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RESEARCH ARTICLE

S100 can be used as a tumor marker in canine mammary tumors

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ABSTRACT

Background: S100 protein has been localized in different normal and tumorous tissues. Furthermore, its use in tumor localization and identification has already been established. **Aims and Objectives:** The aim of this study is to determine the localization and distribution of S100 immunoreactive cells in different canine mammary tumors (CMTs). **Materials and Methods:** The localization of S100 protein was demonstrated in sections of ten different classifications of CMTs and one normal lactating mammary gland by immunohistochemistry. 10 CMT samples were obtained from tumor resections in different veterinary clinics and hospital in Metro Manila, Philippines, and one normal lactating mammary gland was obtained from an anatomy cadaver for comparison. **Results:** S100 was observed to be positive in the endothelial cells of blood vessels in all the samples which make it suitable as a marker for CMT. Other cells that have been observed to be moderate-to-high immunoreactivity are spindle-shaped cells, chondrocytes and stromal cells which may serve as tumor markers. A positive immunoreactivity on some neuroendocrine cells and epithelial cells was also observed in neuroendocrine differentiated carcinoma. **Conclusion:** Based on the results in 10 CMT, this study suggests that S100 immunohistochemistry can be used as a marker to confirm CMT.

KEY WORDS: *Canis familiaris*; Immunohistochemistry; Mammary Gland Tumors; S-100 Protein

INTRODUCTION

S100 protein, named from their solubility in a 100% saturated solution of ammonium sulfate at neutral pH,[1] is one of the commonly used tumors markers used in immunohistochemistry.[2] It is a multigenic family of non-ubiquitous Ca^{2+} - modulated proteins of the EF-hand type expressed in vertebrates exclusively and implicated in intracellular and extracellular regulatory activities.[3] It has been well established that the genes for several S100 proteins are associated with cell differentiation, malignant transformation, and cell cycle growth, and that their expression levels are varied according to various growth- and growth-inhibitory conditions.[4] S100 has been demonstrated by immunohistochemistry in healthy organs in animals such as in the spleens of bovine,[5] sheep,[6] and Philippine swamp buffalo,[7] in the kidneys of sheep and goat,[8] Philippine swamp buffalo,[9] rat,[10] goldfish,[11] saltwater fishes, frog, lizards and ostrich,[12] in testis of poultry and rabbits,[13] Philippine swamp buffalo,[14] and cat,[14] epididymis of poultry and rabbits,[13] and in ovary of rat.[15] In addition, it was also immunohistochemically demonstrated in pathologic conditions in humans such as papillary carcinoma of thyroid,[16] renal cell carcinoma,[17] and nerve sheath tumors such as neurilemoma, neurofibromas, and myxoid sheath nerve tumor[2] and in animals such as canine amelanotic melanoma.[18] It has also been expressed in cases of acute and chronic inflammatory disorders, gastrointestinal inflammation, lung disorders, primary tumour such as melanoma, head-and-neck cancers, and breast cancer.[19]

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Canine mammary tumors (CMTs) are second to skin tumors as most frequent neoplasm in dogs,[20] wherein approximately half of its cases are malignant, but prevalence is low in countries that routinely perform ovariectomy or ovariohysterectomy.[21] In addition, these tumors were comparatively studied and said to have molecular and biological similarities with the human mammary tumors.[22-24] Based on this similarities, different classification methods of CMTs have been proposed in the literature, some of them are adapted from human classification systems.[25-28] Although diagnosis as well as prognosis in cases of mammary tumors remains a challenge, researches done on this subject had been of great help and had been an interest of research in the field of pathology and oncology.

Some of the diagnostic tools that are routinely used in tumors include physical examination, radiographic screening, and histopathological diagnosis through the routine use of hematoyxlin and eosin staining in surgical biopsy or aspiration biopsy, but there may be factors that may alter these morphologically-based diagnostics that may lead to confusion and to an erroneous diagnosis of malignancy.[29] Thus, there have been suggestions on the use of immunohistochemistry which have been proven to be a valuable technique in research and diagnostic histopathology and cytology for the identification and classification of tumor cells using a wide variety of markers.[30,31]

As there are many different tumor markers, each are indicative of particular disease process, and they are used in oncology to help detect the presence of cancer. Particularly with S100 protein, diseases that are associated with altered expression levels of S100 can be classified into four categories as follows: Neurologic disorders, neoplastic disorders, cardiac diseases, and inflammatory diseases. S100 can be found in body fluids, including serum, urine, seminal plasma, saliva, sputum, cerebrospinal fluid and feces and abscess fluid, principally associated with active disease states. According to Donato *et al.*,[19] S100A8 or S100A9 complex and S100B are considered biomarkers for particular disease processes. An elevated level of a tumor marker can indicate cancer, but there can also be other causes of the elevation. Different forms of cancer exhibit dramatic changes in the expression of S100 proteins such as S100B, S100A2, S100A4, S100A6, and S100P.[32] For example, elevated levels of S100A4 (metastasin) are associated with poor survival rates in human breast cancer patients and have been found to induce metastasis in mouse models. Another example is a high secretion of S100B in malignant melanoma, reflecting tumor load, stage, and prognosis.[33] According to Sedaghat and Notopoulos,[34] other members of the S100 protein family may prove to be useful biomarkers in future applications and may S100 protein-targeted therapies emerge as useful opportunities in specific clinical settings. Despite the emergence of newer markers reflecting differentiation, proliferation, immunomodulation, and other relevant processes, according to Ohsie *et al.*,[35] S100B remains to be the most sensitive immunohistochemical marker of melanoma. According to Schmitt and Bacchi,[2] S-100 protein is also useful as a tumor marker in diagnostic immunocytochemistry. In their study, they detected S-100 protein by the immunoperoxidase technique in a heterogeneous group of 159 tumors to determine whether this marker may be of value in facilitating immunocytochemical diagnosis. The results have shown that S100 was widely distributed and demonstrated the strongest degrees of reactivity. S100 was identified in virtually all nerve sheath tumors such as neurilemoma, neurofibromas, myxoid sheath nerve tumor, and also in tumors of controversial histogenesis such as granular cell tumors. In addition, the great majority of carcinomas in their study did not express S100, with only two cases of breast carcinoma displaying focal S100 staining. Despite its presence in a wide array of cell types, it was concluded that S100 protein continues to be an extremely useful marker, especially for soft tissue and peripheral nervous system tumors.

By recognizing and understanding the signatures of normal cells and how these become cancerous, technology can provide important insights into the etiology of cancer that can be useful for early detection, diagnosis, and treatment. Therefore, biomarkers could be an invaluable tool for cancer detection, diagnosis, prognosis, and therapy selection.[36] One of the advent laboratory diagnostic tools, immunohistochemistry, was used in this study, particularly in detecting S100 protein in mammary tumors. Since there had been challenges in diagnosing CMT, as a possible option to do immunohistochemistry in addition to the routine diagnostic method of hematoxylin and eosin staining for histopathology, this study could have a potential for further investigation for more precise diagnosis. When S100 protein will be proven to be a good tumor marker in the tumorous mammary organ in dogs, this study could be useful to veterinarians, practitioners, clinicians, and pathologists. This study could also be a reference for future researchers who are interested in developing more accurate diagnostic tools for various diseases such as tumors and further studying on other potential tumor markers. In addition, as there have been previous studies on S100 proteins in human specimens and in other animal species, this study could be of help in decreasing the limitation of its use in the veterinary field and may also serve as a diagnostic tool in the future and may be further studied for its use in classifying and prognosis of CMT.

