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SURVIVIN AND p53 EXPRESSION IN FELINE ORAL SQUAMOUS CELL CARCINOMA AND CORRELATION WITH PROGNOSIS

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Squamous cell carcinoma (SCC), the most common oral neoplasm of cats, demonstrates aggressive local invasion and has a poor prognosis. In humans, mutation of the p53 gene, crucial in cell cycle arrest and induction of apoptosis in damaged cells, is common in neoplasms. Survivin, an inhibitor of apoptosis, is frequently overexpressed in many types of human cancer. Studies suggest that wild-type p53 inhibits survivin expression, while mutated p53 does not. The purposes of this study included immunohistochemical examination of survivin and p53 expression in feline oral SCC and determination of a correlation between p53 mutation and survivin overexpression, as well as comparison with survival time. Survivin expression was noted in 80% (24/30) of cases, while 43.3% (13/30) of cases were positive for p53. No statistically significant correlation was noted between p53 and survivin expression, even when corrected for age, breed, and sex; and survival time was not affected.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Squamous cell carcinoma is the most common oral neoplasm of cats. The majority of these neoplasms demonstrate aggressive local invasion and have a poor prognosis and long-term survival, despite treatment with surgical excision; radiation; or chemotherapy. Similarly, oral squamous cell carcinoma is a common malignant neoplasm in human medicine and has a poor long-term survival rate. Apoptosis, or programmed cell death, is a mechanism to maintain tissue homeostasis. Loss of apoptotic mechanisms is believed to promote tumorigenesis by augmenting mutations and contributing to genetic instability. The protein p53 has a crucial role in cell cycle arrest and induction of apoptosis in damaged cells. Mutation of the p53 gene is the most common genetic aberration reported in human neoplasia, suggesting that loss of wild-type p53 is an important step in tumor development. Mutation of p53 may also contribute to growth of neoplastic cells and resistance to chemotherapy. Another mechanism that may result in loss of apoptotic mechanisms in neoplastic cells is expression of survivin, a recently recognized member of the inhibitor of apoptosis family. Although present in fetal tissues, survivin is not highly expressed in human terminally differentiated tissues. Studies in a variety of human neoplasms indicate that increased survivin expression is often correlated with poorly differentiated neoplastic cells, a more

aggressive tumor, poor response to therapy, faster time to recurrence, and shorter survival time. Several recent studies have found that survivin is overexpressed in human oral and laryngeal squamous cell carcinomas, particularly those with a poorly differentiated phenotype, aggressive biological behavior, and those that metastasize. Survivin expression was also increased in pre-malignant tissues that advanced to malignant oral squamous cell carcinoma. There may be a correlation between loss of wild-type p53 and overexpression of survivin in human neoplasms, as some studies suggest that survivin is normally suppressed by wild- type p53. Indeed, a correlation between elevated p53 accumulation and high survivin expression was found in one recent study of human laryngeal squamous cell carcinoma.

The goals of this study were to establish survivin expression and to confirm mutant-type p53 expression in feline oral squamous cell carcinomas. In addition, survivin expression and mutant-type p53 accumulation in tumor cells were assessed to determine if a relationship exists between loss of wild-type p53 and survivin expression. Finally, these factors were compared to survival times to ascertain if expression of survivin or p53 could be used as prognostic indicators in feline oral squamous cell carcinoma.

CHAPTER 2

LITERATURE REVIEW

Feline Oral Squamous Cell Carcinoma

Overview

Squamous cell carcinoma (SCC) is the most common oral neoplasm in cats. No breed or sex predilection has been identified; however, older cats are more commonly affected, with the average age reported to be approximately twelve years. The tongue and gingiva are the most commonly affected sites.[1, 2] Less frequently, tumors occur in the tonsils, palate, and pharynx.[1] When gingiva is affected, the neoplasm can invade underlying structures, such as the mandible, maxilla, or hard palate, and result in periosteal proliferation.[2]

Clinical Signs

Cats with oral SCC often present with a facial mass and excessive salivation, dysphagia, halitosis, or loose teeth. Non-specific signs may include weight loss and anorexia. Although the duration of clinical signs is variable, most cats have only a short history of signs;[1, 2] but the neoplasm is often advanced at the time of presentation.[1]

Gross Features

Lingual tumors occur on the ventral surface of the tongue, near the frenulum, and are often ulcerated.[2] Although lingual tumors often appear as small, white nodules, they are frequently more extensive on palpation;[1] however, the dorsal aspect of the tongue typically remains unaffected.[2] The mucosa of the mandible and maxilla is also a common site for oral SCC. Because oral SCC of the mucosa often invades underlying bone, the mass may appear to be a bony tumor.[2] Gingival tumors often have an ulcerated surface.[1] Adjacent teeth may become loose.[2]

Histologic Features

Oral SCC can be definitively diagnosed by biopsy and histopathology.[1, 2] Infralingual tumors are frequently composed of a well-differentiated squamous epithelium that forms keratin pearls; mitotic figures are frequent. A scirrhous response is induced by invasion of neoplastic cells into the adjacent soft tissue. Gingival tumors often invade underlying bone, resulting in bony proliferation and remodeling. Gingival tumors may have fewer keratin pearls and a lower mitotic rate than lingual tumors.[1] However, histologic grading of the tumor is not useful in predicting outcome.[2]

Metastasis

Oral squamous cell carcinomas in cats do not frequently metastasize. When metastasis occurs, it is typically via lymphatics to the regional lymph nodes.[1] Metastasis to the lungs is rare.[2]

Etiology

The etiopathogenesis is not well understood, but the ventral distribution of tumors suggests possible prolonged exposure to carcinogens. Infection, trauma, and impaction of material may be involved in the development of oral squamous cell carcinoma.[1] Although one study suggested a correlation between oral squamous cell carcinoma and infection with feline immunodeficiency virus,[3] all cats were negative for feline immunodeficiency virus in another study. [4] A recent study suggests several factors may be associated with increased risk of developing oral squamous cell carcinoma, including flea collar use, feeding canned food or tuna, and exposure to environmental tobacco smoke.[5]

Treatment

Oral squamous cell carcinoma in cats has a poor prognosis, despite the treatment method employed. Combination therapy for the treatment of squamous cell carcinoma appears to be most effective.[2, 6] However, one study found no significant difference between treatment modalities and survival time.[7] Surgical excision alone results in a one year survival time of less than 20%.[1] Even with radical excision, such as mandibulectomy or maxillectomy, recurrence is frequent,[2] with a median survival time less than two months.[7] Addition of radiation therapy following mandibulectomy in one report improved the median survival time to fourteen months.[8] Other studies have also found that combination therapy resulted in a somewhat increased survival time.[9, 10, 11] However, in one study, radiation therapy failed to result in palliation and had a high rate of side effects in cats with inoperable oral squamous cell carcinomas.[6] Use of a

liposomal cisplatin analogue was also ineffective in one study.[12] A recent article suggested that use of the cyclooxygenase inhibitor piroxicam may be beneficial, at least in some cases of feline oral squamous cell carcinoma.[13] Feline oral squamous cell carcinomas were found to overexpress epidermal growth factor receptor, suggesting that this may be a beneficial target for therapy.[14]

Human Oral Squamous Cell Carcinoma

Cats have been suggested as an animal model for human oral squamous cell carcinoma.[15] Oral squamous cell carcinoma occurs frequently in humans and is the tenth most common malignancy diagnosed in the U.S. Despite advances in treatment, the five year survival rate remains poor, estimated at 50%.[16] Mutations in p53 and overexpression of survivin occur frequently in human oral squamous cell carcinoma and appear to be involved in tumorigenesis.

Apoptosis

Overview

Apoptosis, or programmed cell death, is important in maintaining tissue homeostasis and occurs normally during embryogenesis, as well as in a variety of pathological conditions. Apoptosis is a coordinated sequence of events, including condensation of the nucleus and cytoplasm, blebbing of the plasma membrane, and packeting of organelles and nuclear particles (apoptotic bodies). Phagocytes or adjacent cells quickly ingest and degrade these apoptotic bodies. In contrast to necrosis, apoptosis typically does not incite inflammation or scarring.[17, 18]

The process of apoptosis includes an initiation phase of caspase activation and an executioner phase that results in caspase-mediated cell death. The initiation phase may occur along extrinsic or intrinsic pathways. The extrinsic pathway is mediated by cell-surface death receptors, such as Fas. When Fas is bound by its ligand, FasL, the Fas molecules group to form a binding site for the Fas-associated death domain (FADD). The death receptor can then bind and activate procaspase-8. These molecules act catalytically on one another to form active caspase-8, thus initiating downstream events. In the intrinsic pathway, increased mitochondrial permeability allows release of proapoptotic molecules, such as cytochrome c. When cytochrome c is released into the cytoplasm, it binds apoptosis activating factor-1 (Apaf-1), thus activating procaspase-9. Activated caspase-9 can then induce a cascade of caspase activation. Other molecules, such as apoptosis-inducing factor (AIF), are released into the cytoplasm to prevent the action of inhibitors of apoptosis. Mitochondrial permeability is controlled by the Bcl-2 family of proteins, and the initiation of apoptosis is dependent, in part, on the balance of these molecules. While members such as Bcl-2 and Bcl-x are antiapoptotic, Bak, Bax, and Bim are proapoptotic. Both the extrinsic and intrinsic routes of caspase initiation result in the activation of the executioner caspases, including caspase-3, caspase-6, and caspase-7. Activated executioner caspases degrade cytoskeletal and nuclear proteins, causing breakdown of cellular structures.[17]

Apoptosis in the Development of Neoplasia

Although cells with damaged DNA are normally eliminated by apoptosis, many cancer cells become resistant to apoptotic mechanisms.[19] Resistance to apoptosis is a

characteristic feature of neoplastic cells,[20] and may contribute to tumor growth, metastasis, and resistance to therapy.[21] The mechanisms of apoptotic resistance that are the focus of this discussion are mutation of p53 and overexpression of the inhibitor of apoptosis, survivin.

p53

Overview

Normally p53 functions to arrest cell cycle progression if DNA damage has occurred, allowing time for DNA repair or inducing apoptosis in severely damaged cells. In human cancer, however, mutation of the p53 gene is the most commonly reported genetic alteration, suggesting that loss of p53 function may play an important role in tumorigenesis and resistance to apoptotic mechanisms. In addition, loss of p53 can result in an increased growth rate of the tumor and resistance to therapy.

