

Identification and Characterization of Canine Mammary Tumors Stem Cells: A Review

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Glands cancers consider the most types of cancers in males or females or both. Many challenges are present in controlling cancers in dogs, the first one is related to populations of tumour cells define as cancer stem cells, cancer cells are resistant to chemotherapy and have the ability to metastasise and returned after successful treatment. Cancer consists of different cells that contain a subtype of cell with stemness characteristics, like high tumourigenicity, the self-renew ability to differentiate and resistance to chemo and radiotherapy. These cells are termed cancer stem cells (CSCs) and are involved in the initiation of tumours, recurrence and metastasis. Canine mammary cell tumours are malignant neoplasms characterized by metastasis and a high infiltration rate. Canine mammary tumours (CMT) occur in female dogs frequently, six to seven (6-7) years old animals ages are more susceptible to the disease, however, it can be diagnosed at 9–11 years of age, which is known as the cancer age, it has the ability to form tumours spheres which are considered a characteristic of stem cells. it is difficult to clarify the histological finding base on Hematoxyline and Eosin stains policy, so biomarkers are recommended like CD44+and CD24-,also Immunohistochemistry . These tumours are composed of mesenchymal cells and/or myoepithelial cells with proliferative luminal epithelial cells, cartilage, and osseous tissues. Annually around the world, between 200-250 mammary gland tumours out of 100.000 female dogs, 50% were malignant. Surgical removal is the treatment of choice, however, it is ineffective because of the metastasis high rate. It is important to focus on the analysis of CSCs high incidences in dogs (bitches) and CSCs isolation and diagnosis.

Keywords: Canine, Mammary tumors, Stem cells, Immune histochemical

تميز وتوصيف الخلايا الجذعية لأورام الثدي في الكلاب : مقالة

الخلاصة

يعتبر سرطان الغدد الثديية في الكلاب اكثر انواع الاورام انتشارا في الكلاب حيث يواجه تحديات كثيرة منها وجود مجموعة من كتل الخلايا الصغيرة التي تسمى بالخلايا الجذعية السرطانية والتي تتميز بمقاومتها للعلاج الكيميائي والاشعاعات وكذلك القدرة على الانتشار وعودة المرض مرة ثانية حتى بعد العلاج الناجح. وبصورة عامة يتكون السرطان من مجموعة من الخلايا المختلفة تحتوي على عدة أنواع فرعية من اورام الخلايا ذات الخصائص الجذعية ، مثل هذه الأورام لها القدرة العالية على التجدد الذاتي والتميز والمقاومة للعلاج الإشعاعي والعلاج الكيميائي كما ويطلق على هذه الخلايا اسم الخلايا البادئة للورم أو الخلايا الجذعية السرطانية والمسؤولة عن حدوث وعودة الاورام الخبيثة. فضلا عن ذلك تتميز اورام الخلايا الثديية للكلاب بالقدرة على الانتشار في الجسم مع ارتفاع معدل التسلل والانتشار كما وتحدث أورام الثدي في إناث الكلاب بشكل متكرر وخصوصا الحيوانات التي تبلغ من العمر (6-7) سنوات حيث تكون أكثر عرضة للإصابة بالمرض ومع ذلك يمكن تشخيصها في سن 9-11 سنة وهذا ما يعرف ب عمر السرطان بالإضافة الى قدرتها على تكوين مايسمى بالكرات spheres و تعتبر سمة من سمات الخلايا الجذعية كما وانه من الصعب الكشف عنها باستخدام ملون الهيماتوكسيلين والأبوزين الاعتيادية بل تحتاج استخدام بعض المؤشرات الحيوية مثل CD44+وCD24 . تتكون الاورام الجذعية من خلايا متعددة منها الخلايا الظهارية العضلية التكاثرية والغضاريف والأنسجة العظمية كما وتم تسجيل ما بين 200-250 حالة من اورام الغدد الثديية حول العالم سنويا من أصل 100.000 أنثى حيث تم تشخيص 50% منها على أنهااورام خبيثة ويعتبر الاستئصال الجراحي هو أفضل خيار للعلاج ، ويعتبر غير فعال لأن معدل الانتشاريكون عالي لذا من المهم التركيز على تحليل عينات من الخلايا الجذعية السرطانية في اناث الكلاب وكذلك التركيز على عزل وتشخيص الخلايا الجذعية السرطانية.

