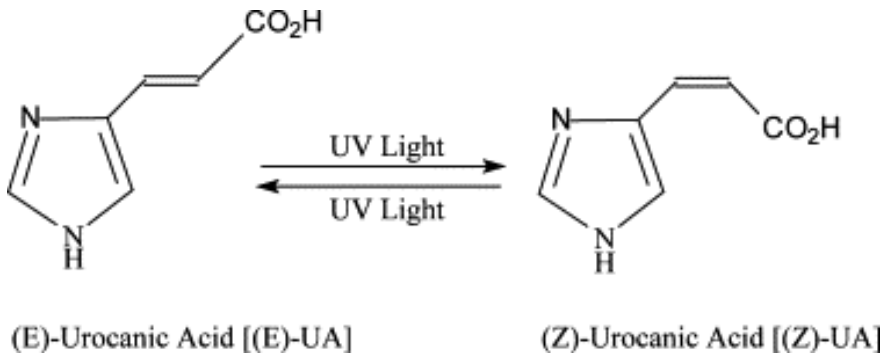

Figure 2. Trans and Cis Isoforms of Urocanic acid. Urocanic acid possesses two isoforms: the trans (E) resting form and the cis (Z) active form. Photoisomerization from the trans to cis forms is induced by UV irradiation. Taken from Mohammad, 2002.

During this time, it was also found that UCA has two forms, the trans (E) and cis (Z) isomers. Trans-UCA is the form normally found in the epidermis under resting conditions, but UV absorption induces isomerization from the trans to cis isomer (Everett

et al., 1961). This isomerization has been shown to be most effective at UV radiation wavelengths less than 320nm (Anglin, Bever, Everett, & Lamb, 1961). Baseline levels of trans-UCA in the epidermis vary widely, but median levels hover at roughly 4 $\mu g/cm^2$ (Norval, McIntyre, Simpson, Howie, & Bardshiri, 1988). In addition to UV irradiation inducing isomerization from trans-UCA to cis-UCA, it also increases the total amount of UCA in the stratum corneum (Hais & Strych, 1969).

Production of UCA requires deamination of histidine via an enzyme known as histidase, or histidine-ammonia lyase (Mehler & Tabor, 1953). The histidine from which UCA is derived, mainly comes from keratohyalin granules located in the stratum corneum (Scott, 1981; Scott, Harding, & Barrett 1982). Histidase is synthesized in the spinosum and granulosum layers of the epidermis and activate when reaching the stratum corneum layer (Norval et al., 1989). This enzyme is known to be present in sufficient quantities only in the stratum corneum layer of the epidermis and in the liver (Mehler & Tabor, 1953). This corresponds to findings that show UCA is only found in appreciable quantities of the stratum corneum layer of the epidermis and in the liver (Baden & Pathak, 1967). Importantly, in the liver, UCA is further broken down via the urocanase enzyme in a degradative pathway (Feinberg & Greenberg, 1958). Therefore, the presence of UCA in the liver can be considered relatively transient and the compound does not accumulate with this hepatic system. Conversely, the enzyme urocanase is not found in sufficient amounts in the epidermis, and thus UCA can accumulate with the epidermal layers, specifically the stratum corneum (Norval et al., 1989). One study illustrated that nearly six to twelve times as much histidine was deaminated into UCA compared to the

amount used for protein synthesis in the epidermis (Baden & Pathak, 1967). The reason for this is that histidase activity is observed to increase with increased UV exposure (Baden & Pathak, 1967). After exposure to UV radiation, levels of the cis-UCA remain increased for up to three weeks before being eliminated by desquamation at the stratum corneum or sweat-based excretion (Norval & El-Ghorr, 2002).

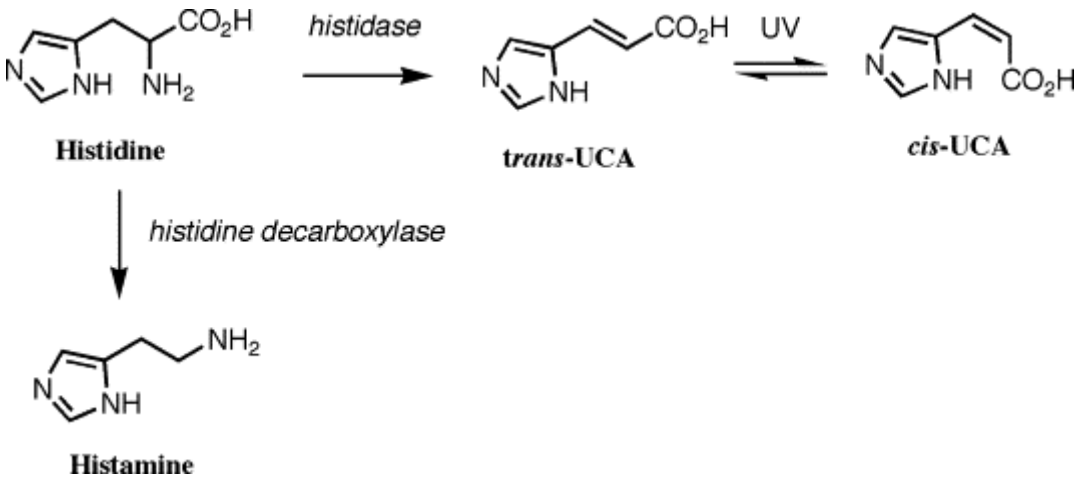

Figure 3. Production of Urocanic Acid by Histidase. Histidase, is responsible for converting the amino acid, Histidine, into Urocanic Acid. Histidase is many found in the stratum corneum layer of the epidermis and in the liver. Histidine can also be converted into Histamine by Histidine Decarboxylase. Taken from Norval & El-Ghorr, 2002.

Health Concerns and Subsequent Withdrawal

Immunosuppression: De Fabo and Noonan

In the early 1980's, a novel finding was published by De Fabo and Noonan showing the immunosuppressive effects of UCA (De Fabo & Noonan, 1983). It was later elucidated that the cis isomer of UCA was responsible for this immunosuppression. The work by De Fabo and Noonan focused on the relationship between UCA and carcinoma induced by UV radiation (De Fabo & Noonan, 1983). Firstly, it is necessary to recognize

that UV radiation plays two main roles in the induction of skin cancer. The first role is neoplastic transformation as a result of genetic mutagenesis from radiation. The second is induction of localized and systemic immunosuppression, which assists malignant cells in invading the immune system. It had been found that UV-radiation, in the range of 250 to 320 nm, resulted in activation of suppressor T-cells and antigen-specific suppressor cells (Kripke, 1984). De Fabo and Noonan were interested in discovering how UV radiation, which does not penetrate beneath the dermal layer of the skin, can result in formation of systemic immune suppression (De Fabo & Noonan, 1983).

As aforementioned above, De Fabo and Noonan had previously found that narrow band UV radiation in the 250 to 32 nm range induced immunosuppression, by observing the systemic suppression of contact hypersensitivity in response to TNCB (2-chloro-l,3,5-trinitrobenzene) in murine models (De Fabo & Noonan, 1983). TNCB is an agent known to induce contact hypersensitivity. They concluded that this suppression occurred independently of epidermal damage and erythema, suggesting that the suppression was not caused by an inflammatory response to UV radiation (De Fabo & Noonan, 1983). De Fabo and Noonan also ruled out antigen-presenting cell (APC) defects and damage to epidermal Langerhans cells by UV radiation (De Fabo & Noonan, 1983). Their hypothesis was that a photoreceptor was present in the epidermis responsible for absorbing UV radiation and subsequently activating a signal transduction cascade that would lead to the production of suppressor immune cells (De Fabo & Noonan, 1983). This hypothesis was further strengthened after De Fabo and Noonan found that removing

the outermost layer of the epidermis, the stratum corneum, prevented induction of systemic immune suppression (De Fabo & Noonan, 1983).

Using this information, De Fabo and Noonan created a model, postulating that a photoreceptor exists in the stratum corneum layer of the epidermis that upon exposure to UV radiation, transduces a biochemical signal that activates T-suppressor and antigen-specific suppressor cells (De Fabo & Noonan, 1983). In this way, the unknown photoreceptor was hypothesized to act similarly to the well-known photoreceptor, Rhodopsin. They ruled out other biochemical compounds found in the stratum corneum, including nucleic acids, lipids, amino acids, and keratin (De Fabo & Noonan, 1983). Components of the stratum corneum were narrowed down until only one realistic possibility remained, UCA. It was found that the absorption of UCA was similar to the action spectrum observed during immunosuppression (De Fabo & Noonan, 1983). Further supporting the likelihood that UCA was the unknown photoreceptor that De Fabo and Noonan were looking for, was the location of UCA in the stratum corneum, the photochemical properties of UCA as a known absorber of UV and radiation.

Thus, De Fabo and Noonan tested this hypothesis using a murine model and found that mice with intact stratum corneum layers but reduced levels of UCA did not display immunosuppression when compared to controls with baseline levels of UCA (De Fabo & Noonan, 1983). The mechanism of action proposed by De Fabo and Noonan was that following UV radiation, UCA is activated and enters systemic circulation, where it results in a decrease of antigen-presenting cells and subsequent activation of suppressor cells upon exposure to antigen (De Fabo & Noonan, 1983). Following this finding,

research investigating the immunosuppressive properties of UCA and the subsequent impact it would have on human health skyrocketed. Numerous studies were also done on the mechanism of action of UCA in immunosuppression and its' relationship to disease pathologies.