MATERIALS AND METHODS

Specimen Sampling

Ten mammary tumor specimens and a normal mammary tissue from dogs regardless of age and breed were studied. Ten mammary tumor specimens were obtained from bitches that underwent surgery at the different veterinary clinics and hospitals within Metro Manila, Philippines from December 2015 to March 2016. Consent from the owners as well as the Veterinarians were obtained before sampling. Normal

mammary tissue were obtained from anatomical cadavers of De La Salle Araneta University. Immediately after surgical resection, tissue samples were collected from the middle portion of the tumor and fixed in 10% buffered formalin for 72 h. The same method was done for the normal lactating mammary gland sample. The fixed samples were then processed routinely using the paraffin technique, sectioned at 5 µm using an 820 rotary microtome (American Optical®, New York) and mounted on ordinary slides for Hematoxylin-Eosin staining to identify various components of the mammary gland or on polysine-coated slides (Thermo Scientific, Massachusetts) for immunohistochemistry.

Immunohistochemistry

The avidin-biotin-peroxidase complex (ABC) method[30] was used to detect immunoreactivity to S-100. Two tissue sections per sample were deparaffinized and washed with 0.1 M tris buffer saline (TBS) at pH 7.6, seven times at 5 min interval. The tissue sections were incubated with 20% normal goat serum (NGS) in TBS for 30 min under room temperature to decrease non-specific staining. Thereafter, the sections were incubated overnight with primary antibody, the polyclonal rabbit anti-S-100 (DakoCytomation, Copenhagen) at 1:2000 dilution with TBS containing 5% NGS at 4°C in a moisture chamber. The sections were washed with TBS four times at 5 min interval; incubated with biotinylated anti-rabbit IgG, 1:500 dilution (Vector Laboratories, California) in TBS containing 1.5% NGS, for 90 min at room temperature; washed in TBS three times at 5 min interval; incubated with ABC (Vector Laboratories, California) for 60 min at room temperature; and rinsed with TBS three times at 5 min interval. The S100 immunoreactivity was visualized through incubation of the tissue sections in a solution of imidazole-HCl buffer containing 0.05% 3,3'-diaminobenzidine, for 8 min at room temperature. Immunoreaction was stopped by washing the tissue sections in TBS 10 times until no further browning of the sections is occurring. The immunostained sections were placed in a covered plastic container to dry overnight at room temperature. Thereafter, the sections were rehydrated in triple distilled water twice, dehydrated in increasing concentrations of ethanol (70%, 90%, 100% and 100%) for 3 min per concentration, cleared in two changes of xylene for 3 min, and mounted with entellan (Eukitt®) (Merck KgaA, Darmstadt). The skin and skeletal muscle were used as a positive and negative control, with the same procedures performed for the canine mammary gland.

Data Gathering

The tumors collected were classified according to the World Health Organization criteria for canine mammary lesions[25-28] based on the most pronounced histological pattern observed in more than 50% of the tumor mass. Whenever tumors displayed multiple morphological patterns, without more prominent growth pattern present in 50% of the tumor mass, lesions were classified as tumors with mixed morphology tumor. Tumor malignancy grade was determined using the Elston and Ellis[37] scoring system based on the assessment of three morphological features: (1) The degree of glandular differentiation assessed using tubular formation, (2) nuclear pleomorphism, and (3) mitotic activity. Each parameter was graded into three categories to which a score of 1–3 was assigned. For evaluating the tubule formation, the tubular structures were scored qualitatively and quantitatively while the proportion occupied by such tubular structures was assessed semiquantitatively. One point is assigned when more than 75% of the area is composed of definite tubules. Two points are allocated for tumors in which between 10% and 75% of the area shows tubule formation. Where tubules occupy 10% or less of the tumor, three points are assigned.[37] On the other hand, in assessing nuclear pleomorphism, one point is appropriate when the tumor nuclei are small regular uniform cells. Two points are given when the nuclei are larger than normal, have more open vesicular nuclei with visible, usually single, nucleoli, and there is moderate variation in size and shape. When there is marked variation in size and shape, especially when there are very large and bizarre nuclei present, three points is given. Finally, for the mitotic count, up to 7 mitosis per 10 high fields are scored 1 point, 8–16 are scored 2 points and more than 17 are scored 3 points.[37] After the scores were added of each category, a number between 3 and 9 would be obtained then using the Elston grade, the following grade was allocated on the following basis: Grade I (well differentiated) is allocated for 3–5 points, Grade II (moderately differentiated) for 6–7 points and Grade III (poorly differentiated) for 8–9 points.

Tumor and normal lactating mammary gland sections were observed under a light microscope to identify S100 immunoreactive cells. The reactions were designated as positive (+) or negative (−). Photomicrographs of representative sections were taken using light microscope with digital camera (Olympus Camedia C-400) (Olympus UK Ltd., Hertfordshire) attachment for documentation.

RESULTS

Ten CMTs were evaluated using the hematoxylin and eosin and classified according to the histological classification of mammary tumors of the dog and cat[28] [Table 1].

Three tumors were benign, six were malignant and one case of dysplasia. One normal mammary gland sample was used for comparative immunohistochemical evaluation. Immunohistochemical reactivity of the different cell types is summarized in Table 2.

Normal Mammary Gland

In the normal mammary gland of dog [Figure 1], the secretory parenchyma is well developed and the connective tissue is reduced. The lumens of the secretory glands and ducts

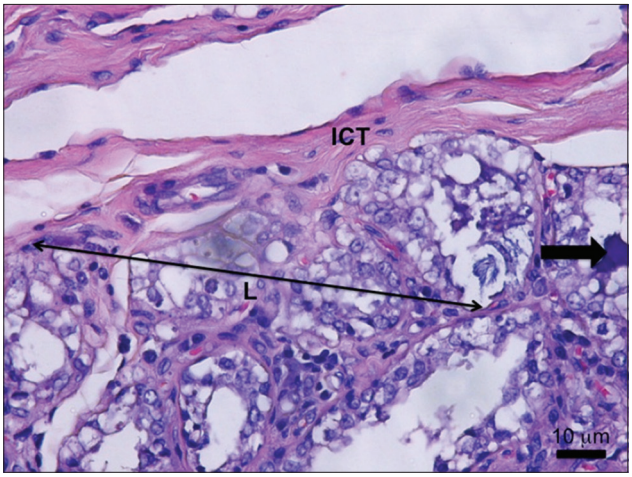

Figure 1: H and E section from the normal lactating mammary gland of dog showing the different structures: Lobule (L), interlobular connective tissue, blood vessel, and secretion (arrow) from the secretory unit