Role of p53 in the Cell Cycle

The cell cycle is divided into the S phase of DNA synthesis, the M phase of mitosis, and gap phases G_1 and G_2 that precede synthesis and mitosis, respectively. Non-dividing cells remain in G_0, a quiescent phase.[19] Transitions between phases are regulated by the cyclin-dependent kinase (CDK) family of protein kinases. Cyclin levels vary during the cell cycle, causing intermittent activation of CDKs.[22]

Checkpoints exist at each stage of the cell cycle to ensure that progress through the cell cycle is orderly and to arrest the cell cycle if DNA damage has occurred. Checkpoints for DNA damage occur at the G_1 to S phase before DNA synthesis and the

G_2 to M phase after DNA replication. Checkpoints are also present during the S and M phases. Cell cycle arrest at the G_1 to S checkpoint after DNA damage has occurred is p53-dependent.[22] A variety of DNA damage can induce p53 activation, such as double-strand breaks and increased amounts of DNA repair substrates; hypoxia and other forms of cellular stress may also activate p53. Although the level of p53 in the cell is normally low because of its short half-life (approximately 20 minutes),[23] the cellular level of p53 rapidly increases after DNA damage has occurred;[22] and the half-life is prolonged.[23]

After p53 is activated, a variety of genes are upregulated to induce cell cycle arrest, resulting in either DNA repair or apoptosis. Activated p21 inhibits CDKs to prevent progression of the cell cycle. In addition, p53 induces *Gadd45* (growth arrest and DNA damage inducible gene) for DNA repair. Although p53 can function at the G_2 to M checkpoint, p53-independent mechanisms also exist for this stage.[22]

The mechanism by which p53 induces apoptosis is not well understood.[24] However, in some cells with severely damaged DNA, p53 induces proapoptotic genes, such as *Bax*, to initiate apoptosis;[22] however, p53-dependent apoptosis can also occur in the absence of *Bax* activation. Another mechanism whereby p53 induces apoptosis is regulation of insulin-like growth factor binding protein 3 (IGF-BP3); other genes that may be affected by p53 include *KILLER/DR5* and *FAS/APO1*. Expression of the antiapoptotic factor Bcl-2 and the proto-oncogene *c-myc* are also suppressed by wild-type p53.[24]

Multiple proteins control p53 upregulation. Mdm2 (murine double minute) provides a negative feedback loop for p53; however, the p19 (ARF) protein can bind Mdm2 to prevent this negative feedback.[22]

p53 and Neoplasia

Originally, p53 was described as an approximately 53 Kd protein that was believed to be a proto-oncogene.[25, 26] However, later studies determined that the p53 examined in the original articles was mutated; wild-type p53 actually inhibits transformation of cells.[27] It is now recognized that p53 is mutated in over half of all human cancers.[28, 29] Loss of tumor suppressor function by p53 can occur in a variety of ways, as noted by Harris and Hollstein, such as "mutation, chromosomal rearrangement and nondisjunction, gene conversion, imprinting, or mitotic recombination;" p53 can also be inactivated by interactions with "cellular proteins or viral oncoproteins"[30] that corrupt the p53 pathway. The majority of mutations are substitutions of single amino acids that result in a missense protein;[28] most of these missense mutations occur in sequences that code for the DNA-binding region of p53.[23] Mutation or inactivation of p53 aids in tumorigenesis by loss of tumor-suppressor function, inhibition of wild-type p53 function by mutant p53 in heterozygous cells and may also confer a growth advantage to neoplastic cells.[24] Mutations in p53 may also increase resistance to chemotherapy by the neoplastic cells.[31] Mutated p53 frequently has an extended half-life and accumulates in the cell; thus, mutated p53 can often be detected with immunohistochemistry.[30]

p53 in Human Oral Squamous Cell Carcinoma

As with many types of human neoplasia, mutation of p53 occurs in approximately one-half of oral squamous cell carcinomas[29] and is the most commonly reported genetic aberration in oral cancer.[32] Mutations detected by polymerase chain reaction (PCR) have been reported to occur in 39%[33] to 42%[34] of cases, while p53 immunohistochemical

expression has been stated to occur in 37.8%[35] to 70%[36] of oral squamous cell carcinoma cases. In addition, a high rate of expression has been found in lymph nodes with metastatic disease, ranging from 51.1%[35] to 72.7%[37] of cases. In contrast, normal oral tissues have a much lower rate of p53 expression. Normal tissues have been reported to have no[38] to 3%[36] p53 positive cells, or be limited to the basal cell layer;[39] one study found that no benign tumors of the head and neck were positive.[38] However, in dysplastic epithelium, p53 immunostaining occurred in the suprabasal layer[39] and was reported at a rate of 52%.[36] Those dysplastic lesions with positive p53 expression tended to advance to carcinoma more quickly than those that were negative.[36] These findings indicate an increase in p53 expression from normal, dysplastic epithelium, to carcinoma and suggest that alterations in p53 may play a role in cancer development and progression.

The prognostic significance of p53 mutation or immunohistochemical overexpression in oral squamous cell carcinoma and squamous cell carcinoma of the head and neck remains unclear. Multiple studies have found an association between p53 mutation[33, 34] or immunohistochemical expression[38, 40, 41, 42, 43, 44] and a shorter survival time. Tumors that were immunohistochemically positive for p53 were also associated with a shorter disease-free interval[35, 36, 41, 45] and higher rate of local recurrence in oral[46, 47] or laryngeal carcinoma.[48] Expression of p53[37, 40] and mutation of the p53 gene[34] have also been found to be associated with metastasis and resistance to chemotherapy[45] and radiation therapy.[47] In some cases, p53 had prognostic significance and a correlation with metastasis only when combined with other factors, such as Bcl-xL, Bax,[37] p21,[49, 50] and mdm2.[49] However, other studies found no statistically significant correlation with

p53 immunohistochemical expression and survival,[51, 52, 53, 54, 55] recurrence, [51, 53, 56] or response to treatment.[51, 57] These studies found rates of expression of p53 from 46%[56] to 78%,[55] similar to other studies. One paper reported an improved survival rate in cases with p53 expression.[58] While the prognostic significance of p53 in oral squamous cell carcinoma remains unclear, the high rate of mutation and immunohistochemical expression suggests its importance in the development and progression of these tumors.

p53 in Feline Oral Squamous Cell Carcinoma

Studies have evaluated p53 expression in feline tumors, although few have evaluated the correlation of p53 immunostaining and prognosis. The feline p53 gene has been sequenced and found to have strong homology with the human sequence (82.1%)[59] and sequences in other species.[60] Feline sequences shared conserved domains with other species; at common sites of mutation in human cancer, feline sequences were identical to the human sequences.[60] Immunohistochemical expression of p53 has been found in feline oral squamous cell carcinoma in 60.0% (3/5)[61] to 77.7% (7/9)[62] of cases. In one study examining p53 aberration in feline oral squamous cell carcinoma and its association with environmental tobacco smoke, 65.2% (15/23) of cases had positive p53 nuclear staining. Although not statistically significant, cats that had been exposed to environmental tobacco smoke were four and a half times more likely to overexpress p53, and those cats that had been exposed for more than five years were seven times more likely to overexpress p53.[63]

p53 in Other Feline Neoplasms

Multiple studies have detected p53 mutations with PCR in a variety of feline neoplasms. In a case of squamous cell carcinoma, a base pair deletion resulted in a frameshift mutation and premature stop codon.[64] Mutations were also found in cases of mammary tumors,[64, 65, 66] bladder spindle cell carcinoma,[67] fibrosarcoma,[64, 67, 68] osteosarcoma,[66] lymphosarcoma,[69] malignant fibrous histiocytoma,[64] and pleomorphic sarcoma.[67] Mutations have been reported in feline vaccine-associated sarcoma;[70, 71] and in one study, p53 gene mutations negatively affected recurrence and survival time.[71]