Immunosuppression: Preliminary Research

The evidence that UCA, specifically cis-UCA, was capable of causing immune defects grew rapidly. Ross et al. utilized a murine model of HSV to show that topical application of subcutaneous injection of cis-UCA to epidermal cells from irradiated mice resulted in immunosuppression of delayed type hypersensitivity responses (DTH) to herpes simplex virus (HSV) (Ross, Howie, Norval, Maingay, & Simpson, 1986; Ross, Howie, Norval, & Maingay, 1987). They also found that the percentage of cis-isomer present correlated with the percentage degree of suppression of DTH, i.e. in a dose-dependent manner (Ross et al., 1986; Ross et al., 1987). Analysis of subsequent production of suppressor immune cells led these researchers to believe that both local and systemic immunosuppressive signals are generated by cis-UCA. In 1988, Norval et al. expanded on this finding by discovering that this immunosuppression is time-sensitive (Norval et al., 1988). They found that the viral infection needed to occur at least more than five hours and less than two weeks post-UV radiation (Norval et al., 1988).

The majority of studies illustrating immunosuppression mediated by UCA were focused on topical application. This was critical as it mimicked the main route of exposure individuals would experience on a daily basis, e.g. through cosmetic products containing UCA. In 1989, Reeve et al. showed that UCA applied topically to UV-irradiated hairless mice resulted in suppression of contact type hypersensitivity to

oxazolone (Reeve, Greenoak, Canfield, Boehm-Wilcox, & Gallagher, 1989). They also investigated the impact of topical application of UCA to hairless mice on progression of malignant growth (Reeve et al., 1989). It was found that mice treated with topical UCA exhibited increased total counts of UV-induced tumor masses and increased degree of malignancy when exposed to chronic doses of UV radiation (Reeve et al., 1989). This illustrated a definitive link between cis-UCA and induction of epidermal malignancy.

The popularity of UCA and its associated sunscreening effects resulted in its addition to commercial sunscreens. In 1991, Reeve et al. performed a study where they showed that solar radiation of areas treated with UCA-containing cosmetics resulted in the formation of cis-UCA (Reeve & Mitchell, 1991). Furthermore, they found that UCA-containing cosmetic lotion applied to the murine epidermis resulted in immunosuppression of contact hypersensitivity to DNFB (Reeve & Mitchell, 1991). A later 1992 study by Gruner et al. illustrated that cis-UCA displayed similar effects to PUVA, in inducing immunosuppression during murine heart allografts (Gruner et al., 1992). Control grafts without either PUVA or cis-UCA treatment observed rejection of the allograft within two weeks' time (Gruner et al., 1992). Groups treated with either PUVA or cis-UCA preventing rejection of the allograft in up to 50% of mice (Gruner et al., 1992).

In the late 1990's, researchers began using anti-cis-UCA antibodies to test the impact of blocking cis-UCA action on immunosuppression. In 1995, Kondo et al. found that an intradermal injection of cis-UCA resulted in immune suppression of CHS when later treated with DNFB (Kondo et al., 1995). For mice that were given anti-cis-UCA

antibodies prior to UV radiation exposure, the amount of immunosuppression was reduced and the CHS response was marginally restored (Kondo et al., 1995. Another study by Finlay-Jones & Hart in 1998 found that commercial sunscreens partially blocked the isomerization of trans-UCA to cis-UCA and subsequently partially blocked systemic immunosuppression (Finlay-Jones & Hart, 1998). Importantly, complete blockage of immunosuppression did not occur unless mice were pre-treated with anti-cis-UCA antibodies (Finlay-Jones & Hart, 1998).

During this period, studies continued to surface demonstrating the immunosuppressive nature of cis-UCA. A study by El-Ghorr et al. in 1997 tested the effects of chronic trans-UCA and cis-UCA on murine epidermal skin health (El-Ghorr & Norval, 1997). Over a four-week period, they found that only mice treated with cis-UCA presented with systemic immunosuppression, reduced thymic weight, and increased lymph node weight (El-Ghorr & Norval, 1997).

Early Evidence for a Mechanism of Action

A Receptor for Cis-UCA

Studies were conducted in the late 1980's demonstrating that minute fluctuations in cis-UCA concentration could result in appreciable amounts of immunosuppression. Concentrations of cis-UCA ranging from less than 1 μg to 1 μg to 100 μg were all adequate to induce immunosuppression in various experimental models (Ross et al., 1986; Noonan & De Fabo, 1992; Norval et al., 1989). This suggested that cis-UCA acted through a specific receptor mechanism.

Cis-UCA and Antigen-Presenting Cells

The immunosuppression induced by cis-UCA also led researchers to hypothesize that the signal transduction pathway cis-UCA activates ultimately acts on antigen-

presenting cells. Because of the role MHC-2 (major histocompatibility complex 2) plays in antigen presentation, many studies were conducted to test this association. Noonan et al. found that application of cis-UCA to epidermal cells from mice displayed up to a 35% decrease in IA (immune-associated, DR) antigen positive, cells (Noonan, De Fabo, & Morrison, 1988). Ross et al. similarly found a decrease in IA (immune-associated, DR) antigen positive, cells, in murine epidermal cells treated with UV radiation and cis-UCA (Ross et al., 1986; Ross et al., 1987). Rasanen et al. further supported these findings by showing a reduced count of HLA-DR positive cells in human epidermal cells treated with cis-UCA. HLA-DR is the MHC Class 2 cell surface receptor found in humans (Rasanen, Jansen, Hyoty, Reunala, & Morrison, 1989). These studies clearly demonstrated the ability of cis-UCA to reduce active MHC Class 2 antigen-presenting cells, either through a downregulation of expression or a reduction of cell count. Multiple theories arose including that cis-UCA causes abnormal migration of antigen-presenting cells from the epidermis, that cis-CUA induces abnormal intracellular functioning of antigen-presenting cells, including antigen uptake, processing, presentation, and intercellular communication (Norval et al., 1989).

A specific subset of antigen-presenting cells, known as Langerhans cells, were of particular interest to researchers due to their location and function in the epidermis. It had been shown that UV radiation could result in loss of Langerhans cells in the epidermis and an accumulation of dendritic cells in surrounding lymph nodes (Laihia & Jansen, 1995). It had also been shown that exposure to cis-UCA mimicked these effects, further supporting the growing theory that UV radiation acts through cis-UCA in modulating

immune function (Laihia & Jansen, 1995). In 1995, Laihia et al. further investigated the role that cis-UCA might play in either of these two processes (Laihia & Jansen, 1995). They found that treating mice with anti-cis-UCA antibodies prior to UV irradiation blocked the loss of Langerhans cells in the epidermis, but did not affect the behavior of dendritic cells (Laihia & Jansen, 1995).

Cis-UCA and Histamine

Recall that UCA is a derivative of the amino acid histidine. Another derivative of histidine is the compound histamine, a regulator of immune function. Histamine, stored in secretory granules in mast cells and basophils, activates H1 and H2-receptors. Activation of H2-receptors results in immunosuppression, particularly through the action of T-suppressor lymphocytes. Norval et. al showed that topical application of histamine caused suppression of DTH following infection of HSV, similar to studies using cis-UCA (Norval et al., 1989). Structural similarities between the two led researchers to hypothesize that cis-UCA may regulate immunosuppression by acting in a similar fashion as histamine and by potentially binding to the same H1 and H2-receptors (Norval et al., 1989). Further research supports the hypothesis that cis-UCA may act similarly to histamine; a 1999 study by Wille et al. found that cis-UCA effectively induced release of mast cell granules, including mast cell TNF-α (Wille, Kydonieus, & Murphy, 1999). Given the complex role that TNF-α plays in modulating immune function, it is highly probable that TNF-α could be a downstream effector of the signal transduction pathway activated by cis-UCA in immunosuppression.

Cis-UCA and DNA Damage

Due to the growing association between cis-UCA and epidermal malignancy, questions arose regarding the potential for UCA to induce DNA damage. Yarosh et al. performed a study assaying the ability of UCA, in both isomeric forms, to bind DNA, induce DNA mutations, and induce DNA repair (Yarosh et al., 1992). Importantly, researchers did not find a conclusive evidence suggesting that cis-UCA or trans-UCA interact with DNA in appreciable amounts (Yarosh et al., 1992). This shifted the view of the mechanism of action of UCA away from DNA interaction.

Cis-UCA and UVA versus UVB Radiation

Most previous research documenting the immunosuppressive properties of cis-UCA have utilized UVB radiation as the source of radiation exposure. A few studies had showed that the physiological behavior of UCA may differ depending on the wavelength of UV radiation used. UVA radiation spans wavelengths 320 to 400 nm, compared to UVB radiation, which spans wavelengths 280 to 320 nm. In a 1997 by Webber et al., researchers found that UVA radiation induced isomerization of trans-UCA to cis-UCA, but did not induce immunosuppression (Webber, Whang, & De Fabo, 1997). Thus, this suggests that isomerization from the trans to cis form of UCA alone is not sufficient to induce isomerization and that other factors are at play. Additionally, it has also been shown that isomerization of UCA from trans to cis forms is more sensitive at short UV wavelengths, i.e. UVB, compared to, longer wavelengths, i.e. UVA (Webber et al., 1997). It is likely that of the two main UV wavelengths individuals are exposed to, UVB is the more harmful. However, a 1998 study by Hanson et al. showed that mice exposed to UVA radiation resulted in higher rates of epidermal photoaging compared to controls

(Hanson & Simon, 1998). This suggests that although UVA radiation may not induce immunosuppression, it may have other harmful effects that have yet to be discovered.