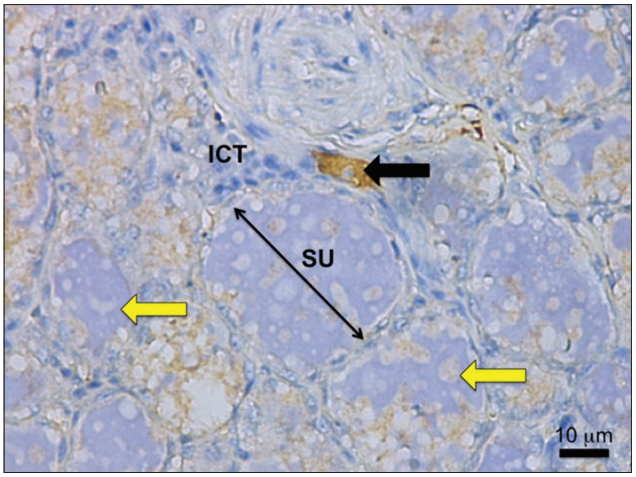

Figure 2: A section of the normal lactating mammary gland showing a positive reaction to S100 of the blood within blood vessel (black arrow) and faint positive staining in milk secretions (yellow arrow). Secretory unit or alveoli interlobular connective tissue

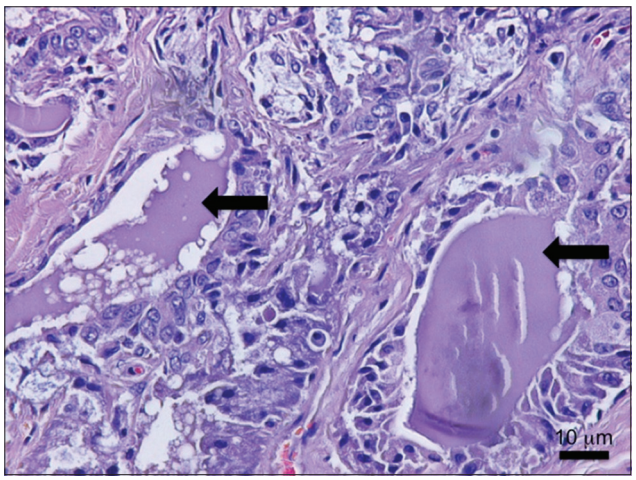

Figure 3: H and E section of the canine mammary tumor with simple adenoma. The ducts contain amorphous secretion (arrows)

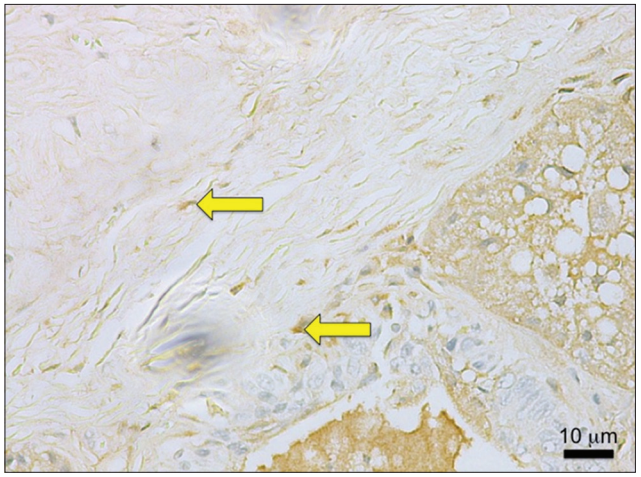

Figure 4: A section of the canine mammary tumor with adenoma showing faint positivity in the stroma and a moderate stain on active fibroblast (yellow arrow) to S100

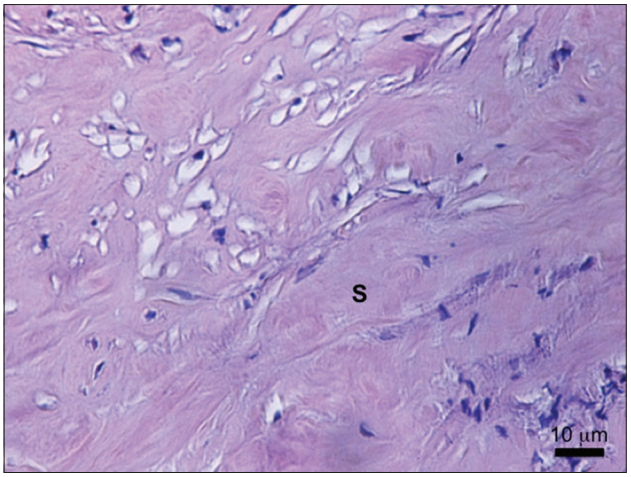

Figure 5: H and E section of the canine mammary tumor with fibroadenoma showing a hyalinized stroma (S)

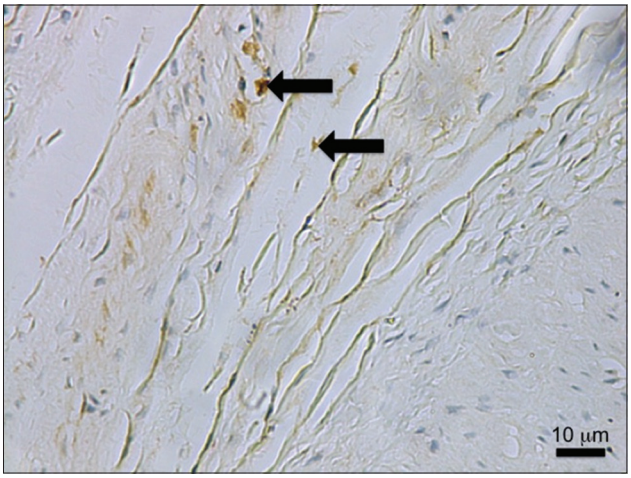

Figure 6: Fibroblasts in the stroma (arrows) showing positive immunoreactivity to S100 in fibroadenoma while the epithelial cells (E) are negative

are filled with secretion. The epithelial and myoepithelial cells were negatively immunostained by S100, and only the endothelial cells of vessels and blood were found to be immunoreactive. There was also a faint brown stain observed in the secretions within alveoli which are milk [Figure 2].

Adenoma

In the hematoxylin and eosin stain, it was described to have epithelial lesion wherein there is lactating adenoma and atypia. There are the presence of cystic ducts containing amorphous secretion [Figure 3] admixed with cellular debris, foamy histiocytes, and inflammatory infiltrates. The ducts are lined by ductal cells and with a tendency to show multi-layering. Most cells show secretory snouts. The ductal cells show large, ovoid, slightly pleomorphic nuclei with prominent nucleoli. Epithelial cells were not reactive to S100, and there was faint positive staining in the stroma and moderate stain on an active fibroblast [Figure 4].