Immunohistochemical staining to detect abnormal p53 accumulations has also been performed in a variety of feline neoplasms. Squamous cell carcinomas had positive Immunoreactivity,[62, 72] including cases in the ear[61] and eye.[61, 73] Other types of neoplasms with positive p53 staining included mammary tumors,[62, 72, 74, 75] adenocarcinoma,[62] osteosarcoma,[62] hemangiosarcoma,[62] and small numbers of melanocytic tumors.[76] Multiple studies have found cases of feline vaccine-associated sarcoma to be positive for p53 immunohistochemistry.[71, 77, 78, 79] In one study of vaccine-associated sarcomas, p53 staining did not correlate with recurrence or survival time; however, those cases with cytoplasmic rather than nuclear staining had a shorter time to recurrence.[77]

p53 in Neoplasms of Other Domestic Species

Mutations in the p53 gene and accumulations of p53 detected by immunohistochemistry have been found in a variety of canine neoplasms. The canine p53 gene has strong homology with the human and murine gene.[80] Multiple studies have

detected p53 mutations in squamous cell carcinoma, with PCR[81] and immunohistochemistry.[61, 72, 82] Other canine neoplasms that have been reported to have p53 mutations include mammary adenocarcinomas, detected by PCR[83, 84, 85, 86, 87, 88, 89, 90] and immunohistochemistry;[82, 91, 92, 93, 94] and intestinal adenocarcinoma,[82] transitional cell carcinoma,[82] nasal adenocarcinoma,[82] and apocrine gland adenocarcinoma[82] determined by immunohistochemistry. Thyroid carcinoma,[95] colon cancer,[96] and adenoma of the circumanal gland[97] were positive with PCR. Hemangiosarcoma,[82] chondrosarcoma,[82] and fibrosarcoma[82] were positive with immunohistochemistry; osteosarcoma has been reported to be positive with both immunohistochemistry[98] and PCR.[96, 99, 100] Cases of lymphoma[96, 101] and leukemia[96] have been reported to be positive with PCR; while one study found positive immunostaining of lymphomas,[82] another study reported that lymphomas were negative.[102] Mast cell tumors have been reported to have positive immunohistochemistry;[82, 103] however, positive staining did not predict survival time.[103] Melanomas were reported to be infrequently positive with immunohistochemistry, and positive staining did not affect survival time.[76]

In other domestic species, p53 immunoreactivity was found in cases of bovine and equine squamous cell carcinoma,[61, 104] including ocular tumors,[73, 105] and ovine squamous cell carcinoma;[104] p53 mutations determined by PCR were also found in equine squamous cell carcinoma.[106] Positive p53 immunoreactivity has been reported in equine lymphoma.[107] While in one study, no p53 staining was detected in bovine lymphoma,[107] another study found frequent p53 mutations in cases of bovine leukemia virus-induced leukemogenesis.[108]

Survivin

Overview

Survivin is a recently described member of the inhibitor of apoptosis family (IAP). Survivin is an approximately 16.5 Kd protein that contains one baculovirus inhibitor of apoptosis repeat; unlike other IAP's, survivin does not contain a carboxyl terminal RING finger.[109] Three splice variants of the survivin gene have been identified: survivin-ΔEx3, survivin-2B,[110] and survivin-3B.[111] While the functions of these splice variants are not well understood, in one experiment, survivin-ΔEx3 maintained its antiapoptotic potential, while survivin-2B had decreased antiapoptotic capability.[110] The function of survivin-3B appears to differ from survivin, and its expression may not be associated with the G_2/ M phase of the cell cycle.[111]

Survivin Functions

Survivin is bifunctional, acting as both a regulator of mitosis during cell division and as an inhibitor of apoptosis.[112] The bifunctional capability of survivin is evolutionarily conserved.[113] An additional function of survivin in response to vascular injury has also been recently identified.[114]

Survivin Function as a Mitosis Regulator

Evidence suggests that survivin has an important function as a mitotic regulator.[115] Survivin expression is cell cycle dependent, and peak expression occurs during the G_2/ M phase of the cell cycle.[116] During cytokinesis, survivin appears to be a member of the chromosomal passenger complex (CPC)[117] and interacts with the other

chromosomal passengers, including Aurora B,[117, 118, 119, 120, 121] inner centromere protein (INCENP),[117, 118, 119. 120, 121,] Borealin,[117, 119] and Dasra A and Dasra B.[118] The CPC has an important checkpoint function to ensure correct chromosome segregation and kinesis; survivin's functions in the CPC are to direct the location of the CPC and control Aurora B kinase function.[115] The importance of survivin in cell division is demonstrated by the multiple cell defects that occur in the absence of survivin, including multinucleated cells, abnormal centrosomes[122] and mitotic spindles,[122, 123] loss of checkpoint signals,[124] and mislocalization of Aurora B.[121]

In addition to its association with the CPC, survivin also associates with the mitotic spindle[116, 125] and centrosomes.[125] Rosa *et al* recently suggested that survivin, independently of Aurora B, has multiple functions throughout the cell cycle, including regulation of microtubule dynamics and nucleation.[126] This model defines the functions of survivin in multiple sites during cell division, including the mitotic spindle,[122, 123, 127] spindle checkpoint,[124, 128] midbody,[129] nucleation and organization of microtubules,[122] and microtubule processes in interphase.[126]

Survivin Function as an Inhibitor of Apoptosis

While the role of survivin in cell division is well established, the mechanism by which survivin functions as an inhibitor of apoptosis is less well understood.[130] While some studies postulated that survivin-like baculovirus IAP repeat (BIR) containing proteins, such as BIR-1 in *Caenorhabditis elegans*, had functions during cytokinesis but lacked a cytoprotective effect,[131] other survivin homologues such as Bir1p in the yeast *Saccharomyces cerevisiae*[113] and deterin in *Drosophila melanogaster*[132] do have an

antiapoptotic function. In addition, survivin lacks some of the structures of other IAP's, such as XIAP.[133] Unlike XIAP, which has been shown to directly bind caspase-3 and caspase-7,[134] the association of survivin with caspases is divisive, with reports both that is does[135] and does not[133] interact directly with caspases. Instead, survivin apparently must associate with cofactors, such as hepatitis B virus X interacting protein (HBXIP)[136] or XIAP,[137] to have an antiapoptotic function.[130] When combined with these molecules, survivin can interfere with caspase-9, interrupt Apaf-1 binding to the apoptosome, and prevent XIAP degradation.[130] Recent evidence suggests that a mitochondrial pool of survivin exists that can be rapidly released into the cytosol after cellular stress occurs. This mitochondrial pool of survivin inhibits caspase-9 activation.[138] The cytoprotective function of survivin is well documented, and its importance as an antiapoptotic protein is validated by the finding that cells with absent or non-functional survivin have mitotic defects or undergo rapid apoptosis.[130]

Survivin Expression in Fetal Tissues

When survivin was first described, a northern blot identified survivin transcripts in fetal kidney and liver, as well as lung and brain; immunohistochemistry has demonstrated survivin in fetal kidney, liver, lung, gastrointestinal tract,[109, 139] and heart.[139] The presence of survivin was also recognized by immunohistochemistry and *in situ* hybridization in fetal stratified epithelial stem cells, endocrine cells of the pancreas, and medulla of the thymus.[139]

Survivin Expression in Adult Tissues

In the original description of survivin, no transcripts were identified in adult human tissues, including peripheral leukocytes, lymph node, spleen, pancreas, kidney, skeletal muscle, liver, lung, brain, or heart; although, small amounts of mRNA were identified in adult thymus and placenta.[109] Survivin has since been found to be expressed at a low level in a variety of normal adult human cells.[140] In human skin, survivin expression was detected in basal keratinocytes[141] and melanocytes.[142] Survivin is also expressed in human[143, 144, 145] and mouse[146] hematopoietic progenitor cells, thymocytes,[127] T cells,[147] and neutrophils.[148] Survivin is expressed in vascular endothelium[149, 150] and other proliferative tissues such as the testis,[151] endometrium,[152] and colonic crypt epithelium.[153]

Survivin Expression in Neoplastic Cells

In the initial description of survivin, immunohistochemistry and *in situ* hybridization demonstrated survivin expression in all cases of lung adenocarcinoma, lung squamous cell carcinoma, pancreatic adenocarcinoma, colonic adenocarcinoma, breast carcinoma, and prostate adenocarcinomas, as well as 55% of high-grade lymphomas examined.[109] Survivin has since been found to be expressed in a wide variety of human neoplasms.[140] The mechanism of increased survivin expression in neoplastic cells is not well understood. Although it has been suggested that increased survivin correlates with an increased proliferative activity of neoplastic cells, survivin appears to be over-expressed independently of mitotic index.[112, 140] In support of this explanation, survivin expression in neoplastic cells often exceeds the number of dividing cells that are

measured by Ki67 expression.[112] However, survivin expression is often positively correlated with the proliferative index in a variety of tumor types.[154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170] In addition to its association with highly proliferative neoplastic cells, survivin also contributes to survival of neoplastic cells via its function as an inhibitor of apoptosis; an inverse relationship between survivin expression and apoptotic rate has been noted in a variety of neoplasms.[156, 158, 160, 164, 167, 171, 172, 173, 174]

Not only is survivin frequently associated with neoplastic cells with a high proliferative rate and low apoptotic index, survivin expression is also correlated with a poor prognosis in multiple types of neoplasms. In many instances, survivin expression corresponds to a more aggressive phenotype, shorter disease-free interval, and decreased overall survival time. However, in some types of cancer, survivin expression appears to be associated with an improved prognosis. These discrepancies may be the result of different detection methods, evaluation of nuclear or cytoplasmic expression, and the presence of survivin splice variants.[140]

Survivin expression may also negatively impact the prognosis because it can impart resistance to therapy on neoplastic cells. Overexpression of survivin in a variety of neoplastic cell lines has been associated with resistance to chemotherapy[175, 176, 177, 178, 179, 180, 181, 182, 183] and radiation.[184, 185] Clinical resistance to chemotherapy has also been noted in cases of ovarian carcinoma that have a high level of survivin expression.[183]

Because survivin is frequently overexpressed in human cancers, is not highly expressed in normal adult tissues, and because it appears to confer resistance to treatment on neoplastic cells, survivin has been explored as a potential target for cancer therapy. Methods that have been investigated to decrease survivin expression or function in

various cell lines include antisense oligonucleotides, ribozymes, small interfering RNA's, dominant negative mutants, and inhibitors of cyclin-dependent kinases.[186] The results of preclinical trials indicate that decreased survivin expression was associated with decreased tumor growth, increased apoptosis of neoplastic cells, and increased sensitivity of tumor cells to chemotherapy or radiation.[187] In addition, survivin appeared not to be associated with toxicity *in vitro* or *in vivo* in xenograft mouse models.[188]