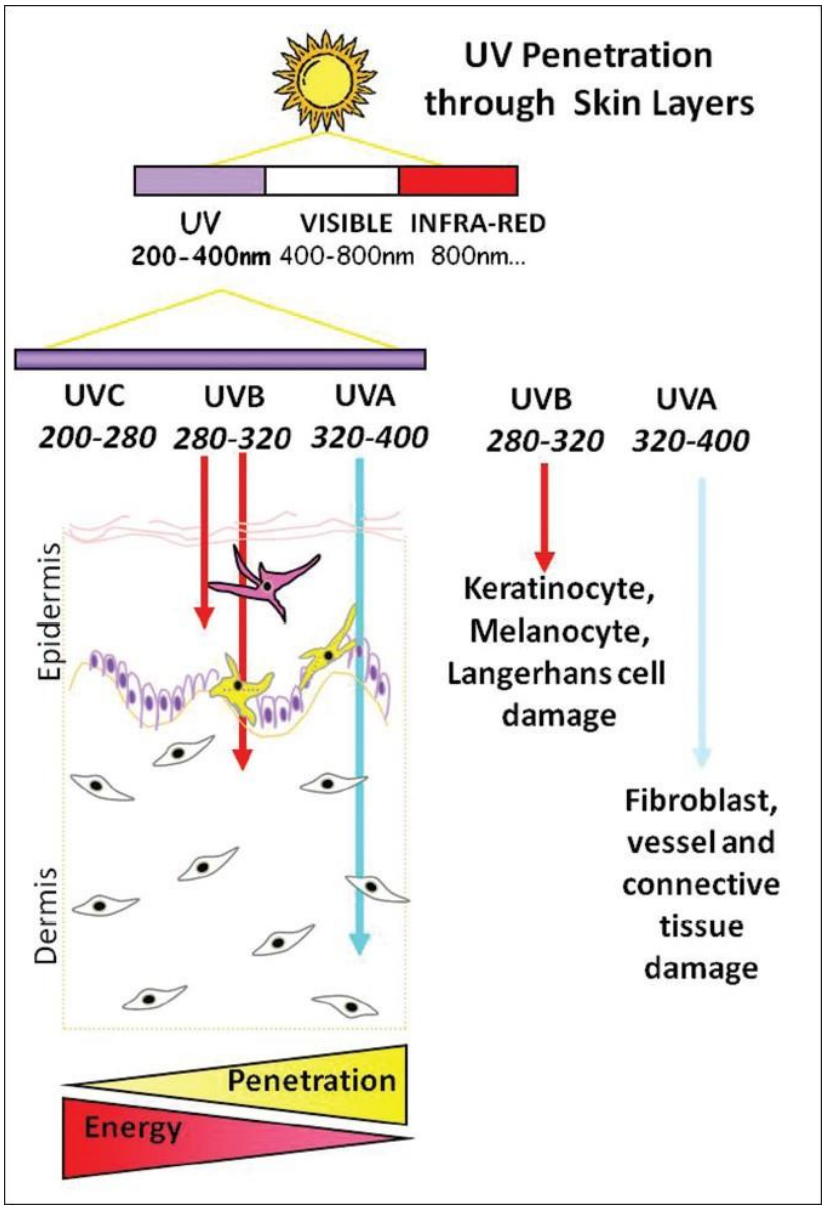

Figure 4. Different Forms of UV Radiation. There are three forms of UV radiation: UVA, UVB, and UVC. UVA ranges from 320-400 nm, UVB ranges from 280-320nm, and UVC ranges from about 100-280nm. In general, UVA and UVB irradiation are the only two forms that make it past the ozone layer. UVA radiation is more penetrating that UVB, reaching the dermis, whereas UVB mainly only penetrates up to the epidermis. Taken from Battie & Verschoore, 2012.

Cis-UCA and cyclic AMP

Numerous theories and hypotheses surrounding the mechanism of action of cis-UCA in the epidermis were being studied during this time period. Another mechanism hypothesized that cis-UCA acted at the intracellular level via cyclic AMP. A 1998 study by Bouscarel tested the effects of both trans-UCA and cis-UCA on cyclic AMP production in three groups of cell lines, human dermal fibroblasts, hamster hepatocytes, and a human adenocarcinoma cell line (Bouscarel et al., 1998). They found that neither isomer of UCA stimulated synthesis of cyclic AMP in any of the three models (Bouscarel et al., 1998). Conversely, they found that cis-UCA alone inhibited synthesis of cyclic AMP in the human dermal fibroblast model and that this inhibition was specific to cyclic AMP synthesis stimulated by prostaglandins (Bouscarel et al., 1998). Another study by Koizumi et al. in 1998 found similar results, showing that cis-UCA inhibited histamine-induced cyclic AMP synthesis in human keratinocytes (Koizumi, Shimizu, Nishino, & Ohkawara, 1998).

Cis-UCA and Cytokines

Another component of the signal transduction pathway of cis-UCA lies in modulation of cytokine activity. A 1999 study by Krulova et al. illustrated that cis-UCA applied topically reduced the likelihood of corneal allograft in a murine model (Krulova, Kuffova, Zajicova, Filipec, & Holan, 1999). Through a further in depth in vitro analysis of the mechanism behind this graft protection, researchers found that cis-UCA induced immunosuppression by activating CD4+ T-lymphocytes and inducing their production of IL-10 (Krulova et al., 1999). This seemed to be highly probable as IL-10 is a known inhibitor of antigen presentation by APC's. However, another 1999 study by Bi et al.

contradicted these findings as they found that cis-UCA actually inhibited expression and subsequent secretion of IL-10 by T-cells (Bi, Xia, & Ni, 1999).

Cis-UCA and an Early Connection to Cancer

Throughout the 1980's and early 1990's, the deleterious effects of cis-UCA on human health were becoming increasingly evident. While a complete understanding of the compound, including its mechanism of action, remained limited, evidence supporting its role in immunosuppression was substantial. An important fact to keep in mind is that the negative effects of UCA arise from its cis form, rather than from its trans isomer, and that UV radiation is the trigger for the isomerization. Marwick raised an interesting question, regarding the reason for an evolutionary adaptation behind a pathway that suppresses the immune system (Marwick, 1995). When an individual experiences a sunburn, components of the "burned" cells appear as foreign invaders to the human immune system. Marwick discusses how in order to prevent autoimmune attacks, the UV radiation activates UCA from its resting trans isomer to its active cis isomer, which then induces immunosuppression to prevent an immune attack against the sunburned epidermal cells (Marwick, 1995). Unfortunately, while UCA may have been evolutionarily adapted to protect against autoimmune attacks during sunburn, it now also has the potential to facilitate immunological evasion by malignant cells created by the same UV radiation (Marwick, 1995). UCA has since been removed from cosmetic ingredients, especially sunscreens.

Research regarding the role of cis-UCA in photocarcinogenesis during this time period remained relatively limited, yet incredibly informative. As an antigen-presenting

cell, Langerhans cells display TAA (tumor-associated antigens) in order to initiate tumor immune responses. In a 1997 by Beissert et al., researchers investigated the role that UCA, either trans-UCA or cis-UCA, play in modulating TAA presentation by Langerhans cells (Beissert et al., 1997). Using a CAF1 murine model immunized against the S1509 spindle cell tumor line, they found that only cis-UCA, not trans-UCA, inhibited antigen presentation activity of epidermal Langerhans cells (Beissert et al., 1997). They also found that only cis-UCA, not trans-UCA, inhibited a delayed-type hypersensitivity response upon antigen exposure (Beissert et al., 1997). Studies of cis-UCA and photocarcinogenesis have also been conducted in human models. In 1998, De Fine et al. measured levels of cis-UCA in patients with skin cancer, patients with previous history of skin cancer, and healthy controls (De Fine et al, 1998). Baseline levels of cis-UCA did not differ significantly between the two groups, however, levels of cis-UCA following a single dose of UV radiation were higher in groups who had skin cancer or previous history of skin cancer, compared to healthy controls (De Fine et al, 1998). This suggests a possibility that individuals suffering from epidermal malignancies experiencing pathological changes that influence current and future exposure to added UV radiation.

In a study by Snellman et al. in 1999, researchers found that there was no significant difference in total UCA and trans-UCA levels in groups of cutaneous malignant melanoma, basal cell carcinoma, and controls (Snellman, Jansen, Rantanen, & Pasanen, 1999). Importantly, trans- to cis-UCA isomerization induced by one dose of UV radiation was lower in skin cancer patients, compared to controls (Snellman et al., 1999).

This further suggests that epidermal responses to future doses of UV radiation are altered in individuals with pathological changes to the skin, including malignant transformations.

Where does UCA stand at the turn of the 20th century?

During the end of the 1990's and the approach of the 21st centuries, a few studies were published that presented alternative viewpoints to the current understanding of UCA. Whereas cis-UCA clearly posed negative effects on epidermal health due to its immunosuppressive properties, the idea of trans-UCA acting as a natural sunscreen still remained. A study done by De Fine et al. in 1996 investigated the photoprotective properties of trans-UCA (De Fine, Wulf, Crosby, & Norval, 1996). They found that topical application of 5% trans-UCA provided a 1.58 sun protection factor in 36 healthy human subjects (De Fine et al., 1996). The authors noted, however, that because the concentration of topical trans-UCA applied was higher than physiological levels of epidermal trans-UCA by about 20-200, it would be unlikely that the main purpose of physiological trans-UCA be based in photoprotection (De Fine et al., 1996). The following year, De Fine et al. continued their research in identifying an endogenous role for UCA and investigated concentrations of UCA in various areas of the epidermis (De Fine et al., 1997). In a study of 36 healthy participants, they found that the percentage of cis-UCA was the highest in body regions exposed to UV radiation (De Fine et al., 1997). These findings suggest that the role of UCA remains in the realm of photobiology and photoprotection, slightly contrasting with their previous work.