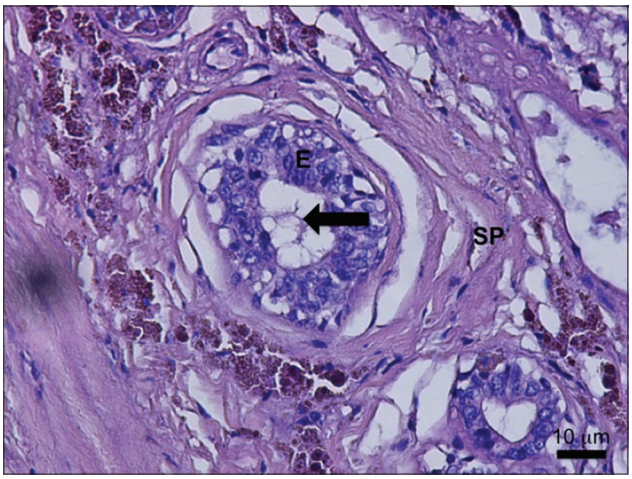

Figure 7: H and E section from the canine mammary tumor with ductal papillomatosis showing the papillary structures (arrow), two cell-type epithelial cells (E) and a spindle-shaped cell or fibroblast

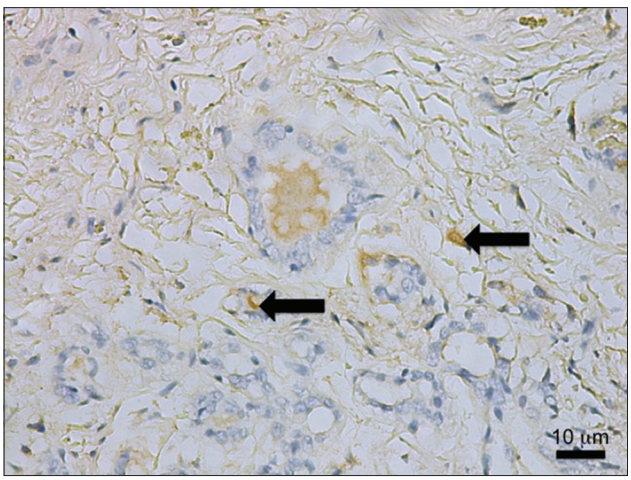

Figure 8: S100 immunostained section of the mammary tumor with ductal papillomatosis showing immunoreactive active fibroblasts (arrows)

Fibroadenoma

Breast tissue shows a solid nodule mostly having hyalinized stroma [Figure 5] with a microscopic focus of necrotic cellular nest along the periphery. A larger focus of necrosis outside the nodule shows large, histiocyte-like cells having numerous, small, intracellular, round hyperchromatic bodies. The background shows abundant necrotic cellular debris and cells with reactive atypia. Fibroblasts in the stroma are immunoreactive to S100 while the epithelial cells are negative. There is also a faint reactivity in the stroma [Figure 6].

Ductal Papillomatosis

Breast tissue shows dilated ducts containing papillary structures [Figure 7] consisting of fibrovascular core superficially lined by two cell-type ductal epithelium. Some fibroblasts [Figure 8] are immunoreactive to S100 with. The epithelial cells were also immunonegative.

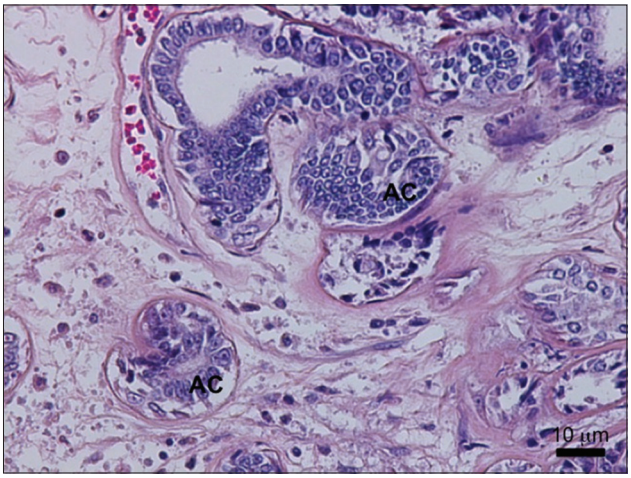

Figure 9: H and E section of a canine mammary tumor with complex carcinoma showing multi-layered atypical cells in the remnant ducts

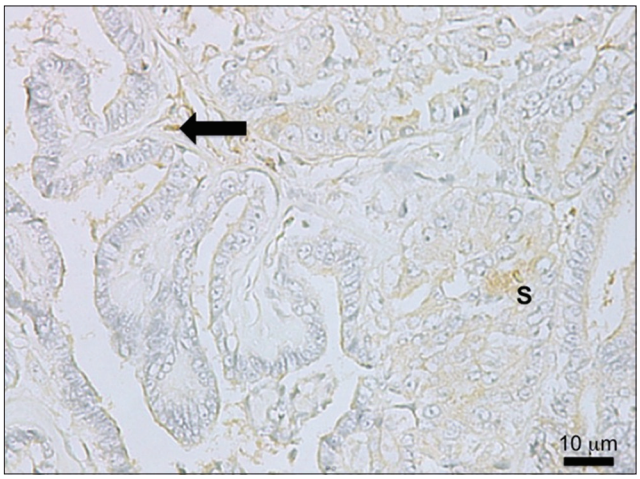

Figure 10: S100 immunostained section of the canine mammary tumor with complex carcinoma showing positive immunoreaction of spindle-shaped cells (arrow) with faint immunostaining in the stroma (S)

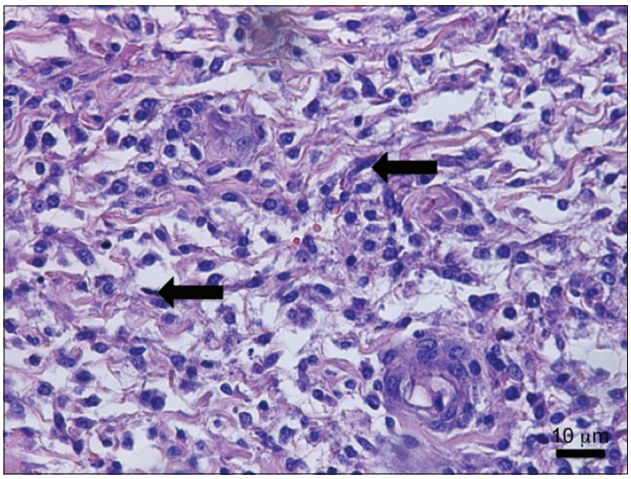

Figure 11: H and E section of the canine mammary tumor with spindle cell carcinoma showing infiltration of spindly cells (arrows)