Introduction

The glands epithelium are consist of cells (basal and luminal cells) which arranged in a monolayer in the mammary duct as hierarchical shape that expresses an α -smooth muscle actin and which looks like a stellate shape all around the acini and depend on progenitors of stem cells in mammary glands (1). Mammary gland undergoes remodeling, both systemic and local signals trigger, epithelial tissue under goes differentiation and proliferation during pregnancy and estrus cycle (2). Cancer stem cells (CSCs) are usually identified in veterinary medicine in osteosarcoma cases via "gold standard methods" in this methods used serial xenotransplantation to limiting dilution of marker and confirming of renewal of CSCs by serial xenotransplantation that is previously re isolated from population of CSC into other recipients (3) between 200-250 mammary gland tumors out of 100.000 female dogs around the world , 50% were malignant (4 and 5). Surface markers CD44+ and CD24- expressions can be detected in CSCs of canine mammary adenocarcinoma (2). Mammary gland tumors are reliant on both progesterone and estrogen levels, thus seen in female dog (6). CMT classifying in to simple and complex pathways, in simple pathway CSCs will differentiate in to either neoplastic myoepithelial which is the abundant one or luminal cells, While in compound pathway it develop to both neoplastic myoepithelial and luminal cells and duct of gland is lined by two types of epithelium simple cuboidal and abistratified cuboidal (7). Little information are demonstrated about the cellular metabolism of CSCs in canine mammary gland tumors (3). In human medicine they demonstrated that fatty and amino acid metabolism have important roles in maintenance of CSCs stemness, for instance proline metabolism is necessary in the self-regeneration ability of cancer stem cells breast cancer in human and dogs consider a good model in study human cancer because of canine mammary

characteristic features like in human. (8). Several factors have the influences the incidence of mammary neoplasms like some chemicals ,bisphenol A and some hormones such as estrogen (9and 10). One of the major issue of canine mammary tumor is the accuracy after surgical intervention but some time surgical intervention is not valuable because of metastasis (11). CSCs were identified in some tumoral tissues as a clusters of cells (spheres) that have a stem cell characteristics , known in their ability for self-regenerations, Features of gene expression, and their ability to diminish tumors and its contain a high percent of proline ,alanine , and glycine amino acids in comparisons to neighboring tissue cells and high percentage of palmitate and palmitoleate which they increase spheres number in cell line and maintain the CSCs stemness (12and 13). Dogs have been proposed as highly valuable animal models for studying tumors of mammary gland in human breast cancer because these animals have correlates to human breast cancer such as biological behavior, histological features and genetics, the inguinal and caudal mammary glands are highly affected and the older dogs more affected with malignant tumor than younger one however dogs with lesions of hyperplasia are highly susceptible and increase risk to develop canine mammary cancer.(14and115).In veterinary medicine, some studies discovers the metabolism of CSCs in canine mammary tumors, these findings seem to be of value for eradicating progression of cancer and the reprogramming of metabolism of canine stem cells, is of value for malignancy, tumorigenesis, recurrence, and drug resistance (6 and16) .

Metabolism of canine stem cells:

The Cancers consists from non-homogenous cells , possess features of stem cells which called cancer stem cells (CSCs) contain progressions and metastasis of tumor there is some evidence refer to cancer as a disease of stem cells while

tumor is consist of a mixture of functional and genetical cells with small number of stem cells (17). There are multiple type of canine stem cells in veterinary medicine such as hepatocellular carcinoma, and pulmonary adenocarcinoma, we can isolate the CSCs from a solid malignant mass like brain, breast, prostate and osteosarcoma (18). Immunodeficient mice sphere-forming cells which comes from CMC cells lines, possess high tumourigenicity, increased expression to CD133 and resist the anticancer drugs, these cells possess the properties of stem cell and CSCs are to toxic effect of chemo and radiotherapy and have ability to regenerate and metastasis (19). cancer metabolism stage represented by deregulation of amino acid and glucose uptake through using of cycle glycolysis biosynthesis, utilize of opportunistic ways of nutrient ,alterations in regulation of gene metabolite-driven, and microenvironment metabolic interactions (20) cancer cells ATP are generated by glycolysis rather than mitochondria oxidative phosphorylation which facilitated synthesis of lipids ,amino acids and nucleotides, prostaglandins have been conserved to regulate cancer stem cells hematopoiesis (21). CSCs can be adapted to many environmental factors, like therapeutic toxicity, hypo nutrition, and oxidative stress ,cancer stem cells metabolism are depend on the type of cancer, like breast, pancreatic and ovarian type (22).