A complete understanding of UCA has yet to be established. As shown here and in previous sections of this paper, scientific understanding regarding UCA has grown immensely but remains limited and inconclusive. Bits and pieces of valuable information regarding UCA has been elucidated, but the entire story behind UCA is still relatively fragmented. Still, in recent years, research around UCA and its role in epidermal health has rapidly resurfaced. For instance, a study by De Fine et al. in 1999 illustrated the differential immunosuppressive effects in individuals of varying pigmentation levels (De Fine, Wulf, Crosby, & Norval, 1996). In a study of 28 health subjects, they found higher rates of trans- to cis-UCA isomerization in lighter pigmented versus heavier pigmented individuals, suggesting than lighter pigmented individuals have higher immunosuppressive risk upon UV exposure (De Fine et al., 1996). This suggests potential interplay between cis-UCA and melanin, a plausible hypothesis given the proximity of the two compounds within the epidermis. Numerous more studies such as these have shown promise in completing our understanding of UCA and have continued well into the 21st century. The following section provides an in-depth analysis of research conducted on UCA after the year 2000, specifically focusing on the connection between UCA and epidermal malignant transformations.

ADVANCEMENTS IN UROCANIC ACID RESEARCH (Post-21st Century)

Molecular Understanding of Urocanic Acid

UCA is Derived from Filaggrin

Although current studies have shown that external additions of UCA have induced immunosuppression through the action of cis-UCA, the normative function of baseline physiological UCA levels remains yet to be discovered. Recall that histidine is the precursor to UCA, converted through the action of the enzyme, histidase. Due to high levels of histidine present in the filaggrin protein in the epidermis, researchers have hypothesized that filaggrin proteins are the original source of histidine used to produce UCA in the stratum corneum (Mildner et al., 2010). In a 2010 study by Mildner et al., investigators found that *in vitro* filaggrin knockout models showed a drop in UCA concentration by over half, when compared to baseline controls (Mildner et al., 2010). Despite the association of cis-UCA with induction of immunosuppression, trans-UCA still remains a potent protector against UV irradiation. Thus, reduced levels of total UCA in filaggrin knockout model resulted in reduced levels of trans-UCA and increased UVB-induced damage, including formation of pyrimidine dimers and activation of apoptotic caspase pathways (Mildner et al., 2010).

A recent 2017 study by Simonsen et al. supports these findings, by showing that individuals irradiated with minimal doses of UVB radiation displayed reduced levels of filaggrin protein, in addition to reduced levels of trans-UCA (Simonsen, Thyssen, Heegaard, Kesiz, & Skov, 2017). Interestingly, UV irradiation has been repeatedly shown to induce isomerization of trans- to cis-UCA, but these results now also suggest that UVB

irradiation reduces total levels of UCA by modulating filaggrin levels (Simonsen et al., 2017). It is possible that this serves as an evolutionary adaptation to cap the total level of cis-UCA that can be formed in response to UVB irradiation.

UCA Activation Independent of Method of Irradiation

In the previous studies of UCA analyzed pre-21st century, recall it was noted that the wavelength most effective in inducing trans- to cis-UCA photoisomerization was UVB, in the 240 to 270 nm range. In recent years, further research has expanded on these findings and discovered that not only is UCA photoisomerization dependent on the wavelength, but it is also dependent on the route of irradiation delivery (Brookman, Chaco, & Sinclair, 2002). A 2002 study by Brookman et al. tested the behavior of both trans-UCA and cis-UCA when using pulsed UV irradiation methods (Brookman, 2002). They found that percentage photoisomerization yielded upon pulsed UV irradiation at 266 nm was similar to percentage photoisomerization yielded with standard methods of UV irradiation (Brookman, 2002).

UCA Activation Dependent on Wavelength and Microenvironment

While the percentage photoisomerization of trans- to cis-UCA has not been shown to differ depending on route of continuous or pulsed UV irradiation, evidence has previously showed that it does depend on wavelength range (Webber et al., 1997). Recent research has also suggested that the photoisomerization of UCA depends on the makeup of its microenvironment. A 2004 study by Wallis et al. has shown that in nonaqueous solvents, rates of trans- to cis-UCA photoisomerization correlates linearly with degree of solvent polarity (Wallis, Smith, & Dunford, 2004). This has important implications for production of any potential future compound containing UCA, particularly those that aim

to isolate trans-UCA for its natural sunscreening properties. Topical formulations that alter the microenvironment of the stratum corneum, specifically those that shift the microenvironment towards increasing polarity, may result in increased photoisomerization of UCA and subsequent immunosuppression (Wallis et al., 2004).

Cis-UCA Induction of Immunosuppression in Human Models

While the evidence surrounding cis-UCA and induction of immunosuppression has gained a sizeable foundation of knowledge in the past fifty years, studies in humans have been relatively limited. Studies that have been conducted in humans seem to corroborate findings concluded from *in vitro* and *in vivo* animal models. A study by Dahl et al. in 2010 found that roughly one-third of patients treated with topical cis-UCA experienced immunosuppression when challenged by DNCB (Dahl, McEwen, & Katz, 2010).

Cis-UCA Effects on Immune Cells

The ability of cis-UCA to induce systemic immunosuppression has been well-documented, particularly through studies showing prevention of allograft rejection and reduced CHS and DTH responses using extended doses of cis-UCA. What has been less studied are acute exposures to cis-UCA and their effects on specific populations of T-lymphocytes, a question investigated by Prater et al. in 2003. They observed immunological changes following one day, five days, and 30 day doses of cis-UCA in a C56BL/6N murine model (Prater, Gogal, De Fabo, Longstreth, & Holladay, 2003). Single day doses of cis-UCA resulted in reduced ability of splenocytes to undergo phagocytosis, five-day doses of cis-UCA resulted in reduced cell counts of mature CD4+ and CD8+ thymocytes and increased cell counts of immature CD4+ CD8+ thymocytes,

and thirty-day doses resulted in reduced total cell counts in the thymus and increased total cell counts in the spleen (Prater et al., 2003b).

In addition to regulation of T-lymphocytes, cis-UCA has continually been shown to modulate the function of Langerhans cells, a key immune cell located in the epidermis (Beissert et al., 2001). However, what has been recently shown is that trans-UCA may also be implicated in modulating immune function. A 2016 study by Bruhs showed that non-irradiated mice deficient in histidine displayed stronger immune reactions when challenged with a TNCB sensitizer (Bruhs, Eckhart, Tschachler, Schwarz, & Schwarz, 2016). Because these were non-irradiated mice, the bulk isomer of UCA remained in the trans form, indicating that trans-UCA may also play a role in modulating Langerhans cells of the epidermis (Bruhs et al., 2016).

Cis-UCA and the 5-HT2A Receptor

The 5-HT2A Receptor is a G-protein Coupled Receptor (GPCR) that binds the ligand, serotonin, and has been implicated in many physiological processes, including neurological functioning, smooth muscle contraction, endocrine functioning, so on and so forth. The link between activation of cis-UCA in the epidermis and the downstream suppression of the immune system remains relatively elusive to this day. New research has hypothesized that cis-UCA acts through the 5-HT2A Receptor to induce systemic immunosuppression (Walterscheid et al., 2006). A 2006 study by Walterscheid et al. has shown that cis-UCA binds 5-HT receptors with high affinity and that both anti-cis-UCA antibodies bind 5-HT and anti-5-HT antibodies bind cis-UCA (Walterscheid et al., 2006). Additionally, they found that both 5-HT2A receptor antagonists and anti-serotonin

antibodies were capable of inhibiting immunosuppression *in vitro*, induced by either UV irradiation or application of cis-UCA (Walterscheid et al., 2006).

Cis-UCA as Modulator of Cytokine Expression

The theory of cis-UCA as a regulator of gene expression, particularly for immunomodulatory intermediates, has grown in popularity in recent years. A 2008 study by Kaneko investigated the effect of cis-UCA on expression of immunomodulatory cytokines using a microarray analysis of human keratinocytes (Kaneko et al., 2008). They found that cis-UCA was responsible for the upregulation of 16 total genes, whose functions were associated with apoptosis, arrest of cell growth, stress pathways, and cytokine production (Kaneko et al., 2008). More specifically, they found that cis-UCA was responsible for increasing the synthesis of PGE2 and cytokines: TNF-α, IL-6, and IL-8. Synthesis of these intermediates correlated linearly with cis-UCA dosages (Kaneko et al., 2008). None of these effects were observed with application of trans-UCA (Kaneko et al., 2008).

Opposition Against UCA as an Inducer of Immunosuppressive Cytokines

Recall that in a select few studies analyzed prior to the turn of the 21st century, researchers showed evidence showing that UCA, specifically cis-UCA, acts an as inductor of immunosuppression by inducing the production of immunosuppressive cytokines. Moving into the 21st century, a few more studies have surfaced showing evidence to oppose this mechanism of action. In a 2001 study by Zak et al., researchers tested the ability of cis-UCA to induce release of immunosuppressive cytokines in a PAM-212 murine keratinocyte cell line (Zak et al., 2001). They found no significant changes in expression of key immunosuppressive cytokines, including IL-10, TGF-β, and

TNF-α (Zak et al., 2001). Thus, this study suggests that induction of expression of immunosuppressive cytokines may not be the mechanism by which cis-UCA exerts its influences on the immune system. These results were supported by another 2001 study performed by Amerio, where researchers conducted a CHS assay on TNF-α receptor knockout mice, treated with UV irradiation or intradermal injection with UCA (Amerio et al., 2001). They found that both local and systemic immunosuppression was still observed in TNF-α receptor knockout mice following either UV irradiation or intradermal cis-UCA injection, indicating that TNF-α activation may not be not required for induction of immunosuppression by cis-UCA (Amerio et al., 2001). However, it is important to recognize that these are the results of a small sample size of studies and further studies should be done to confirm the preciseness and accurateness of these conclusions.