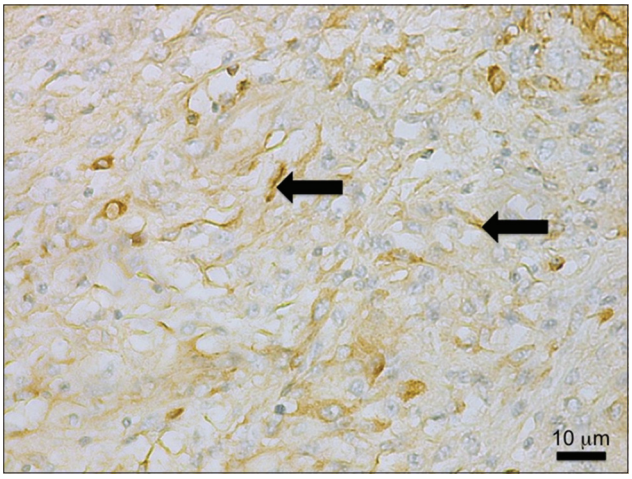

Figure 12: S100 immunostained section of a canine mammary tumor with spindle cell carcinoma showing immunoreactive spindle-shaped cells (arrows)

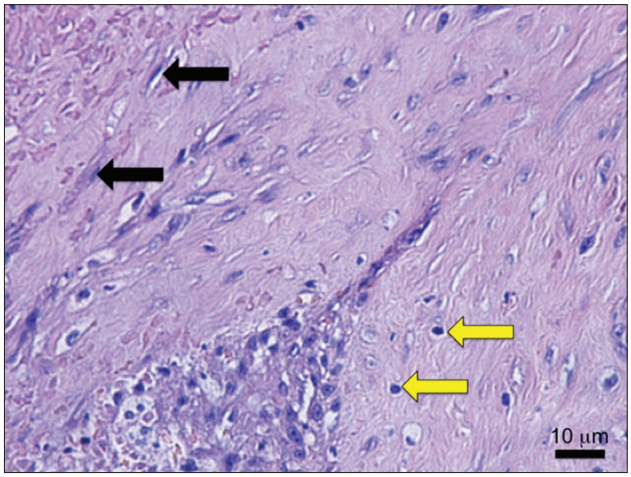

Figure 13: H and E section of a canine mammary tumor with chondrosarcoma showing spindle-shaped cells (black arrow) or fibroblasts and cartilage cells (yellow arrow) (×400)

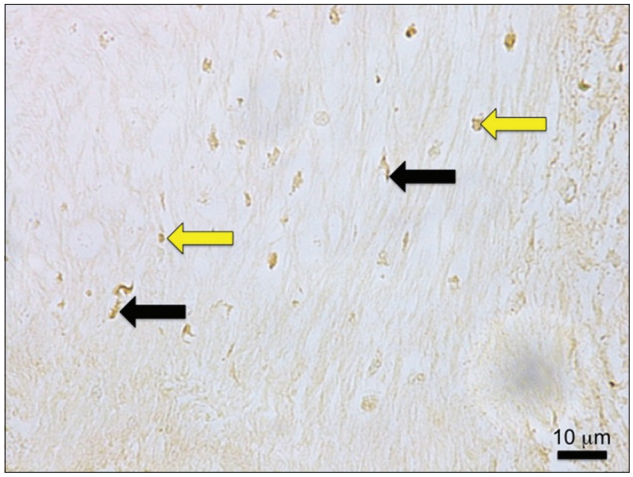

Figure 14: S100 immunostained section of a mammary tumor with chondrosarcoma showing immunoreactivity of spindle-shaped cells (black arrow) or fibroblasts and cartilage cells (yellow arrow)

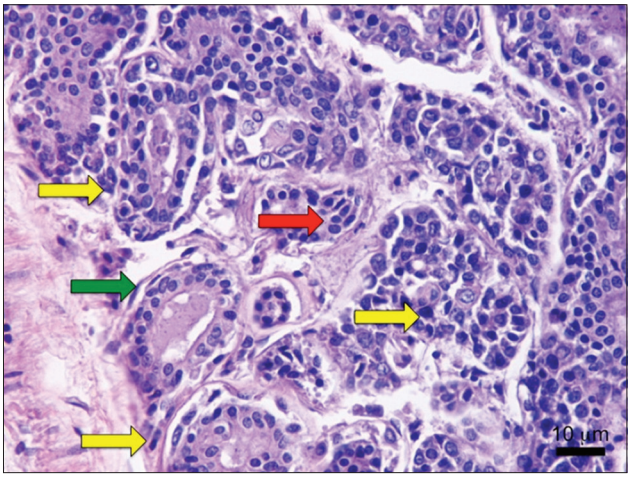

Figure 15: H and E section of the canine mammary tumor with neuroendocrine differentiated carcinoma showing the different cells: myoepithelial cells (green arrow), epithelial cells (red arrow), and neuroendocrine cells (yellow arrows)

Complex Carcinoma

Breast tissue with overlying skin shows focal ulcer. The base of the ulcer shows a remnant gland or duct lined by multilayered atypical cells having large, ovoid, pleomorphic nuclei with prominent nucleoli and moderate, thick, eosinophilic cytoplasm [Figure 9]. These cells freely infiltrate the ulcer base and stroma and can be seen within endothelial-lined spaces. In immunostaining of S100, spindle-shaped cells (SP) [Figure 10] are immunoreactive with faint immunostaining in the stroma.

Spindle Cell Carcinoma

There is fibromuscular tissue with widespread infiltration of neoplastic spindly cells [Figure 11] occurring in random stream and whorls. These cells possess small to medium-sized

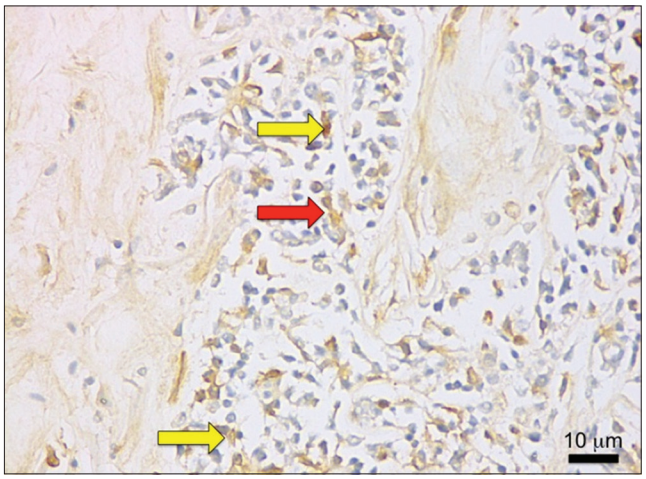

Figure 16: Section of the canine mammary tumor with carcinoma showing some neuroendocrine cells (yellow arrows) and epithelial cells (red arrows) that are positive in S100 immunostaining

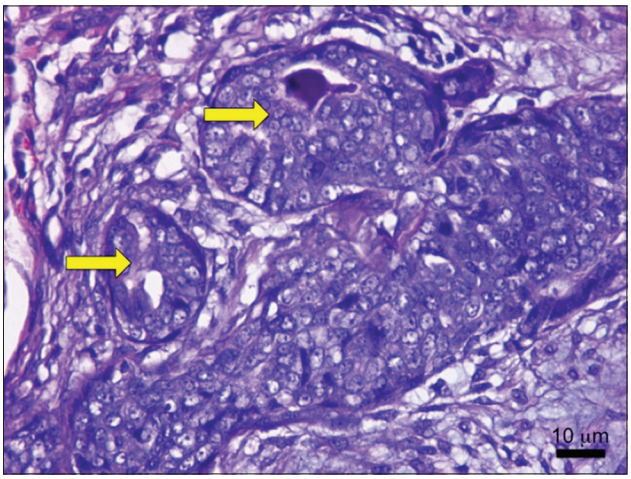

Figure 19: H and E section of a canine mammary tumor with carcinosarcoma showing carcinomatous ductal cells (yellow arrows)