Survivin Expression in Human Oral Squamous Cell Carcinoma

Survivin has been found to be overexpressed in human oral, oropharyngeal, and laryngeal neoplasms in multiple studies utilizing immunohistochemistry, PCR, and western blotting. With IHC staining for survivin, normal oral epithelial tissues were negative,[189, 190] except for occasional basal and suprabasal cells.[191, 192, 193] In contrast, premalignant lesions, such as leukoplakia[189, 194] and epithelial dysplasia,[195] demonstrated rates of survivin expression ranging from 14.2% to 97.0%.[189, 194, 195] In one study, the group of precancerous lesions that did not progress to oral SCC had a low rate of survivin expression (33%), while the group of precancerous lesions that progressed to malignancies had a much higher percentage of survivin positive cases (94%). The number of survivin positive cells within these cases was also higher.[193] In multiple studies, rates of survivin IHC expression in oral and oropharyngeal SCC have varied but always exceeded 50% of the cases studied. The percentages of survivin positive cases of oral SCC have been reported as follows: 50%,[191] 55.3%,[194] 56%,[190] 58%,[189] 82.7%,[192] 98%,[195] and 100%.[193, 196, 197] Quantitative real-time PCR (qRT-PCR) has also identified survivin transcripts in oral SCC.[198]

Survivin expression in oral SCC can often be predictive of tumors with a more aggressive phenotype or greater likelihood of lymph node metastasis. Survivin expression has been noted more frequently in poorly differentiated tumors[192, 194] and those with a higher grade of malignancy.[190, 199] A statistically significant increase in survivin expression has been noted in cases of oral SCC that had lymph node metastasis compared to those without lymph node metastasis.[190, 191, 194] Survivin expression was also significantly higher in cases of oral SCC with distant metastasis.[191] In some studies, survivin expression in oral SCC has had prognostic significance, although the results are conflicting. Survivin expression resulted in a statistically significant decrease in survival time in patients with oral SCC in some studies.[192, 195, 197] In one study, overall survivin expression had an apparent, but statistically insignificant, correlation with shorter survival times, while those cases with predominantly cytoplasmic rather than nuclear survivin expression were significantly associated with decreased survival time.[198] Survivin mRNA expression in lymph nodes with metastases was also a negative prognostic factor.[200] However, one study found that survivin expression was predictive of an improved outcome in oral SCC patients following radiation therapy.[201]

Survivin expression has been reported in 74.6%[202] to 98.5%[203] of laryngeal SCC. In both of these studies, survivin expression correlated with poor prognosis,[202, 203] and in one study was also associated with lymph node metastasis.[202]

Survivin Expression in Veterinary Species

Studies investigating surviving expression in companion animals are limited. Based on PCR, fetal feline and canine survivin mRNA have close homology with the

human transcript (Johnson ME, unpublished data). In canine tissues, survivin transcripts were found in normal adult tissues, including heart, lung, liver, kidney, spleen, gastrointestinal tract, and testis. The study also found survivin mRNA in three malignant canine neoplasms.[204] In a study of canine mast cell tumors, most of the tumors demonstrated a low rate of nuclear, cytoplasmic, or mixed expression of survivin; expression of survivin did not correlate with clinical outcome. In this study in canines, a canine oral squamous cell carcinoma had diffuse positive staining for survivin, and this tissue was utilized as the positive control. A small population of lymphoid cells in multiple tissues also stained; however, no staining was observed in a variety of other tissues.[205] RT-PCR demonstrated survivin transcripts in 2 cases of canine transitional cell carcinoma; canine testicle was used as the positive control tissue.[206] To the author's knowledge, no additional information on survivin expression in felines or other domestic animals has been published.

Correlation of p53 and Survivin

Overview

Evidence indicates that survivin expression is normally suppressed by p53. However, since *p53* mutation occurs commonly in human neoplasms, loss of p53 function may correlate with increased survivin expression and may contribute to the progression of cancer.

Role of p53 in the Suppression of Survivin

In various human neoplastic cell lines, both exogenous and endogenous p53 protein resulted in suppression of the survivin gene at the level of transcription[207, 208, 209] or protein expression.[208] Suppression of the survivin gene occurred independently of cell cycle arrest[208, 209] or p53-induced expression of p21^{waf1}.[208] In one study, p53 appeared to exert a suppressive effect on the survivin gene without direct DNA binding. Instead, the method of suppression was proposed to be through deacetylation of the promoter region of the survivin gene.[209] However, in another study, wild-type p53 bound directly to the promoter region of the survivin gene; these authors proposed that a p53-Sin3-HDAC (histone deacetylase) complex bound the promoter region of the survivin gene and inhibited E2F binding. This study also suggested that p53 can suppress the survivin gene indirectly by activation of p21^{waf1}.[208]

Correlation of p53 and Survivin in Cancer Development

Mutant p53 lacked the ability to suppress activity of the survivin promoter in cell lines[208, 209] and did not bind the survivin gene promoter *in vivo*,[208] suggesting a correlation between loss of wild-type p53 and overexpression of survivin in the development of cancer.[207, 209] In transgenic mice, keratinocytes that overexpressed survivin were resistant to UVB-induced apoptosis; loss of p53 further contributed to resistance to apoptosis. This study proposed that survivin expression in conjunction with the loss of a p53 allele contributed to the occurrence of skin cancer in human patients.[210] Another study found that mutation of p53 in melanocytes led to increased survivin expression in the development of melanoma.[211] In cases of laryngeal squamous cell

carcinoma, the degree of survivin expression was significantly higher in those cases that were immunohistochemically positive for mutant p53.[203] A relationship between survivin and p53 expression was also noted in cases of experimentally-induced buccal pouch squamous cell carcinomas in hamsters.[212] Correlations between survivin expression and mutant p53 have also been noted in cases of human ovarian carcinoma,[213] breast cancer,[214, 215] gastric carcinoma,[216] pancreatic adenocarcinoma,[217] and non-small cell lung cancer.[218]

CHAPTER 3

MATERIALS AND METHODS

Cases and Control Tissues

Archived cases of feline oral squamous cell carcinoma from Mississippi State University College of Veterinary Medicine diagnostic laboratory service were reviewed. Case data was available for January, 1999, to December, 2000, and July, 2005, to October, 2007 (because of discontinuity of databases). Cases with tissue that had been decalcified or had an insufficient quantity of tissue were eliminated. Hematoxylin and eosin (H&E) stained slides of all cases were reviewed to confirm the diagnosis. Thirty cases of feline oral squamous cell carcinoma were included in the study. Questionnaires were submitted to the referring veterinarian concerning treatment, date of death, and cause of death for the cats included in the study. For control tissue, an adult, neutered male cat that was humanely euthanized at the local humane society for unrelated reasons was donated. The cat was necropsied shortly after euthanasia. Tissues from all major organs and oral tissues were placed in 10% neutral buffered formalin for histologic evaluation or were fresh frozen. In addition, feline testicles from cats routinely neutered at the humane society or Mississippi State University College of Veterinary Medicine's mobile spay/ neuter service were placed in 10% neutral buffered formalin or were fresh frozen. A gravid uterus from a female cat undergoing routine ovariohysterectomy was

donated by a local veterinarian; fetal tissues were placed in 10% neutral buffered formalin for histologic evaluation or were fresh frozen. Tissues were fixed for approximately 24 hours before processing.

Survivin Immunohistochemistry

Formalin-fixed and paraffin-embedded tissues were cut to 5 μm and mounted on charged microscope slides (Fisher Scientific, Pittsburgh, PA); slides were processed within 24 hours of being cut. Slides were placed in a 70° C oven overnight. Slides were then deparaffinized in 3 washes of xylene (Fisher Scientific, Pittsburgh, PA) for 5 minutes each and rehydrated in graded ethanols (3 washes of 100% ethanol, 2 washes of 95% ethanol, and 1 wash of 80% ethanol). Slides were then rinsed in distilled water for 5 minutes. Target retrieval was performed by immersing slides in target retrieval solution (Dako, Carpinteria, CA) and placing them in a steam cooker (Black and Decker, Hunt Valley, MD) for 20 minutes. Slides were then allowed to cool in the target retrieval buffer for 20 minutes at room temperature and rinsed in distilled water for 1 minute and Tris buffered saline (Dako, Carpinteria, CA) for 1 minute. Slides were placed on an autostainer (model S3400, Dako, Carpinteria, CA). All of the steps were performed at room temperature; rinsing was performed between each step on the autostainer. Endogenous peroxidase activity was blocked by covering slides with peroxidase blocking reagent (Dako, Carpinteria, CA) for 5 minutes; slides were then covered with universal protein block (Dako, Carpinteria, CA) for 20 minutes. Slides were incubated for 45 minutes with a rabbit anti-human polyclonal survivin antibody (LifeSpan Biosciences, Seattle, WA) at a dilution of 1:800 in antibody diluent (Dako, Carpinteria, CA). Slides

were then incubated with a biotinylated anti-rabbit, anti-mouse link, using the LSAB2 HRP system (Dako, Carpinteria, CA) for 30 minutes, followed by streptavidin HRP (Dako, Carpinteria, CA) for 30 minutes. Slides were developed with 3,3'-diaminobenzidene (DAB) chromagen (Dako, Carpinteria, CA) for 10 minutes and counterstained with hematoxylin (Sigma-Aldrich, St. Louis, MO) for 2 minutes. After being removed from the autostainer and rinsed in distilled water for 1 minute, slides were counterstained with bluing solution for 10 seconds and rinsed with distilled water for 1 minute. Slides were dehydrated in serial ethanols and xylene (Fisher Scientific, Pittsburgh, PA) and then coverslipped.