Cis-UCA and Permethrin

Permethrin is an insecticide commonly used topically to treat conditions such as scabies and lice. Permethrin is also a known inhibitor of CHS; given these immunosuppressive properties, researchers have hypothesized connections between permethrin and cis-UCA (Prater, Blaylock, & Holladay, 2003). A 2003 study was conducted by Prater et al. investigation levels of CHS inhibition mice treated with intradermal cis-UCA, topical permethrin, or both intradermal cis-UCA and topical permethrin (Prater et al., 2003a). They found that independent application of either intradermal cis-UCA or topical permethrin resulted in inhibition of CHS, with application of both chemicals resulting in an even greater inhibition of CHS (Prater et al., 2003a). Moreover, it was found that TNF-α receptor knockout mice experienced only partial

abrogation of inhibition of CHS when given either of the three treatment method (Prater et al., 2003a). It was also found that INF-y receptor knockout mice experienced partially abrogated inhibition of CHS only when given either cis-UCA or permethrin alone (Prater et al., 2003a). This abrogation was not observed with combined cis-UCA and permethrin in IFN-γ knockout mice (Prater et al., 2003a). These findings suggest that TNF-α and INF-y are not necessarily required in the signal transduction cascade initiated by cis-UCA, but are likely still involved in the pathway. This also suggests that IFN-γ may play a bigger role in inhibition of CHS response when induced by both cis-UCA and permethrin.

UCA and Acidification

Up until the turn of the 21st century, the main understanding of UCA's function within the epidermis has focused on trans-UCA as a potential natural sunscreen and cis-UCA as a regulator of UV-induced immunosuppression. Recent studies have shown that the function of UCA within the epidermis may be broader than originally theorized. A study by Krien et al. in 2000 suggested that UCA may play important roles in the differentiation of the stratum granulosum to the stratum corneum layer and in regulation of enzymatic processes within the stratum corneum (Krien & Kermici, 2000). By analyzing the proton flux via electrode of forearm skin in 12 healthy subjects, they found that the pH of the of the stratum corneum correlated with levels of UCA (Krien & Kermici, 2000). In regards to enzymatic processes, this suggests that UCA likely contributes to the acidic microenvironment of the stratum corneum. Furthermore, because of the pH-dependent activation of the histidase enzyme, researchers concluded that the

production of UCA in the stratum corneum is potentially a self-regulated process (Krien & Kermici, 2000).

Importantly, a conclusive understanding of UCA's role in acidification of the stratum corneum remains fragmented. Still, other studies have shown that UCA is unnecessary for the acidification of the stratum corneum. Rather, these findings assert that sPLA2 and NHE1 are the mediators responsible for modulating the pH stratum corneum (Fluhr et al., 2010). They show that abrogation of sPLA2 and NHE1 pathways reduces acidification of the stratum corneum with resultant increases in pH (Fluhr et al., 2010).

Current Roles in Epithelial Health

Physiological Role of Endogenous UCA

While the effects of excess UCA applied to both *in vitro* and *in vivo* biological systems have been the focus on most UCA studies throughout the past fifty years, the normal, physiological role of UCA has remained rather elusive. Speculation that UCA functions as an endogenous photoprotectant still remains, a theory further studied by Barresi et al. in 2011. These investigators utilized histidase-deficient mice to study the role of UCA in a murine model (Barresi et al., 2011). Mice lacking histidase, and thus lacking UCA, were observed to have higher levels of pyrimidine dimer formations and apoptotic cell death activity, where treatment with topical UCA to mice lacking histidase reversed these effects (Barresi et al., 2011).

Cis-UCA and Phototherapy

Phototherapy, also referred to as light therapy, consists of regulated forms of exposure to specific wavelengths of light to prevent, treat, and manage certain disease

pathologies, particularly those related to dermatological function. Despite the negative reputation cis-UCA has earned in regards to its immunosuppressive properties, UCA has been hypothesized to serve important roles in the physiological effects associated with phototherapy (Beissert & Schwarz, 2002). We have already seen that cis-UCA is capable of suppressing immune reactions to sensitizer agents, including suppression of CHS and DTH. The ability of cis-UCA to participate in phototherapeutic pathways has been attributed to the likelihood that it reduces inflammation (Beissert & Schwarz, 2002).

Phototherapy indications

NB–UV-B	PUVA	UV-A1
Common	Common	Common
Psoriasis	Psoriasis	Morphea
Vitiligo	Vitiligo	Lichen sclerosis
Atopic dermatitis	Atopic dermatitis	Atopic dermatitis
Mycosis fungoides	Mycosis fungoides	Less common
Pruritus (associated with renal disease, polycythemia vera)	Less common	Cutaneous graft-versus-host disease
Less common	Alopecia areata	Cutaneous mastocytosis
Acquired perforating dermatosis	Cutaneous graft-versus-host disease	Granuloma annulare
Chronic urticarial	Cutaneous mastocytosis	Lichen planus
Cutaneous graft-versus-host disease	Polymorphous light eruption	Mycosis fungoides
Polymorphous light eruption	Dermatitis herpetiformis	Necrobiosis lipoidica
Cutaneous mastocytosis	Dyshidrotic eczema	Pityriasis lichenoides
Granuloma annulare	Granuloma annulare	Sarcoidosis
Lichen planus	Histiocytosis	Systemic lupus erythematosus
Lichen simplex chronicus	Lichen planus	
Lymphomatoid papulosis	Lichen sclerosis	
Parapsoriasis	Morphea	
Pityriasis lichenoides	Pityriasis rosea	
Pityriasis rosea	Urticaria	
Pityriasis rubra pilaris		
Seborrheic dermatitis		

Adapted from Walker D, Jacobe H. Phototherapy in the age of biologics. Semin Cutan Med Surg 2011;30(4):190–8; with permission.

Table 2. Indications for Phototherapy. A specific form of phototherapy is UV phototherapy, which utilizes various wavelengths within the UV spectrum to treat diseases, particularly dermatologic conditions. There are three main types of UV phototherapy: narrowband UVB, psoralen and UVA, and UVA1. Taken from Totonchy & Chiu, 2014.

Ocular Epithelial Health

Though immunosuppression is deleteriously associated with certain disease pathologies, such as malignant transformation, it can have potently beneficial effects on treatment and management of other conditions that require inflammation reduction. Some ocular conditions serve as prime examples, including uveitis, blepharitis, and keratitis. Inflammation associated with these conditions is not only potentially damaging to ocular function, but results in extreme pain and uncomfortability for patients. A 2009 study by Viiri et al. illustrated that cis-UCA may serve as a promising therapeutic agent against inflammation in human ocular cells, specifically corneal and conjunctival cells (Viiri et al., 2009). They found that application of cis-UCA to an *in vitro* assay of these cell populations abrogated the pro-inflammatory effects associated with UVB irradiation and resulted in decreased secretion of IL-6, IL-8, and reduced apoptotic caspase pathways (Viiri et al., 2009).

In addition to inhibiting secretion of pro-inflammatory mediators in ocular cells, cis-UCA has also been shown to block ocular inflammation through inhibition of the SAPK/JNK pathway (Jauhonen et al., 2011). Normally, part of the pro-inflammatory pathway induced by UVB irradiation on ocular cells facilitates increased expression of c-Jun, c-Fos, and NF-KB (Jauhonen et al., 2011). In a 2011 study by Jauhonen et al., researchers found that an *in vitro* application of cis-UCA inhibited binding of transcription factors and reduced gene expression of c-Jun and c-Fos in human corneal cells, not NF-KB (Jauhonen et al., 2011). Because of the anti-inflammatory effects of cis-UCA in response to UVB irradiation, researchers speculate it has high potential to become a potent therapeutic to control inflammation within this organ system (Jauhonen

et al., 2011). Importantly, these results differ from the effects of cis-UCA often observed in the epidermis. Thus, it is important to recognize that the effects of cis-UCA may vary across organ systems and this should be taken into consideration when developing new therapeutics.

UCA and Cancer

While cis-UCA has been traditionally associated with UV-induced immunosuppression that has deleterious associations with malignant epidermal transformations, researchers have unveiled an anti-proliferative effect associated between cis-UCA and bladder cancer (Arentsen et al., 2012). Application of cis-UCA in a rat bladder cancer cell line and a murine bladder cancer model showed reduction of cellular proliferation in the cell lines and reduction of tumor growth in the *in vivo* tumor model (Arentsen et al., 2012). This application was specific to non-muscle invasive bladder cancer with cis-UCA delivered through intravesicular agents (Arentsen et al., 2012). Thus, cis-UCA may not be a viable treatment option for muscle invasive bladder cancers.

In addition to potential as a therapeutic for bladder cancer, UCA has also shown promise as a treatment for lung cancer as well. This has been combined with a relatively new form of therapeutic delivery, aerosol delivery, due to its less invasive and non-viral based delivery system. A 2006 study by Jin et al. utilized a novel urocanic-acid-modified chitosan (UAC) treatment method delivered through aerosol gene delivery to a lung cancer murine model (Jin et al., 2006). The urocanic acid-based delivery method is used to deliver the PDCD4 gene to cancer cells in mice lungs (Jin et al., 2006). Because of the ability of PDCD4 overexpression to activate apoptotic cellular pathways in other forms of cancer, including breast cancer, it was employed to test its ability to activate apoptotic

pathways in lung cancer cells (Jin et al., 2006). It was found that mice treated with the UAC-method carrying the PDCD4 gene displayed higher rates of cancer cell apoptosis, reduced rates of cellular growth, and inhibits angiogenesis (Jin et al., 2006).