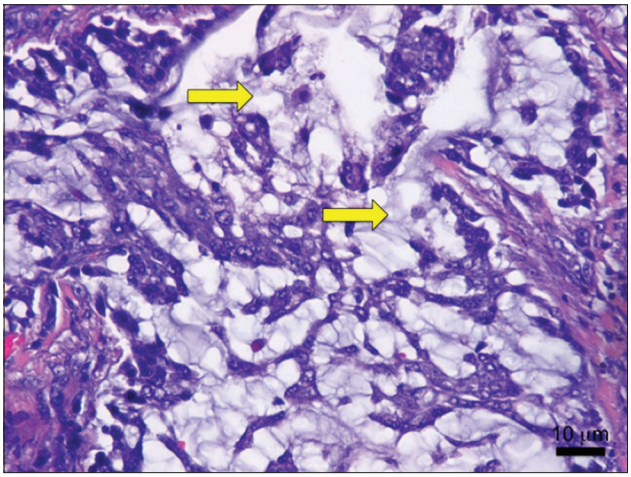

Figure 17: H and E section of a canine mammary tumor with mucinous carcinoma showing mucinous secretions (yellow arrows) in the ducts

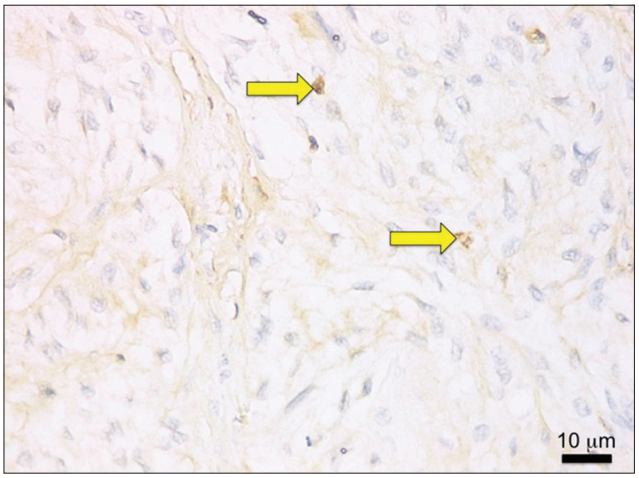

Figure 20: S100 immunostained section of a canine mammary tumor with carcinosarcoma showing positive reactions of some stromal cells (arrows)

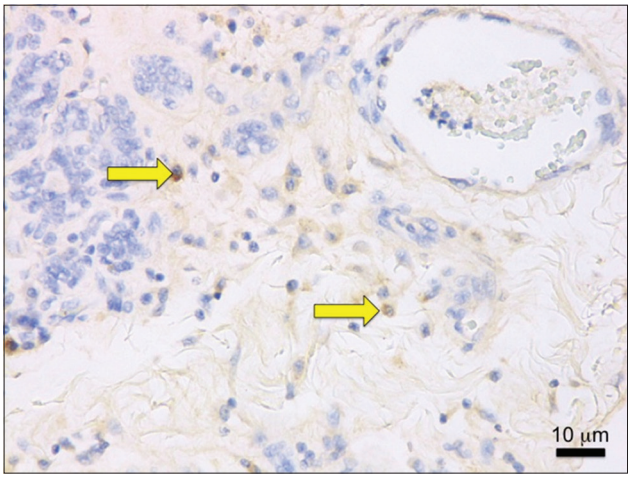

Figure 18: S100 immunostained section of a canine mammary tumor with mucinous carcinoma showing faint immunoreactivity of some stromal cells (arrow)

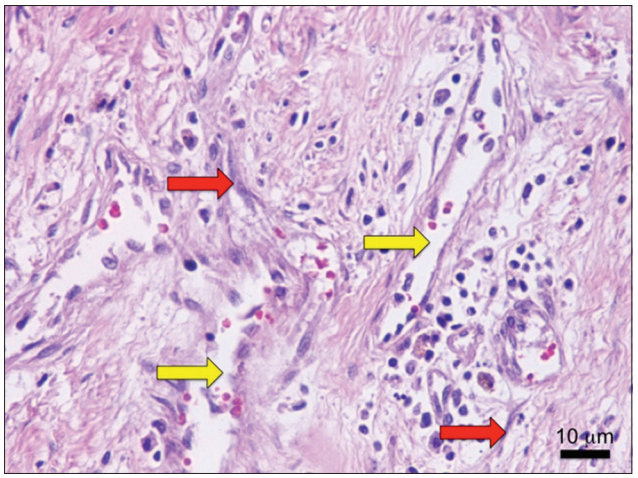

Figure 21: H and E section of a canine mammary tumor with tubular adenosis showing proliferation of tubular glands (yellow arrows) separated by fibroconnective stroma. Myoepithelial cells (red arrows) are evident

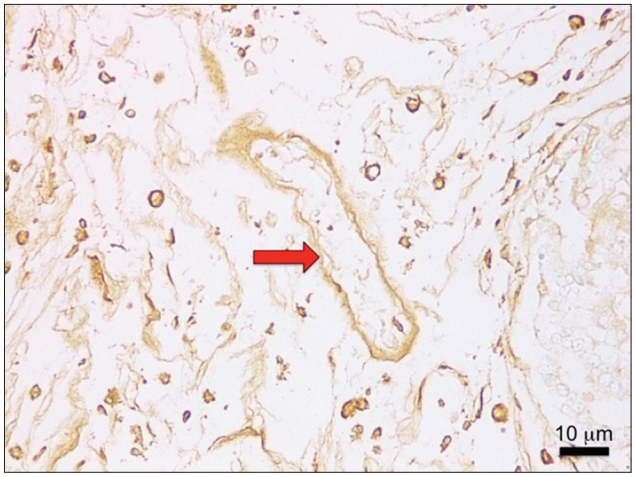

Figure 22: S100 immunostained section of a canine mammary tumor with tubular adenosis showing highly immunoreactive stromal cells (arrow)

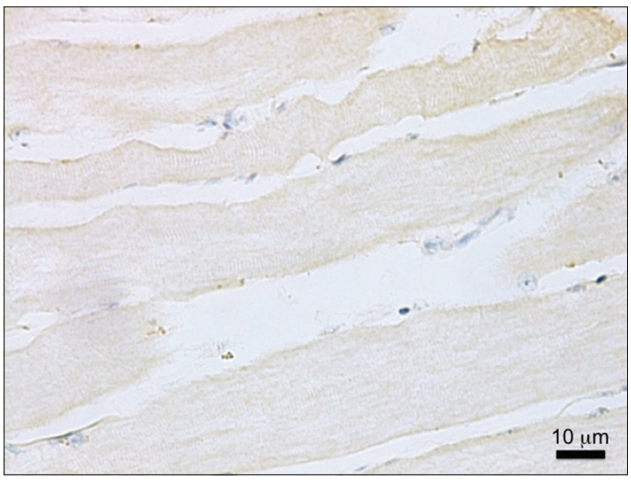

Figure 23: S100 immunostained skeletal muscle as negative control showing negative immunoreaction of cells

ovoid, slightly pleomorphic nuclei and fibrillary, eosinophilic cytoplasm. In S100 immunostaining, SPs [Figure 12] are immunoreactive with faint staining in the stroma.