Classification of canine mammary tumors CMTs:

Classification of CMTs is based on tumor features like origin of cell, morphology and molecular markers , World Health Organization (WHO) combines descriptive morphology, histogenetic classification and prognostic elements in their classification (10,23and 24). Cellular mediators and inflammatory cells are important constitute of tumors and its contribute to development of cancer, cyclooxygenase-2 (COX-2) is consider as deregulated inflammatory

mediator (3and 25). Most canine mammary tumors are of epithelial origin (simple adenoma and carcinoma),composed of myoepithelial and epithelial tissues (complex adenoma and carcinoma). the term ‘adenocarcinoma’ was firstly described by WHO classification in papillary and tubular epithelial malignant tumors adenocarcinoma is just like ‘a carcinoma of which the glandular arrangement with a lumen and can be divided into papillary and tubular types (23). differentiation between benign and malignant cancer is based on many characteristics like morphology and metastasis ect. and grading of cancer is important assessment of malignancy (27and28)diagnosis of malignant tumors as benign may cause a problem in 10% of the mammary tumors' in the female dog Generally benign tumors are not invasive or destructive and encapsulated, while Necrosis, nuclear and cellular polymorphism, loss of differentiation, high micro vessel density, high mitotic index, and discontinuous basal membranes are characteristic of malignant tumors (3). In general classification of CMT ,clarify by (WHO) classification as showed in table (1).

Table1: Canine mammary tumors classification, World Health Organization (WHO) (23).

Malignant tumors	
Complex carcinoma	This tumor is consist of epithelial and myoepithelial cells.
Non-infiltrating carcinoma	epithelial tumor with malignant characteristic ,there is no invasion of basement membrane.
Tubulo papillary carcinoma	This tumor formed by tubules and / or papillary projections.
Solid carcinoma	The tumor is arranged in solid sheets, nests or cords .
Anaplastic carcinoma	A highly infiltration of pleomorphic epithelial cells .
Simple carcinoma	This tumor composed of one cell kind, myo epithelial cells or luminal epithelial cells and probably haematogenous and lymphatic spread .
Special types of carcinomas	
Mucinous carcinoma	characterized by production of abundant amounts mucins
Lipid-rich carcinoma	A tumor characterized by abundant vacuolated cytoplasm in cells that filled with ahuge amount of neutral lipid.
Spindle cell carcinoma	A tumor consisted of spindle cells arranged in epithelial patterns.
Squamous cell carcinoma	A tumor consist of solid cords and sheets of cells.
Benign tumors	
Simple adenoma	A tumor of uniform clusters and cords of Basaloid epithelial cells.
Fibro adenoma	tumor of a mixture of stromal cells and luminal epithelial cells, or mixed with myoepithelial cells
Adenoma	well-differentiated tumor of luminal epithelial or myoepithelial cells.
Complex adenoma	myo epithelial and luminal epithelial tumor .
Benign mixed tumor	benign cells like epithelial components myoepithelial , luminal and mesenchymal cells producing bone

	,cartilage , and fat .
Duct papilloma	
Mammary dysplasia\ hyperplasia	mammary duct dilated progressively .
Ductal hyperplasia	Ducts Hyperplasia and stroma .
Duct ectasia	
Gynecomastia	an proliferation of intra ductal epithelial cells consist of non-neoplastic lesion, with total or partial disappearance of the lumen by hyperplasia epithelium.
Lobular hyperplasia	
Adenosis	Ductules proliferation it is non-neoplastic.
Epithelial hyperplasia	Epithelial cells proliferation of within intralobular ductules(non neoplastic).