UCA and Skin Conditions

Oedema and Erythema

Oedema and erythema are both forms of epidermal inflammation. Due to the immunosuppressive properties of cis-UCA, studies have been conducted investigating the potential of UCA to act as an anti-inflammatory therapeutic agent. In a 2012 study by Laihia et al., researchers found that topical application to cis-UCA to murine models with epidermal inflammation showed reduced signs of inflammation by the end of the treatment period (Laihia, Taimen, Kujari & Leino, 2012). Importantly, results showed cis-UCA displayed stronger acute anti-inflammatory properties compared to standard anti-inflammatory treatment agents, including hydrocortisone (Laihia et al., 2012). If formulated safely and effectively, cis-UCA may serve as a potent target for treating inflammatory skin conditions.

Atopic Dermatitis

Atopic dermatitis, also referred to as eczema, is an inflammatory skin condition that causes skin to become itchy, red, swollen, dry, so on and so forth. The current understanding of cis-UCA as an immunosuppressor has led to many studies being conducted regarding therapeutic potential of cis-UCA in treating pro-inflammatory conditions. In phase 1 and 2 double-blinded randomized control clinical trials conducted by Peltonen et al. in 2014, the therapeutic safety and efficacy of topical application of cis-UCA was tested in 16 subjects (Peltonen et al., 2014). Results showed reduced inflammation in groups receiving the cis-UCA treatment, compared to groups receiving

placebo. Furthermore, no toxicity was observed and the treatment itself was tolerated well by the subjects receiving it (Peltonen et al., 2014).

Chronic Spontaneous Urticaria

Chronic Spontaneous Urticaria is an inflammatory skin condition associated with hives and often angioedema. Because previous research has shown that high levels of filaggrin protein have been associated with increased severity of CSU, studies have been conducted investigating the role that cis-UCA, a derivative of filaggrin, may play in disease progression (Pham et al., 2017). It was found that cis-UCA was able to increase degranulation of basophils and mast cells, which is hypothesized to contribute to the pro-inflammatory symptoms of CSU (Pham et al., 2017). It was also found that CSU patients possessed higher levels of cis-UCA compared to trans-UCA, indicating that the high levels of cis-UCA may be responsible for symptoms such as the redness and itching of hives (Pham et al., 2017). Targeting cis-UCA to reduce its epidermal levels may serve as a potential therapeutic pathway for treating CSU.

UCA and Vitamin D

Due to the dependence of Vitamin D production on UVB radiation from sunlight, considerable interest has arisen concerning the potential interplay between cis-UCA and Vitamin D synthesis in the epidermis. A 2016 study by Landeck et al. found that a sample of 28 contact dermatitis patients treated with sub-erythemal UVB irradiation displayed the expected conversion of trans- to cis-UCA in addition to increased 25-hydroxyvitamin D levels (Landeck et al., 2016). The levels of vitamin D were shown to be inversely proportional to levels of trans-UCA, as expected (Landeck et al., 2016).

UCA and Autoimmune Encephalomyelitis

Autoimmune encephalomyelitis encompasses a wide variety of diseases that involve immune attack against neurological cells. It has been shown that UVB irradiation protects against experimentally-induced autoimmune encephalomyelitis, prompting researchers to speculate whether the immunosuppressive properties of cis-UCA are involved in this process. In a recent 2017 study by Irving et al., researchers tested protection against experimentally-induced autoimmune encephalomyelitis using UVB irradiation, both isomeric forms of UCA, and a control treatment (Irving, Marling, Plum, & DeLuca, 2017). They found that UVB irradiation protected against disease progression by nearly 80%, where cis-UCA levels were prominent (Irving et al., 2017). However, increasing cis-UCA levels further did not confer any additional protection, suggesting that other mechanisms induced by UVB irradiation are responsible for protection against experimentally-induced autoimmune encephalomyelitis (Irving et al., 2017).

DISCUSSION OF THERAPEUTIC APPLICATIONS

Potential Role as Therapeutic Target for Skin Cancer

UCA as a Measure of Commercial Sunscreen Efficacy

From SPF (sun protection factor) 15 to SPF 100, sunscreen has served as one of the main methods of risk reduction and prevention of skin cancer for generations. Though most current studies discussing the potential roles of UCA as a therapeutic target for skin cancer have focused on molecular mechanisms, one study provided a novel suggestion to use UCA as a marker for current commercial sunscreen efficacy (Van Der Molen et al., 2000). Using both *in vivo* models of healthy human volunteers and *ex vivo* models of human skin, researchers determined that effectiveness of sunscreens measured by sun protection factor correlated with percentage protection against photoisomerization from trans-UCA to cis-UCA (Van Der Molen et al., 2000). In addition, they found that broad-spectrum sunscreens provided greater protection against UCA photoisomerization, indicating that sunscreen UV absorption spectrums may be of greater priority compared to penetration characteristics (Van Der Molen et al., 2000).

A 2005 study by McLoone et al. have also corroborated this theory by investigating the rates of trans- to cis-UCA photoisomerization in five healthy human subjects (McLoone et al., 2005). They found that maximal production of cis-UCA from the trans isomer occurred in the UVB range of 280 to 310 nm (McLoone et al., 2005). This encompasses a UV spectrum wider than those currently specified for protection against UV-induced erythema. Speculation has grown in recent years surrounding a gap between the ability of commercial sunscreens to protect against UV-induced erythema and UV-induced immunosuppression. The requirement of a broad spectrum formulation

to reduce UCA photoisomerization may serve as the solution for that gap (McLoone et al., 2005).

A decade into the new century, research has expanded on the initial idea of using cis-UCA as a measure for the effectiveness of commercial sunscreens. Recently, research has focused on employing non-invasive means of measuring UCA levels through Raman spectroscopy as, which is both time and cost efficient for researchers but more importantly, safe and effective for study participants. A 2008 study by Egawa et al. found that confocal Raman spectroscopy effectively measured the levels of UCA, especially trans-UCA in this study, in an *in vivo* model of about 20 subjects (Egawa & Iwaki, 2008). Importantly, application of sunscreen decreased the effects of UV radiation on trans-UCA levels, and this was accurately captured by the Raman spectroscopy method (Egawa & Iwaki, 2008). A few years later, Egawa et al. expanded on these findings and tested the ability of the Raman spectroscopy to measure both cis-UCA and trans-UCA levels specifically within the stratum corneum, validated using stratum corneum paper strip samples (Egawa, Nomura, & Iwaki, 2008). The rose in levels of cis-UCA and fall in trans-UCA levels following UV irradiation were correctly measured by Raman spectroscopy (Egawa et al., 2008).

Use of IL-12 to Modulate cis-UCA

While trans-UCA has been relatively well-established as a protective mediator against the effects of UV irradiation, cis-UCA has been implicated in UV-induced immunosuppression associated with enabling malignant evasion of the immune system. One potential target to prevent this evasion is targeting the cytokine IL-12, an immunological mediator with anti-immunosuppressive properties, as an indirect means of

targeting cis-UCA (Beissert et al., 2001). A 2001 study by Beissert et al. investigated the ability of IL-12 to inhibit the immunosuppressive properties of cis-UC in both *in vitro* and *in vivo* models (Beissert et al., 2001). They found that application of IL-12 was capable of suppressing immunosuppression induced by cis-UCA *in vitro* in Langerhans Cells, enabling the LCs to regain function of antigen-presentation (Beissert et al., 2001). They also found that application of anti-cis-UCA antibodies to mice with skin cancer reduced tumor yield in this *in vivo* murine model, indicating that inhibition of cis-UCA may serve as a potent target for preventing, treating, and managing carcinoma induced by UV irradiation (Beissert et al., 2001).

UCA and Reactive Oxygen Species

Recognition of the beneficial effects of trans-UCA as a protectant against the effects of UV irradiation has led to research focused on isolating this isomer and ensuring maintenance of the trans form once it has reached the epidermis. Reactive oxygen species (ROS) are highly reactive compounds derived from molecular oxygen (O2) that are associated with a number of deleterious effects, from the cellular level in induction of oxidative damage and stress to pathological levels with neurocognitive decline and cancer progression. Recent research has provided evidence suggesting trans-UCA is capable of quenching reactive oxygen species produced by UV irradiation. A 2002 study by Haralampus-Grynaviski et al. confirmed the production of reaction oxygen species by UVA irradiation in a cholesterol hydroperoxide assay followed by quenching of these reactive oxygen species by application of trans-UCA (Haralampus-Grynaviski et al., 2002). A later 2011 study by Tiwari et al. also concluded that UCA could serve as a

hydroxyl radical scavenger, contributing to the theory that UCA acts as a physiological antioxidant (Tiwari & Chand Mishra, 2011).

The development of a trans-UCA therapeutic is challenging, particularly because of the importance of maintaining UCA in the trans form and preventing photoisomerization to the cis form. Interestingly, whereas we had seen above that trans-UCA was implicated in quenching of reactive oxygen species, the cis form of UCA has been shown to contribute to formation of reaction species under UV irradiation (Menon & Morrison, 2002). A 2002 study by Menon et al. illustrated that cis-UCA was capable of inducing the development singlet reactive oxygen species following UCA irradiation (Menon & Morrison, 2002). A later 2011 study by Kaneko et al reported that cis-UCA was capable of generating reactive oxygen species in a human keratinocyte cell line, which was associated with the ability of cis-UCA to increase prostaglandin production and promote cell apoptosis (Kaneko et al., 2011).