Chondrosarcoma

There is mesenchymal lesion and tumors made up of neoplastic chondroid [Figure 13] occurring in irregular islands with intervening highly cellular areas and pockets of necrosis. The cartilage cells and spindle-shaped cell are both immunoreactive to S100 [Figure 14].

Carcinoma with Neuroendocrine Differentiation

Breast tissue shows a mass made up of small, uniform, hyperchromatic cells in trabeculated nests separated by delicate fibroconnective tissue. Cells possess small, round, compact, hyperchromatic nuclei, and scant cytoplasm

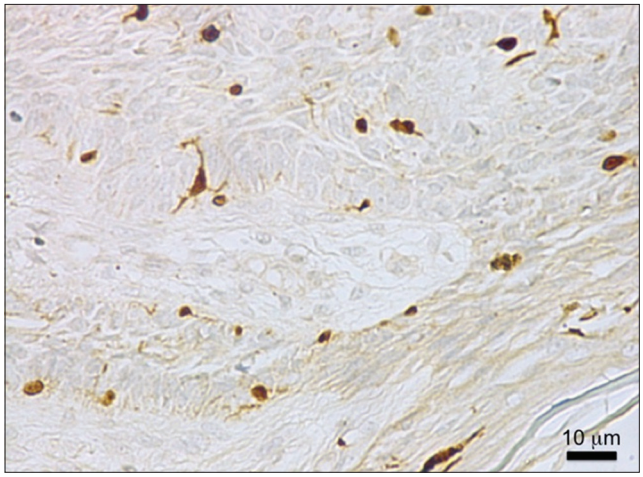

Figure 24: Immunostained section of skin as positive control showing highly immunoreactive melanocytes (stained brown)

[Figure 15]. In S100 immunostaining, neuroendocrine cells and luminal epithelial cells are immunoreactive [Figure 16].

Mucinous Carcinoma

In H and E staining, breast tissue shows dilated ducts lined by multilayered neoplastic cells. The ducts contain abundant extracellular mucin within which float the neoplastic cells [Figure 17]. In S100 immunostaining, there is faint staining of some stromal cells [Figure 18]. No epithelial and myoepithelial cell immunoreactivity to S100 has been observed.

Invasive Ductal Carcinoma with Chondromyxoid Sarcoma or Carcinosarcoma

Breast tissue shows an admixture of ductal and chondromyxoid elements. The ductal cells which are carcinomatous possess large, ovoid, pleomorphic nuclei, and polygonal cytoplasmic occurring in solid nests with the glandular formation [Figure 19]. These carcinomatous cells are randomly distributed within abundant chondromyxoid stroma forming nodular masses [Figure 19]. In S100 immunostaining, some stromal cells were found to be positive [Figure 20].

Tubular Adenosis

There is proliferation of crowded tubular to complex glands separated by delicate and scanty fibroconnective intervening stroma [Figure 21]. Some glands form simple intertwined tuftings or short papillations while intraluminal bridging and fenestrations are seen in others. Myoepithelial cells are still evident. In S100 immunostaining, epithelial cells are negative while the stromal cells are highly immunoreactive [Figure 22].

For the controls used in the study, the negative control used is a section of skeletal muscle [Figure 23] and the

positive control is a section from the skin [Figure 24]. These controls were used to indicate that the immunohistochemical procedure is optimized and working.

DISCUSSION

S100 has also been demonstrated in specific normal organs of other species such as in buffaloes,[7,9] sheep and goat,[6] rats,[15] poultry and rabbits,[13] and humans.[38] In these studies, the endothelial of capillaries, arteries, veins, and lymphatic vessels are regularly S100 protein immunoreactive. This is similarly observed in the endothelial cells of the blood vessels (BVs) in the normal lactating mammary and in the ten cases of CMTs wherein the endothelial cells of the BV particularly the arterioles are also immunoreactive with moderate to intense stain. Although normal BVs generally are not reactive with either monoclonal or polyclonal anti-S100 antibodies, weak labeling of a minority of endothelial cells are still noted.[39] The immunoreactivity of these endothelial cells in both normal and tumorous mammary may be due to the regulatory activity of extracellular S100 proteins on the endothelial and vascular smooth muscle in order to participate in innate and adaptive immune responses, cell migration, and chemotaxis, tissue development and repair and leukocyte and tumor cell invasion.[19] Since the endothelial cells of BVs in both normal and tumorous mammary are observed to positively react to S100, these cells cannot be used as markers for CMT.

There was also faint positive immunostaining observed on the milk secretions within the alveoli of a normal lactating mammary. This may imply the possible presence or expression of S100B in canine milk since S100B are normally found in small amounts in human milk.[40]

The ten classifications of CMTs studied were all positive to S100 with notable differences in cells that were immunoreactive. The cells that are constantly observed moderately positive to S100 are the SPs and stromal cells as particularly observed in the majority of the obtained specimens, in cases of adenoma, fibroadenoma, ductal papillomatosis, complex carcinoma, spindle cell carcinoma, chondrosarcoma, mucinous carcinoma, carcinosarcoma, and tubular adenosis. These SPs and irregularly-shaped cells in the stroma are identified as fibrocytes and fibroblasts as confirmed in the visualization of these cells in the hematoxylin and eosin staining based on its morphology.

Fibrocytes are Commonly Reactive to S-100

Fibrocytes are the most common cells of the connective tissue or stroma. They are generally elongated and spindle-shaped, with processes that contact adjacent cells and fibers, maintaining the connective tissue matrix by forming the fibers and constantly renewing the ground substance. On the other hand, fibroblasts have a larger, more euchromatic nucleus and more abundant, basophilic, cytoplasm than the fibrocyte and they are more active in the connective tissue matrix fibroblasts may arise directly from mesenchymal cells or are transformed from fibrocytes under the influence of microenvironmental factors such as cytokines.[41] Fibroblasts have also been found to be a major cell type in the tumor stroma,[42,43] which was also observed in this study. Based on the study by Kalluri and Zeisberg,[42] fibroblasts are associated with cancer cells at all stages of cancer progression and that

Table 1: Classification (according to WHO) of 10 CMT

Type of tumor	*n*
Benign tumors	3
Simple adenoma	1
Fibroadenoma	1
Ductal papillomatosis	1
Malignant tumors	6
Complex carcinoma	1
Spindle cell carcinoma	1
Chondrosarcoma	1
Carcinoma	1
Mucinous carcinoma	1
Carcinosarcoma	1
Hyperplasia and dysplasia	1
Adenosis	1