Sections of donated feline fetus and feline testicle served as positive controls. Sections of donated feline brain and skeletal muscle were used as negative controls. An additional negative control was performed by incubating slides of feline fetus and feline testicle in a non-relevant antibody (Dako, Carpinteria, CA).

p53 Immunohistochemistry

Formalin-fixed and paraffin-embedded tissues were cut to 5 µm and mounted on charged microscope slides (Fisher Scientific, Pittsburgh, PA). Tissues were cut sequentially and were processed within 24 hours of being cut. Slides were placed in a 70° C oven overnight. Slides were then deparaffinized in 3 washes of xylene (Fisher Scientific, Pittsburgh, PA) for 5 minutes each and rehydrated in graded ethanols (3 washes of 100% ethanol, 2 washes of 95% ethanol, and 1 wash of 80% ethanol). Slides were then rinsed in distilled water for 5 minutes. Endogenous peroxidase activity was blocked by placing slides in a peroxidase blocker (Peroxidazed 1, Biocare Medical,

Concord, CA) for 5 minutes. For target retrieval, slides were immersed in a target retrieval solution (Reveal, Biocare Medical, Concord, CA) and placed in a decloaking chamber (Biocare Medical, Concord, CA) for 30 minutes. Slides then remained in the retrieval solution at room temperature for 20 minutes and were rinsed in 2 washes of distilled water for 3 minutes each. Slides were next placed on an autostainer (Dako, Carpinteria, CA); the steps were performed at room temperature with rinses between each step. Avidin and biotin blocking solutions (Biocare Medical, Concord, CA) were placed on the slides for 10 minutes each. A universal blocking agent (Background Sniper, Biocare Medical, Concord, CA) was applied to the slides for 15 minutes. Slides were then incubated with a rabbit anti-human polyclonal (CM1) p53 antibody (Biocare Medical, Concord, CA) diluted to 1:100 in van Gogh yellow (Biocare Medical, Concord, CA) for 30 minutes. Slides were next incubated with a biotinylated anti-rabbit, anti-mouse link using the LSAB2 HRP system (Dako, Carpinteria, CA) for 10 minutes, followed by streptavidin HRP (Dako, Carpinteria, CA) for 10 minutes. Slides were developed with 3,3'-diaminobenzidene (DAB) chromagen (Dako, Carpinteria, CA) for 5 minutes and counterstained with hematoxylin (Sigma-Aldrich, St. Louis, MO) for 2 minutes. After being removed from the autostainer and rinsed in distilled water for 1 minute, slides were counterstained with bluing solution for 10 seconds and rinsed with distilled water for 1 minute. Slides were dehydrated in serial ethanols and xylene (Fisher Scientific, Pittsburgh, PA), then coverslipped.

A commercially purchased human breast carcinoma slide (Newcomer Supply, Middleton, WI) served as a positive control. Normal adult feline skin and oral mucosa from the donated cat served as negative controls. An additional negative control was a

human breast carcinoma slide incubated with a non-relevant antibody (Dako, Carpinteria, CA).

Slide Interpretation

For both survivin and p53 immunohistochemistry, the slides were reviewed independently by two pathologists (HR and MJ) without knowledge of the survival data or results of the other immunohistochemical stain; when the score of a slide differed, the case was reviewed and a consensus score was reached. Tissues with fewer than 10% positive cells were considered negative. Cases with 10-50% positive cells were considered moderately positive, while those with greater than 50% positive cells were considered strongly positive.

Statistical analysis

SAS (Cary, NC) statistical analysis software was utilized in this study. A weighted kappa agreement test was performed to measure the degree of agreement between the immunohistochemical scores assigned by each pathologist (HR and MJ). The Cochran-Mantel-Haenszel test (Modified Ridit) was utilized to determine if a correlation existed between p53 mutation and survivin expression, as well as to correct for age, breed, sex, and neuter status. The Cox proportional hazard test was used in evaluation of p53 expression and survivin expression in relation to survival time.

CHAPTER 4

RESULTS

Case Data

Thirty cases of feline oral squamous cell carcinoma were included in this retrospective study (Table 1). The age was known for 28 cats; the average age was 13.3 years (median 13.5 years; range 7-18 years). The sex was known for all of the cases; 16 were neutered males, 3 were intact males, 6 were spayed females, and 5 were intact females. Twenty-six of the cats were domestic shorthairs, and 2 were domestic longhairs; the breed was not reported for 2 cases. Tumors were reported in a variety of locations within the oral cavity. Nine tumors were located in the tongue or sublingual region; 9 were in the mandible or maxilla. Eight of the tumors were in an unspecified site within the oral cavity. One case each was reported in the pharynx, palatoglossal arch, gingiva of the left commissure, and hard palate. One case included a section of regional lymph node (case 14).

Questionnaires concerning date and cause of death were returned for 15 of the cases. Of 14 cases, the average survival time was 34.6 days (median 31 days; range 0- 69 days). Four of the cats received palliative therapy; surgical debulking was reported in 2 cases. Additionally, in one case, the tumor was surgically debulked, and the cat was treated with a non-steroidal anti-inflammatory; the cat was still alive 126 days after

diagnosis. Twelve of the cats were euthanized because of the oral neoplasm, and 1 cat died naturally from the neoplasm. The cause of death was not reported for 1 case. In addition, 1 cat was euthanized and 1 cat had a natural death related to the neoplasm; however, the date of death was not reported for these cases. One cat was reportedly feline immunodeficiency virus positive (case 4).

Survivin Immunohistochemistry

Eighty percent (24/ 30) of the cases of feline oral squamous cell carcinoma were positive for survivin with immunohistochemistry (Figure 1); of these, 13 (43.3%) were moderately positive, and 11 (36.7%) were strongly positive. In the majority of the cases, immunohistochemical staining was limited to the cytoplasm; however, in 5 cases (cases 13, 18, 19, 26, 28), moderate nuclear staining was also noted (Figure 2). Mitotic figures were frequently positive (Figure 1), even in those cases that were considered survivin negative. In sections of the tumors, the surrounding stroma and inflammatory cells were often negative, but occasionally demonstrated mild cytoplasmic staining. Sections of lymph node from case 14 contained survivin-positive neoplastic cells (Figure 3). Six (20%) cases were negative (Figure 4). Multiple cell types in sections of feline fetus had strong cytoplasmic staining (Figure 5). In sections of feline testicle, seminiferous tubular epithelium was strongly positive, while the supporting stroma was negative (Figure 6). Sections of adult feline brain were largely negative (Figure 7), with occasional mild background staining; sections of adult feline skeletal muscle were negative (Figure 8). Sections of feline fetus (Figure 9) and testicle (Figure 10) processed with an irrelevant

control antibody were diffusely negative. The weighted kappa agreement between the two pathologist's scores was 0.83 ($p < .0001$).

p53 Immunohistochemistry

Thirteen of thirty cases (43.3%) of cases were positive with p53 immunohistochemistry (Figure 11); 4 (13.3%) were moderately positive, and 9 (30%) were strongly positive. All cases demonstrated a strong nuclear reaction; no background staining was observed. Sections of lymph node from case 14 contained p53-positive neoplastic cells (Figure 12). Seventeen cases (56.7%) were negative (Figure 13). Slides of the purchased human breast carcinoma had a strong nuclear reaction with p53 immunohistochemistry (Figure 14). Sections of normal adult feline skin and oral mucosa were negative, except for occasional basal cells of the epidermis or mucosa (Figure 15); a small number of these cells also had cytoplasmic staining with the irrelevant control antibody (Figure 16). Sections of the human breast carcinoma processed with the irrelevant control antibody were diffusely negative (Figure 17). Both pathologists were in 100% agreement on the scores for the p53 immunohistochemistry.

Statistical Analysis

No significant differences were noted between p53 mutation and degree of survivin overexpression. No statistically significant difference was determined between p53 and survivin when corrected for age, breed, sex, or neuter status. The immunohistochemical scores for p53 and survivin also had no statistically significant effect on survival time.

Table 1

Case signalment, survival time, survivin score, and p53 score.

Case	Age (years)	Sex	Breed	Mass Location	Survival time (days)	Survivin score	p53 score
1	8	MN	DLH	Oral		++	-
2	16	F	N/R	Palatoglossal arch		+	++
3	10	F	DSH	Tongue/ sublingual		++	+
4	11	MN	DSH	Commissure		+	-
5	14	MN	DSH	Tongue/ sublingual		++	++
6	14	MN	DLH	Oral		+	+
7	17	FS	DSH	Oral		++	+
8	14	FS	DSH	Oral	0	+	-
9	12	F	N/R	Tongue/ sublingual		+	-
10	11	MN	DSH	Pharyngeal	69	-	-
11	N/R	M	DSH	Tongue/ sublingual		+	-
12	N/R	F	DSH	Tongue/ sublingual		++	-
13	15	MN	DSH	Mandible/ maxilla	34	++	-
14	11	MN	DSH	Mandible/ maxilla	28	++	++
15	13	MN	DSH	Oral	68	++	++
16	17	FS	DSH	Mandible/ maxilla	0	+	+
17	14	MN	DSH	Tongue/ sublingual	3	-	-
18	11	MN	DSH	Tongue/ sublingual		+	-
19	14	MN	DSH	Mandible/ maxilla	56	+	-
20	11	FS	DSH	Mandible/ maxilla	24	-	-
21	15	MN	DSH	Mandible/ maxilla	26	+	-
22	7	MN	DSH	Hard palate		-	-
23	14	F	DSH	Mandible/ maxilla		++	-
24	7	M	DSH	Oral		-	++
25	13	MN	DSH	Oral	67	-	++
26	18	MN	DSH	Mandible/ maxilla	45	+	++
27	13	MN	DSH	Tongue/ sublingual	24	+	++
28	17	FS	DSH	Tongue/ sublingual		++	-
29	12	M	DSH	Mandible/ maxilla	>126	+	-
30	17	FS	DSH	Oral	40	++	++

N/R: not reported. MN: male neutered; M: intact male; FS: female spayed; F: intact female. DSH: domestic shorthair; DLH: domestic longhair. Oral: unspecified location. Survivin and p53 score: (-) <10%; (+) 10-50%; (++) >50%.