UCA and DNA Photoproducts

DNA damage, particularly the production of DNA photoproducts including pyrimidine dimers, plays a primary role in cancerous transformations within the epidermis. For the most part, the two processes induced by UV irradiation that have been linked to cancer progression, DNA damage with photoproduct production and immunosuppression induced by cis-UCA have remained relatively independent of one another. Recent research has suggested that a link may exist between these two processes (Snellman, Xu, Pasanen, Laihia, & Hemminki, 2002). If this is the case, individuals with high concentrations of epidermal cis-UCA combined with defective DNA repair mechanisms would suffer from an even greater risk of UV-induced skin cancer compared

to possessing either risk factor alone. Although data on this process remains somewhat limited, a 2002 study by Snellman et al. found that a few potential links between processes involving DNA photoproducts, including DNA photoproduct repair mechanisms, and concentration of cis-UCA within the epidermis (Snellman et al., 2002).

UCA and Tumor Growth

While most research has supported the link between cis-UCA and progression of tumor growth, a few, select studies have shown otherwise. In a 2002 study, Macve & Norval found that injection fibrosarcoma (FS) cells into a murine model irradiated with UVA radiation resulted in proliferation of tumor cells, compared to non-irradiated controls (Macve & Norval, 2002). Importantly, these observations were not replicated when cis-UCA was applied topically of injected intradermally in place of UVA irradiation, suggesting that cis-UCA is not the mediator by which UV radiation induces tumor cell proliferation (Macve & Norval, 2002). Additionally, it was found that application of anti-cis-UCA antibodies did not reverse the effects originally seen in mice irradiated with UVA radiation, further suggesting other pathways are responsible for tumor growth upon exposure to UV radiation (Macve & Norval, 2002).

UCA as a Biomarker

The relative physiological increase of UCA, particularly total UCA and cis-UCA content, following exposure to UV irradiation has led to speculation regarding the use of cis-UCA as a biomarker for UV exposure. A 2005 study by Sastry et al. found that minimal erythemal doses of UV radiation resulted in nearly five-fold increase in UCA biomarker, measured using HPLC (high-performance liquid chromatography) analysis of human urine samples (Sastry et al., 2005). The UCA biomarkers was defined as a UCA

ratio, a ratio of cis-UCA to trans-UCA, or as the amount of cis-UCA corrected for urine volume (Sastry et al., 2005).

Because of the damaging effects of cis-UCA on the immune system and its association with cancerous progression in the epidermis, levels of cis-UCA can also potentially serve as a useful biomarker when monitoring the safety of UV-associated activities. Excessive overexposure to sunlight has long been established as a risk factor for developing skin cancer, but use of artificial UV exposure such as through commercial suntanning has been posed as an even greater risk factor. Despite warnings regarding this activity, commercial suntanning remains an activity highly engaged in by the public. Part of this may stem from a lack of a direct biomarker linked to increased risk of skin cancer in that can be measured in individuals who engage in commercial suntanning. In a 2002 study by Ruegemer et al., researchers found that total UCA and cis-UCA content increased in UV-exposed skin, even prior to any clinically observable effects including changes in skin pigmentation and observed sunburn (Ruegemer et al., 2002).

Antigen Viability Facilitates Direction of UCA as Therapeutic Target

As mentioned previously, exposure to UV radiation causes the activation of two different pathways associated with cancer pathogenesis: DNA damage with photoproduct development and activation of cis-UCA and immunosuppression. Recent research has shown that the viability of the antigen may determine which of these pathways dominates and thus will direct treatment strategies used to prevent or treat cancerous transformations (Kim et al., 2003). A novel study by Kim et al. in 2003 applied either anti-cis-UCA antibodies or endonucleases to a murine model to identify which of these treatments would induce immunosuppression of DTH (Kim et al., 2003). If mice treated with anti-

cis-UCA antibodies displayed abrogation of DTH suppression, it would indicate that the cis-UCA pathway was dominant. Conversely, if application of endonucleases resulted in blocking DTH suppression, one could reasonably conclude that the DNA damage-associated pathway was dominant. Researchers found that anti-cis-UCA antibodies were only capable of abrogating DTH suppression when live antigen sensitizers were used to induce DTH (Kim et al., 2003). Conversely, it was found that endonucleases were only capable of blocking DTH suppression when non-live antigen sensitizers were used to induce DTH (Kim et al., 2003). Thus, when designing therapeutics to inhibit immunosuppression, whether in the context of cancer or other conditions, properties of the antigen need to be taken into consideration for the treatment to be effective.

The Return of the 5-HT2 Receptors

In the previous section analyzing post-2000 early research regarding the role of UCA in both physiological and pathological pathways, some studies suggested that cis-UCA was capable of binding to 5-HT2A receptors and potentially served as an important focal point of the cis-UCA immunosuppression signal transduction cascade. Modern day research has expanded on these findings, which has not only confirmed that cis-UCA is capable of binding serotonin receptors, but that this binding is responsible for cis-UCA-mediated immunosuppression and that blocking this binding could abrogate inhibition of the immune response (Ulrich, 2007). In a 200 7 paper by Ulrich, researchers found that use of a selective serotonin receptor antagonist, originally to be used as a control measure, was able to inhibit the immunosuppressive properties of cis-UCA (Ulrich, 2007). To further investigate upon this finding, they found that application of excess amounts of cis-UCA was able to displace bound cis-UCA or bound 5-HT to human

serotonin receptors, whereas 5-HT receptor antagonists were able to inhibit this binding entirely (Ulrich, 2007).

These findings were also supported in a 2010 study by Sreevidya et al., where serotonin receptor antagonists were shown to reduce UV-induced immunosuppression and UV-induced carcinogenesis, in addition to promoting DNA repair (Sreevidya et al., 2010). Furthermore, researchers found that the ability of serotonin receptor antagonists to promote DNA repair was blocked when applied to mice deficient in DNA repair enzymes, indicating that serotonin receptor antagonists interact with DNA repair mechanisms (Sreevidya et al., 2010).

UCA Mimic

Although the normal form of cis-UCA has been generally shown to have deleterious effects within the epidermis and systemically in regards to the immune system, researchers have begun testing the possibility of modifying UCA into a form with only beneficial effects. In a recent 2015 study, Ito et al. prepared urocanic acid-based chitin nanofibers and found that application of these UCA nanofibers protected against sunburn in a murine model (Ito et al., 2015). Other studies have also supported this theory of developing modified-UCA therapeutics that can be used to protect against UV irradiation, without the immunosuppressive effects observed by using normal form cis-UCA (Mollet et al., 2017).

Non-Melanoma Malignancy

Recall that non-melanoma malignancies refer to skin cancers that do not derive from melanocytes, namely base cell and squamous cell carcinomas. To test the

association of cis-UCA levels to progression of non-melanoma malignancies in a human model, Decara et al. analyzed UCA content in UV-exposed and non-UV-exposed skin biopsies of BCC and SCC patients (Decara et al., 2008). Whereas no significant differences were found in levels of total UCA concentration in UV-exposed skin sites between skin cancer patients and healthy controls, increased concentrations of cis-UCA was found specifically in SCC patients, compared to BCC patients and healthy controls (Decara et al., 2008). Thus, this suggests that progression of SCC may be linked to cis-UCA induced immunosuppression.

Individuals deficient in the histidase gene are expected to have low levels of UCA. Thus, researchers have begun to investigate the genetic-environmental relationship in individuals with histidase deficiency and exposure to UV irradiation, and its subsequent impact on developing non-melanoma skin cancer. In a large 2008 population-based study, Welsh et al. found that individuals with variation in the histidase gene who are exposed to higher lifetime sunburn counts and higher lifetime UV exposures had higher risks of developing non-melanoma skin cancers compared to individuals without variation in the histidase gene (Welsh et al., 2008).

Importantly, the connection between UCA and non-melanoma malignancies is not yet fully understood. While some studies have found connections between UCA and non-melanoma malignancies, both negative and positive, others suggest that no connection exists. In a 2001 study by De Simone et al., researchers found that the levels of total UCA or cis-UCA did not vary significantly between individuals who had non-melanoma skin cancers versus healthy controls (De Simone et al., 2001).

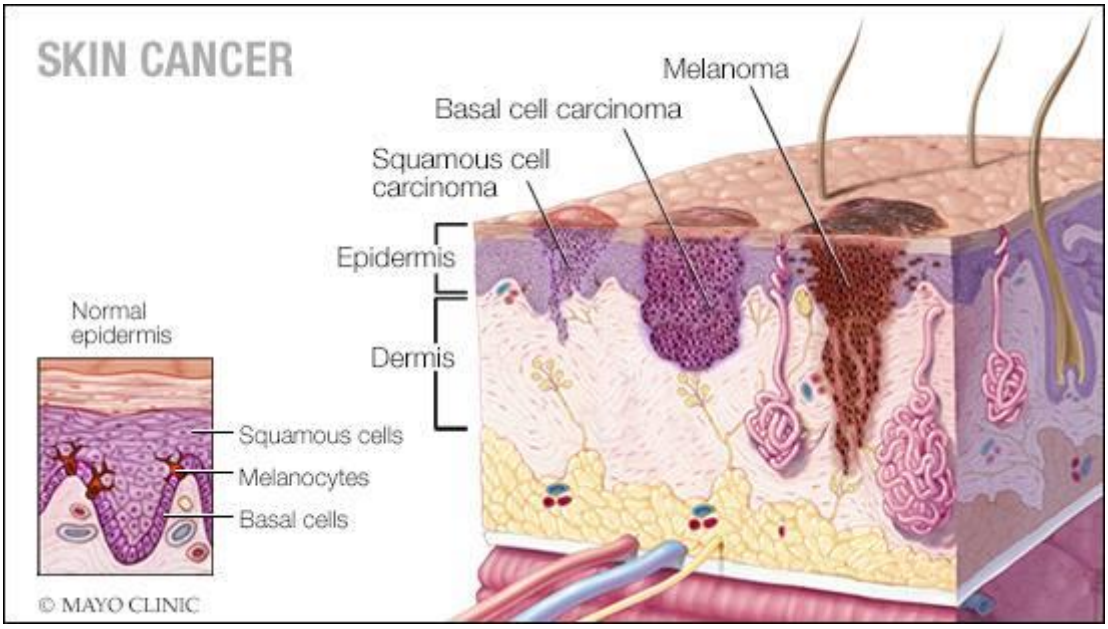

Figure 5. Different Forms of Skin Cancer. Skin cancers are categorized by their cell of origin. There are two main types of skin cancer: melanoma and non-melanoma skin cancer. Non-melanoma skin cancers can be further broken in basal cell carcinoma and squamous cell carcinoma. Basal cell carcinoma is the most common, followed by squamous cell carcinoma. Melanoma is the deadliest form of skin cancer. Taken from Kelly, 2018.