WHO: World Health Organization, CMT: Canine mammary tumors

Table 2: Immunohistochemical reactivity of cell types for S100 in 10 CMTs and in normal lactating mammary gland

Type of tumor	Cell types or structures and immunoreactivity	
	Positive	Negative
Normal lactating mammary gland	En	E, R, P, SP
Benign tumors		
Simple adenoma	En, SC	E, R, P
Fibroadenoma	En, SP, SC	E, R, P
Ductal papillomatosis	En, SP	E, R, P
Malignant tumors		
Complex carcinoma	En, SC	E, R, P
Spindle cell carcinoma	En, SP	E, R, P
Chondrosarcoma	En, SP, C	E, R, P
Carcinoma with endocrine differentiation	En, E, NEC	S, R, P
Mucinous carcinoma	En, SC	E, R, P
Carcinosarcoma	En, SC	E, R, P
Hyperplasia and dysplasia		
Adenosis	En, SC	E, R, P

SP: Spindle-shaped cells, C: Cartilage cells, SC: Stromal cells, E: Epithelial alveolar or ductal cells, R: Resting myoepithelial cells, P: Proliferative suprabasal myoepithelial cells, En: Endothelial cell of blood vessel, NEC: Neuroendocrine cells. CMTs: Canine mammary tumors

their structural and functional contributions to this process are beginning to emerge. This suggests that production of growth factors, chemokines, and extracellular matrix by fibroblasts facilitates the angiogenic recruitment of certain cells. The impact of fibroblasts on tumor growth and progression has been the subject of the intensive investigation recently.[44] As observed in this study, fibroblasts, fibrocytes and cartilage cells, which are cell types of mesenchymal origin, were positive to S100. The immunoreactivity of these cells to S100 may be explained by the expression of specific members of S100 in relation to their functions, particularly in mammary tumors. Some members of S100 protein family that have been noted in breast cancer are S100A4, S100A7, S100A8, S100A9, S100A6 and S100A11.[34] These members of S100 have been said to exist as homodimers within cells[3] and that upregulated gene expression of these proteins may imply the presence of a pathological condition such as tumor since overexpression of particular S100s has been found to be associated with tumorigenesis.[19] One of the members of S100 is closely associated with fibroblasts, and that is the fibroblast-specific protein 1, also named as S100A4 which can be expressed by different cell types of mesenchymal origin.[45] S100A4 expression found in the stroma had been found to contribute to metastatic dissemination.[46] In human breast cancer cells, S100A4 overexpression is associated with increased migratory capacity since it was discovered that S100A4 has angiogenic effects.[47] The explanation on this angiogenic effect of S100A4 is due to the interaction of this protein with Annexin II, and endothelial plasminogen co-receptor, and accelerated tPA-mediated plasminogen activation. This resulting local plasmin formation was found to contribute to tumor-induced angiogenesis and metastasis.[48] Thus, both the angiogenic functions of fibroblasts and its expression of S100A4 visualized by immunoreactivity in S100 immunostaining may have an indication of an undergoing tumorigenesis in the tissue.

Chondrocytes are Moderately Reactive to S-100

Other cells that have been found to be moderately immunoreactive are chondrocytes, which were observed in the chondrosarcoma. These chondrocytes were positive to S100 probably due to its relation with fibroblasts as stated in a reference that in certain situations, fibroblasts may differentiate into chondroblasts which are active cartilage cells that form the matrix of cartilage and becomes chondrocytes when less inactive.[41] Another possible explanation would be due to the other intracellular function of S100A4 at nanomolar levels, which stimulates matrix metalloproteinase 13 release from chondrocytes in a receptor for advanced glycation end products-mediated manner.[49] Relatively, other members of S100, S100A8, and S100A9, also found in breast cancer, form a heterodimer complex implicated in regulating cell proliferation and in the metastatic process such as increasing the motility of cancer cells and facilitating the homing of migrating cells to pre-metastatic "niches" within the targets tissues.[50]

Neuroendocrine Differentiation Reactivity to S-100

In one rare case, carcinoma with neuroendocrine differentiation, there were some neuroendocrine cells that were positive to S100. Neuroendocrine tumors of the breast are rare, accounting for <0.1% of all breast cancer and <1% of all neuroendocrine tumor.[51] Neuroendocrine tumors are slow-growing tumors derived from neuroendocrine cells which are present throughout the body.[52] These S100-positive neuroendocrine cells may be due to the extracellular function of another member of S100 family, the S100B, which is expressed in astrocytes, Schwann cells, melanocytes, associate satellite cells, certain neuronal population and few other cell types. The positive immunoreaction of these neuroendocrine cells to S100 may be due to the sophisticated interrelationship of neuronal cells and glial cells.[53]

Luminal Epithelium is Variably Reactive to S-100

Finally, luminal epithelial cells in the normal lactating canine mammary and in 9 CMTs were observed to be negative to S100 except for carcinoma with neuroendocrine differentiation wherein the epithelial cells were positive. This observation was parallel with the result in other a related study wherein some luminal epithelial cells were positive to S100 in some cases of human breast cancer.[54]

This study was conducted to detect S100 immunohistochemically in CMT. In addition to the gold standard procedure, hematoxylin, and eosin staining, for histopathological diagnosis of tumors, the use of immunohistochemical staining was employed to determine the immunoreactivity of the different cells in CMT. 10 CMT samples were obtained from surgical resection in the different veterinary clinics and hospital in Metro Manila, Philippines, and a normal mammary sample was also obtained for comparison. The samples were processed for hematoxylin and eosin staining and S100 immunostaining. The hematoxylin and eosin stained sections were histopathologically diagnosed and classified according to the histological classifications of the mammary tumors of the dogs and cat. The malignant tumors were further graded using the Elston and Ellis method.

The S100 immunostaining of the sections showed that there is a moderate immunoreactive of the endothelial cells of the BVs in the normal lactating mammary and in the 10 cases of CMT. These cases of CMTs were all positive to S100. The cells that were found to be moderate to highly immunoreactive to S100 are SPs, chondrocytes, and stromal cells. While, in specific classification of CMT, carcinoma with neuroendocrine differentiation, some neuroendocrine cells were positive as well as in luminal epithelial cells. These positive staining of S100 in many cells was probably due to the intracellular and extracellular function of S100 in pathological conditions such as tumors.

CONCLUSION

S100 is detected in CMTs by the use of immunohistochemistry. The SPs, chondrocytes, and stromal cells are the cells that are immunoreactive to S100, therefore, these cells may possibly be used as markers for a tumor. In contrast, the endothelial cells of the BVs, which are positive both in normal mammary and in CMT, cannot be used as a marker. Although the ten CMTs are positive to S100, there are some differences regarding the cells that are positive. This difference is particular to a case, a carcinoma with neuroendocrine differentiation, wherein its luminal ductal epithelial cells and neuroendocrine cells are positive while those cells in other classifications of mammary tumors are negative. Thus, in the study of 10 classifications of CMT, immunohistochemical staining of S100 can be used as a marker in CMT.

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