CHAPTER 5

DISCUSSION

In this retrospective study, 30 cases of feline oral SCC were examined for immunohistochemical expression of p53 and survivin. Slightly less than one-half of cases were positive with p53 immunohistochemistry, indicating mutation of the p53 gene in these cases. The majority of cases were positive with survivin immunohistochemistry. This immunohistochemical staining profile is consistent with reported cases of human oral SCC, suggesting that cats may potentially serve as a model for the human disease. However, other mechanisms besides p53 mutation and survivin overexpression may also contribute to the tumorigenesis of oral SCC.

In human cell lines and in certain cancer types, mutant p53 lacked the ability to suppress survivin expression, and p53 mutation was significantly associated with increased survivin expression. No statistically significant differences were noted between p53 and survivin immunohistochemical staining in these cases of feline oral SCC. In addition, p53 mutation and survivin overexpression are often associated with a decreased survival time and poor overall prognosis in human cases of oral SCC. In this study, no significant differences were noted in survival time when compared with p53 or survivin immunostaining. However, the number of cases for which survival data was available was small. In addition, none of the cats received aggressive treatment, such as

radiation or chemotherapy, and most were euthanized because of the neoplasm. The lack of extensive treatment and variable time of euthanasia could have contributed to the short average survival time, making statistical analysis difficult.

However, to the author's knowledge, there are no published reports of survivin immunohistochemical staining in feline tissues. Both feline fetus and testicle proved to be useful positive controls. Survivin immunostaining of feline tissues was reliable, as evidenced by the strong agreement between the pathologists when scoring the slides.

In future studies, larger numbers of cases with more uniform clinical treatment would be more ideal for statistical analysis of survival time. In addition, further techniques, such as PCR, could be used to identify the presence of p53 gene mutations or survivin overexpression. Other methods, such as evaluation of proliferative index and apoptotic index, may aid in further elucidating the role of survivin in tumors of veterinary species.

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APPENDIX

IMMUNOHISTOCHEMISTRY PHOTOMICROGRAPHS

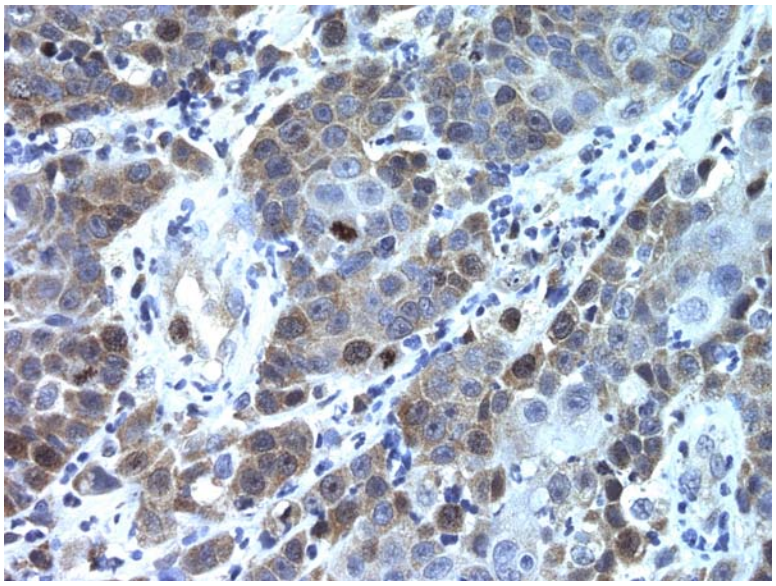

Figure 1

Feline oral SCC, survivin IHC positive.

Cytoplasmic staining of neoplastic cells; mitotic figures positive. Case 30, 40X.

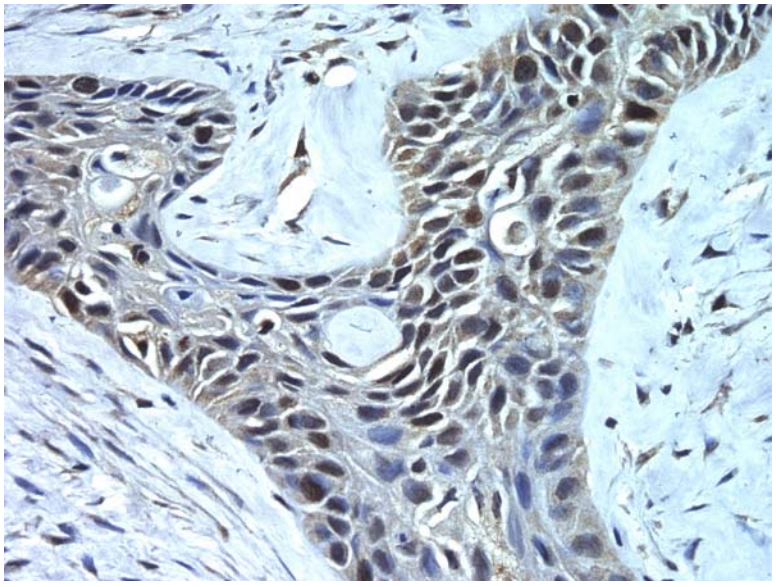

Figure 2

Feline oral SCC, survivin IHC, nuclear staining.

Strong nuclear staining of neoplastic cells. Case 13, 40X.

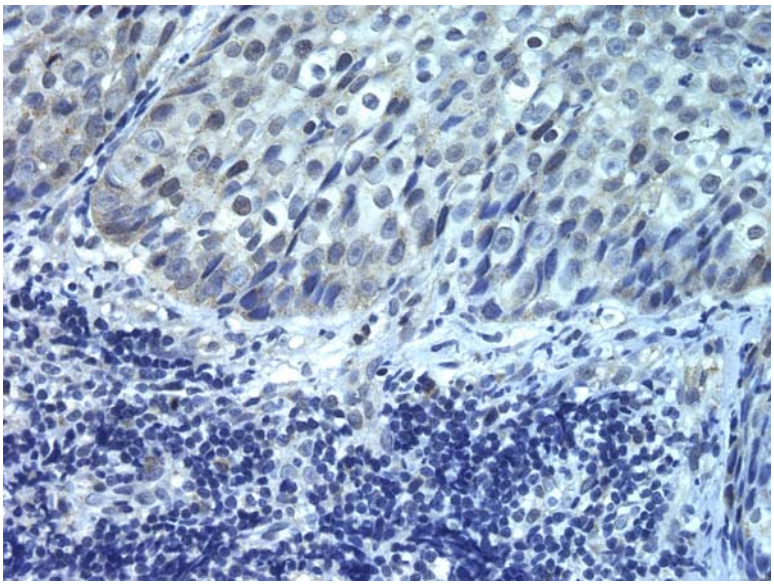

Figure 3

Feline lymph node, survivin IHC.

Metastatic squamous cells in lymph node positive. Case 14, 40X.

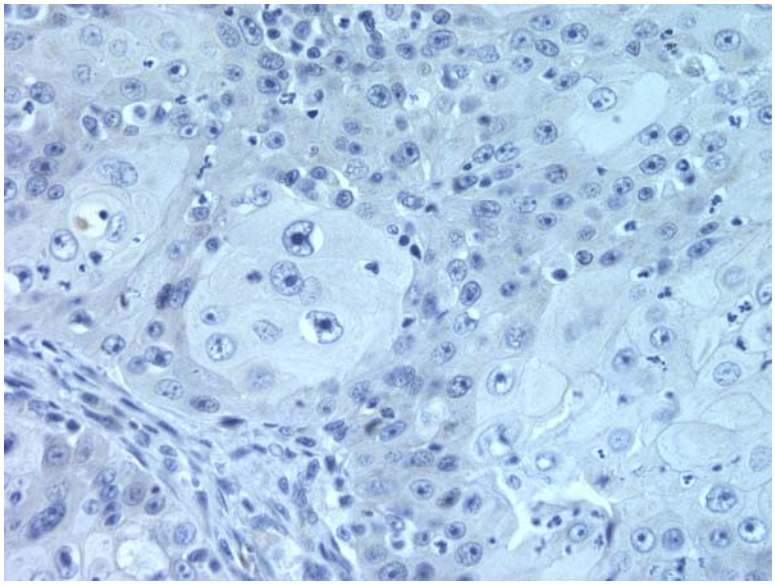

Figure 4

Feline oral SCC, survivin IHC negative.

Case 25, 40X.

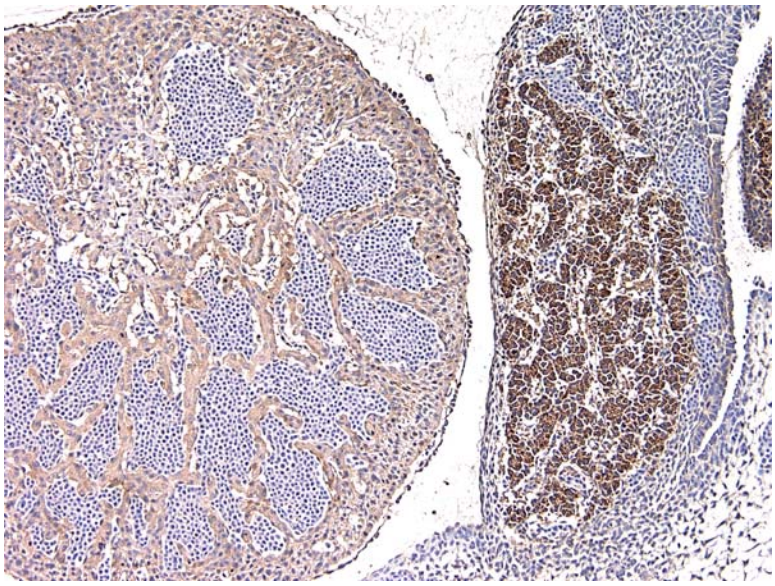

Figure 5

Feline fetus, survivin IHC.