Melanoma Malignancy

Melanoma refers to cancerous transformations in melanocyte cells found in the skin. The role of cis-UCA in melanoma malignancies remains under debate, but recent research has begun to shed light on potential relationships between the two. A 2010 study by Laihia et al. tested the protodynamic concept, involving the ability of cis-UCA to acidify epidermal microenvironments, in melanoma cancer cell lines and observed the resultant effects (Laihia et al., 2010). Interestingly, application of cis-UCA reduced cell counts of human melanoma, cervical carcinoma, and fibrosarcoma cell lines through activation of caspase pathways and induction of apoptosis (Laihia et al., 2010). This is

one of the few studies that have suggested cis-UCA can serve as an anti-cancerous therapeutic agent.

Particularly in the realm of melanoma malignancies, UCA has generally been shown to have more protective effects, compared to deleterious effects. The reasons for this remain unknown, but possibilities include variation in the interaction of UCA with specific populations of tumor cells, such as melanocytes compared to keratinocytes, and variation in the way melanoma and non-melanoma malignancies interact with the immune system. A case-control study by Thyssen et al. in 2018 tested a growing theory that filaggrin contributes to protection against skin cancer, partially through the filaggrin derivative UCA, and that loss of the filaggrin protein through loss-of-function mutations would likely increase the risk of malignant transformations in the epidermis (Thyssen et al., 2018). However, they found that a loss of function mutation in the filaggrin gene was not significantly associated with risk of developing melanoma (Thyssen et al., 2018).

CONCLUSION

Despite being a key player within the epidermis, with roles in both physiological skin health and in modulation of the immune system, Urocanic acid remains a compound that few have heard about. After its discovery in the 1950's as a novel, endogenous photoprotectant, many believed it would be the key to developing cosmetics and therapeutics to radically reduce rates of skin cancer. Unfortunately, the discovery that the cis-form of Urocanic acid mediates immunosuppression and could contribute to cancer cell evasion of the immune system, quickly dispelled those beliefs. After that time, scientific investigation around Urocanic acid took a large dip, with only a few studies being published every so often. The beginning of the 21st century came with a resurgence in research around the endogenous photoprotectant that many had forgotten about. New findings, including UCA's derivation from the filaggrin protein, downregulation of T-lymphocytes and Langerhans cells by cis-UCA, and interaction with serotonin receptors have begun clarifying the physiological role of endogenous UCA, and provide insight into methods that therapeutics can be adapted to treat epidermal malignancies. In addition to serving a potential measure for efficacy of commercial sunscreens, theories of using the IL-12 cytokine to suppress cis-UCA function holds great potential as a means of controlling tumor growth in in vivo murine cancer models. Research showing that UCA may serve as a biomarker for various skin conditions, including risk of skin cancer, hold great promise in improving detection and prevention strategies. Still, a full molecular understanding of UCA's interactions with the integumentary system and the immune system is far from being complete. A few studies pointing in a particular direction are

unfortunately not enough to draw definitive conclusions from. Even more, contradictory evidence from various studies make drawing conclusions immensely more difficult. A prime example analyzed was the effect of UCA on reactive oxygen species, a key cell stressor and risk factor for cancerous transformations. Some research has shown that trans-UCA is capable of acting as an antioxidant, whereas others have shown cis-UCA acting as a contributor to the formation of reactive oxygen species. Clearly, if we are to target UCA as a therapeutic for skin cancer, the differences in effects between trans-UCA and cis-UCA must be fully elucidated. Another prime example was the varying evidence regarding UCA and forms of skin cancer. Cis-UCA seemed to be deleteriously associated with non-melanoma skin cancers, thus suggesting cis-UCA should be inhibited if targeted for therapeutic intervention. On the other hand, cis-UCA seemed to be protectively associated with melanoma skin cancers, which is, in general, in opposition to a large majority of the evidence we have seen in regards to cis-UCA and cancerous development. Thus, current research has provided extensive knowledge and direction for defining the epidermal and immunological pathways UCA plays a role in, but further research must focus on solidifying this understanding if UCA is ever to be targeted as a therapeutic for melanoma and non-melanoma malignancies.

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CURRICULUM VITAE

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1994

EDUCATION:

- **University of California Berkeley** **August 2016**
  Bachelor of Arts in Molecular and Cell Biology and Minor in Nutritional Science
- **Boston University School of Medicine** **Expected May 2019**
  Master of Science in Medical Science
- **Boston University School of Public Healt** **Expected May 2019**
  Master of Public Health: Epidemiology and Biostatistics

EXPERIENCE:

- **Varsity Tutors** **June 2017 - June 2018**
  MCAT Tutor
  Facilitated learning for pre-medical students, by implementing individual-tailored study plans and aiding with mastering of core content in preparation for the MCAT Exam. Encouraged active participation in learning and individual growth as a student and young professional.
- **Falcon Critical Care Transport** **January 2016 - May 2017**
  Emergency Medical Technician
  Ensured safe and reliable transport and patient care during interfacility transports. Skilled in medical and trauma emergency care. Experience working under CCT RN. Assisted in training of new employees.
- **Berkeley Medical Reserve Corps** **August 2015 - February 2017**
  Volunteer Emergency Medical Technician
  Led patient care services and ensured safety of the UC Berkeley Campus and community by providing medical care in event of emergency, at large-scale campus events, and public health initiatives.
- **UCSF Diabetes Center Koliwad Lab** **May - December 2016**
  Laboratory Research Assistant
  Contributed to diabetes and obesity-associated metabolic disease research under MD studying association between metabolic syndrome and human microbiome composition via 16S rRNA sequencing and meta-analysis. Skills include DNA/RNA extraction, qPCR, statistical analysis, etc.
- **UC Berkeley Hellerstein Lab** **July 2015 - May 2016**
  Undergraduate Research Assistant

Engaged in UCSF research studies measuring endogenous glucose production using deuterium labeled glucose. Project duties include isolation and derivatization of glucose from human plasma samples and percent enrichment analysis from GC-Mass Spec.

❖ **Alta Bates Medical Association** **August 2014 - March 2016**
Surgical Pre-Op/Cardiology Clinical/Gift Shop Volunteer
Demonstrated initiative in supporting pre-op RNs and staff with duties including safe escorting of patients through surgical preparation processes, preparing changing rooms, gurneys, preoperative patient packets, delivering laboratory specimens, and discharging post-op patients as requested.

LEADERSHIP & ACTIVITIES:

- **Boston Healthcare for the Homeless** **January - December 2018**
Meal Service
Collaborated with staff to effectively provide meals to local homeless and underserved populations.
- **UC Berkeley Human Anatomy Lab** **August - December 2015**
Undergraduate Student Instructor
Led small group discussions and teaching of fellow undergraduate students in human anatomy laboratory curricula presented through using models, human cadavers, and wet specimens.
- **UC Berkeley Issues: Berkeley Medical Journal** **August 2014 - May 2015**
Writer
Writer for UC Berkeley's medical and health sciences undergraduate publication on advancements in biomedical research, modern day medical practice, and public health.
- **University of California Berkeley Cheer Team** **April 2014 - May 2015**
Spirit Squad Member and Social Media Chair
Performed at and support all home football, basketball, and campus sporting events in addition to rallies, alumni, and community events. Educated and shared mission of Cal Spirit to Cal community and promote alumni relations through social media outlets.
- **UC Berkeley American Red Cross at Cal** **September 2013 - January 2015**
Blood Services Coordinator
Hosted and planned monthly campus blood drives, including coordinating campus publicity and sponsorships. Organized biweekly meetings for committee members and training of volunteers.
- **UC Berkeley Suitcase Clinic** **August - December 2014**
Meal Service and Health Service
Actively engaged in serving meals, shelter, and rehabilitation services to local homeless and underserved populations. Facilitated group discussions regarding homelessness and needs of underserved communities.
- **American Red Cross at Cal/Bay Area Chapter** **April - December 2014**

First Aid/CPR/AED Instructor
Taught courses training individuals as lay responders with proficiency in CPR, AED usage, and first aid.

HONORS & AWARDS:

- *UC Berkeley Incentive Awards Program Scholar*
- *Boston University GMS BI751 Biochemistry Award*
- *Boston University Women's Council Scholarship 2018-2019*
- *Boston University School of Public Health Dual Degree Scholar*

SKILLS:

- Exceptional communication and people skills, emphasizing diversity and teamwork and a quick learner with excellent time management, organizational skills and hard work ethic.
- Goal oriented thinker upholding high level of professionalism, respect, and reliability.
- Strong science background including coursework in human anatomy and physiology with labs, biochemistry, nutritional science, histology, etc.
- Language: Proficient in conversational Chinese (Mandarin).
- Strong background in SAS, R, Prism, and Excel statistical platforms.