Multiple fetal tissues positive. 10X.

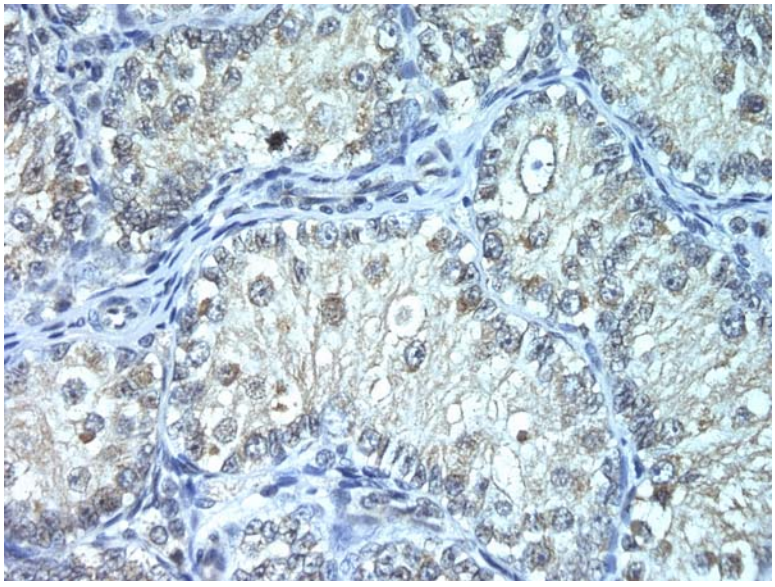

Figure 6

Feline testicle, survivin IHC.

Cytoplasmic staining in seminiferous tubules. 40X.

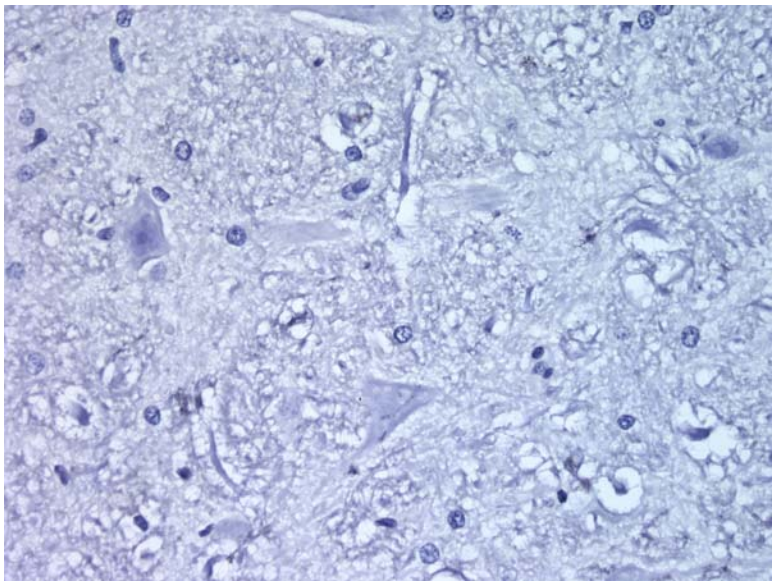

Figure 7

Feline brain, survivin IHC.

No staining. 40X.

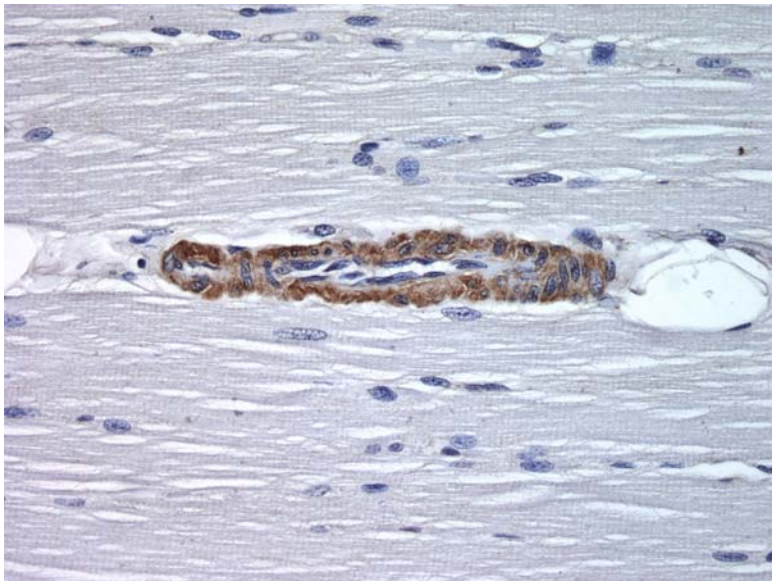

Figure 8

Feline skeletal muscle, survivin IHC.

Skeletal muscle negative; blood vessel endothelium positive. 40X.

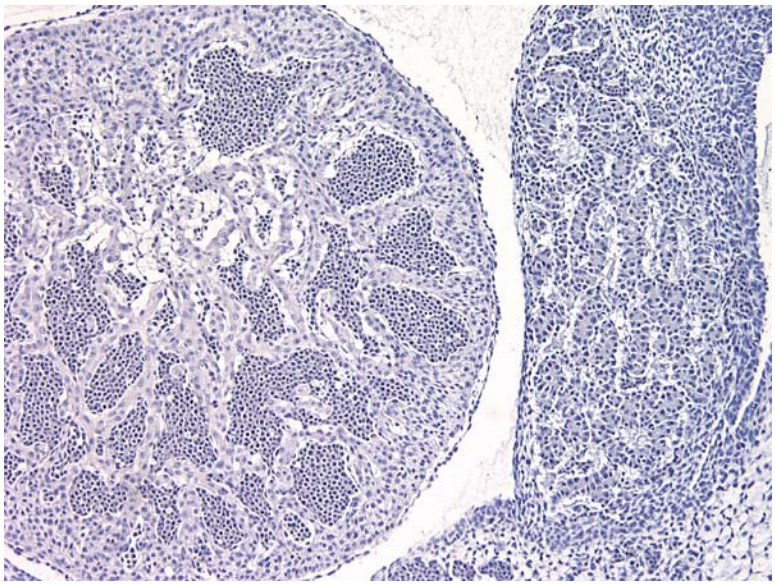

Figure 9

Feline fetus, negative control IHC.

No staining. 10X.

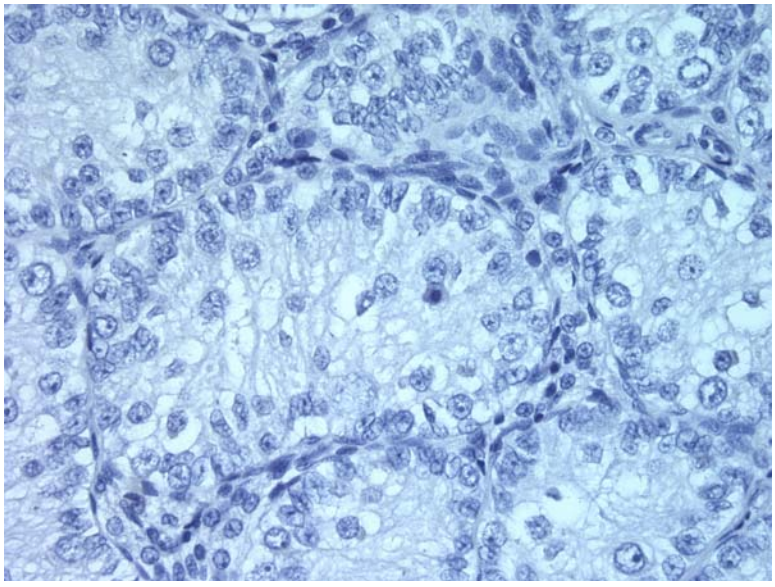

Figure 10

Feline testicle, negative control IHC.

No staining. 10X.

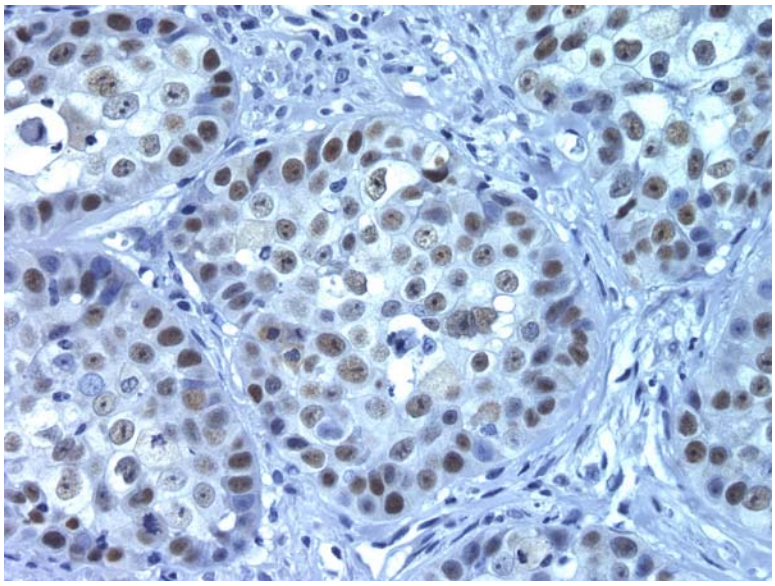

Figure 11

Feline oral SCC, p53 IHC positive.

Nuclear staining of neoplastic cells. Case 5, 40X.

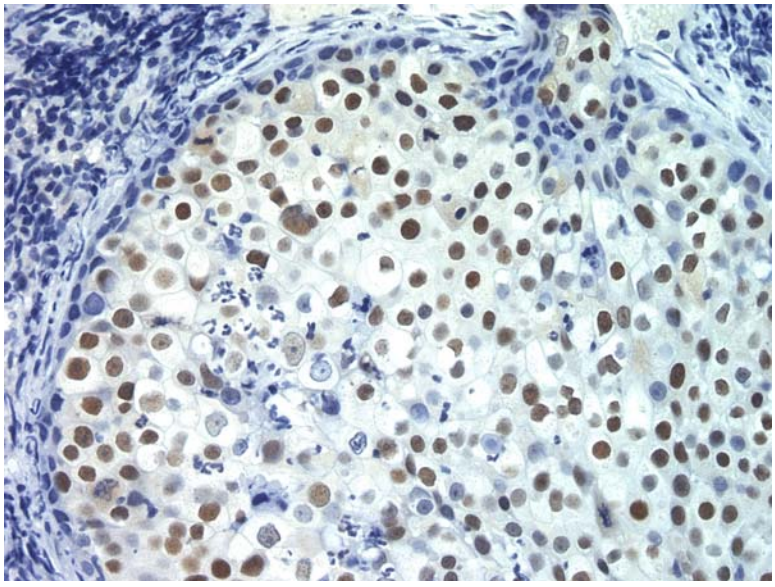

Figure 12

Feline lymph node, p53 IHC.

Metastatic squamous cells in lymph node positive. Case 14, 40X.

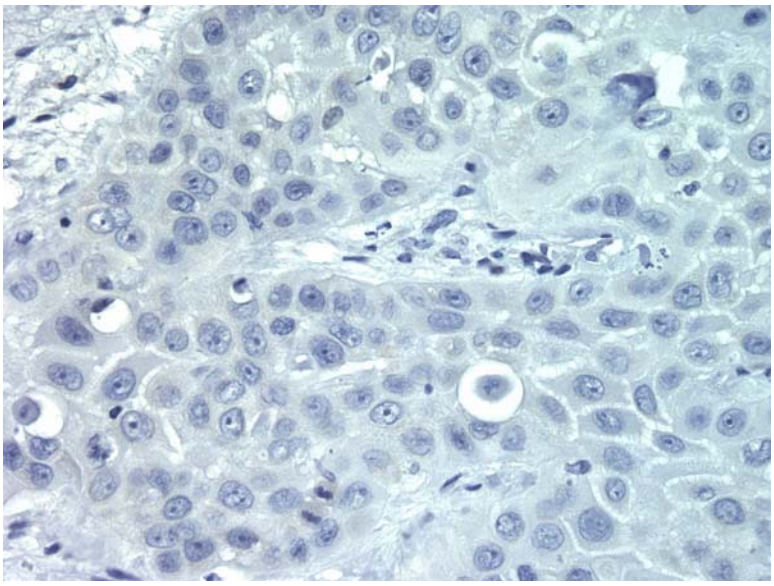

Figure 13

Feline oral SCC, p53 IHC negative.

Case 23, 40X.

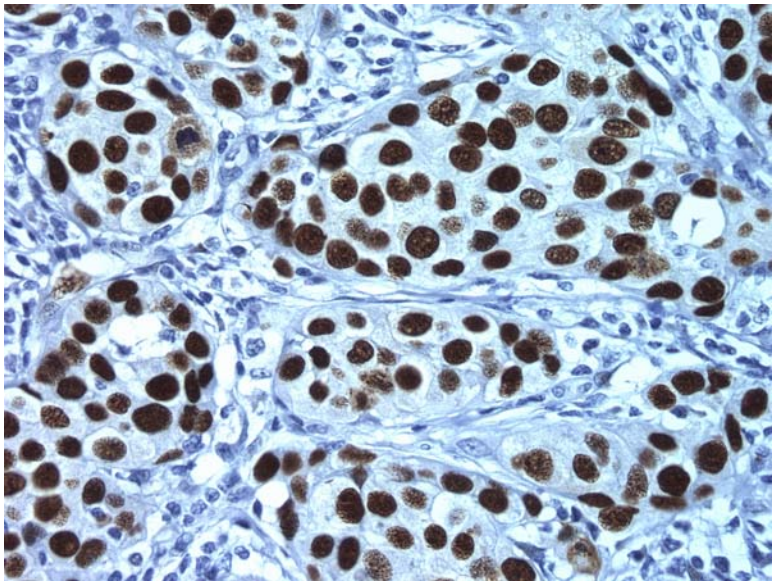

Figure 14

Human breast carcinoma, p53 IHC.

Strong nuclear staining of neoplastic cells. 40X.

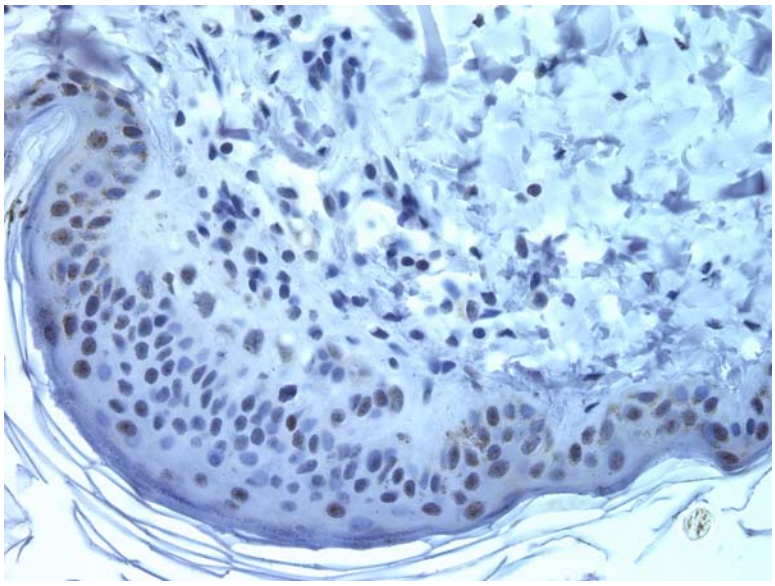

Figure 15

Feline skin, p53 IHC.

Occasional cytoplasmic staining of epithelial cells. 40X.

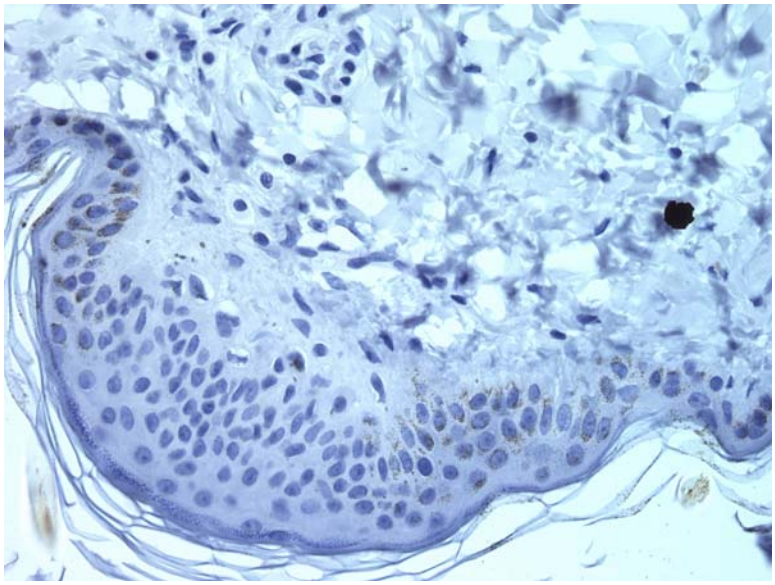

Figure 16

Feline skin, negative control IHC.

Occasional non-specific staining of epithelial cells. 40X.

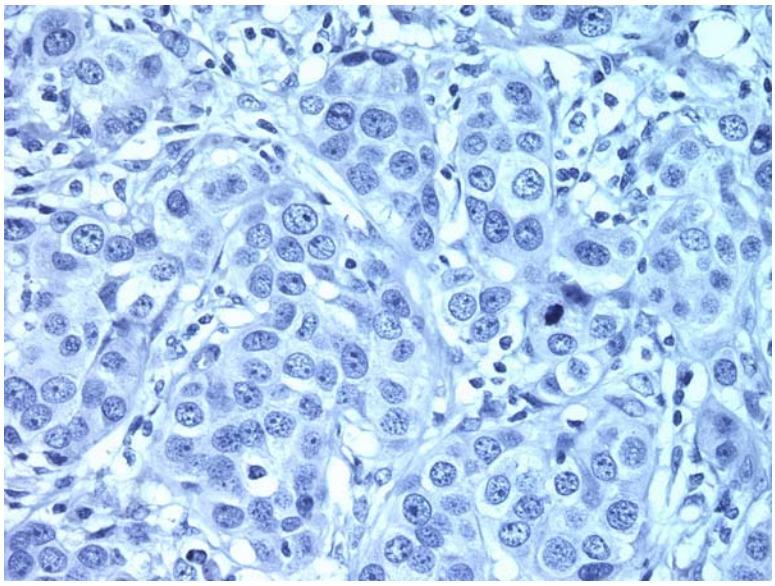

Figure 17

Human breast carcinoma, negative control IHC.

No staining. 40X.