Canine mammary tumors (CMTs) risk factors:

mammary tumors is a disease initiated from accumulation of multiple genetic factor such as alter cellular functions and environmental factors, genetic susceptibility , penetrant autosomal dominant genes , in addition to age, nutritional, many growth factors and steroid hormone and its receptors like progesterone and estrogen play important role in development of cancer and growth hormone also affect cancer developing we found that progestins and Endogenous progesterone help in increase of Growth Hormone level in neoplastic normal glands of dogs(25and26) also mammographic density , late menopause, hormone replacement therapy, body mass index , previous exposure to radiation therapy, exposure to diethylstilbestrol , lifestyle factors like alcohol consumption and physical activity, also Nutritional factors like diet rich in lipid leads to increase ability of synthesis of steroidal hormone from adipose tissue which is consider important component of estrogens which is in turn leads to increase risk of

tumorigenesis(29).

Origin of cancer and CSCs

Some study suggested that origin of cancer and cancer stem cells are from small populations of cancer cells that have ability to initiation tumor these cells named CSCs which have some characteristic of normal stem cells like self-regeneration capacity and extensive proliferation with differentiation ability the first isolation is from leukemia (30).mutations occurred in germ line and somatic cells as a result of errors made during repair, DNA damage or replication(6 and31). CSCs termed tumors-initiating cells (TICs) are generated either from differentiated cells or from mutational events in normal tissue stem cells(32)Tumor cells present at the apex of a pyramids tissue cancer which organized as tree-like pyramids (33and 34) Figure 1. mixed tumors consist of epithelial components like myoepithelial cells, luminal or mesenchymal cells such as chondrocytes and osteoblasts but the cellular origin remains unclear (35) most of CMT in veterinary medicine is of epithelial origin while

other cell types observed in mixed tumors originated from CSCs (36) there are several methods used to identification of canine mammary tumors stem cells and their functional characteristic reviewed by(37) such as sensitivity to drugs ,xenotransplantation, and targeting therapy.

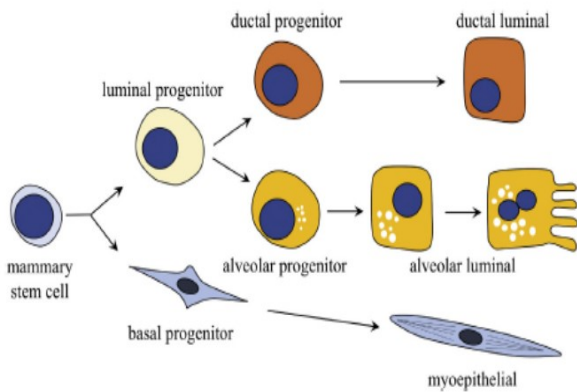


Figure 1: A diagram of epithelial mammary pyramids of dogs (37)

Isolation of canine cancer stem cells

Stem cells differ from somatic cells in which have ability to self-regeneration, culture cells split from tissue used to proliferation of progenitors on low attachment plates in serum supplemented with B27, in process of tumor developing needs formation of new blood vessels for supplying nutrition to new mass so some biomarker is used in detection of growth of tumor called von Willebrand factor and vascular endothelial growth factor(6,38). first canine mammary CSC isolated by Cocola et al in vitro by culturing of CSC on collagen gels when injected into the mice they induced tumor growth cells within the floating spheres possessed multi lineage differentiation potential and have ability to regenerate colonies(39and 40) . The easy and useful method for CSC isolation is sphere culturing system with some limitations may exist like inaccurate sphere calculations called larger spheres due to aggregation of smaller spheres and difficulties preparation single-cell suspensions

(41and42).Flow cytometry is important methods of isolation CSC from samples of human breast cancer based on functional properties and expression to specific biomarkers (43and44). Identification of CSCs by using a sphere-forming assay with surface markers expression such as CD44+ and CD24-, there are a number of marker and genes associated with tumor diagnosis in mammary tumors like: EGF receptor (EGFR), BRCA gene mutations, Ki-67,p63,matrix metallo proteinases, mucins, heat-shock proteins, maspin, and CO-2,in CSCs there are two way in determining development of tumor : deterministic in which cancer cells originated from Cancer stem cells,The way was stem cells symmetrically divided (27,44).CD133 is well known as a CSC marker and strong associated with cancer path way its a trans membrane protein expressed on the surface of hematopoietic stem cells and promotes cancer invasion ,progression and migration also consider a potent diagnostic marker in melanoma and prostate cancer (47,48and49). the most biomarkers of canine mammary tumors are endothelial growth factor , antigen Ki-67, HER-2, COX-2 and progesterone receptor which can be diagnosed in both tissue and serum samples (50and51).

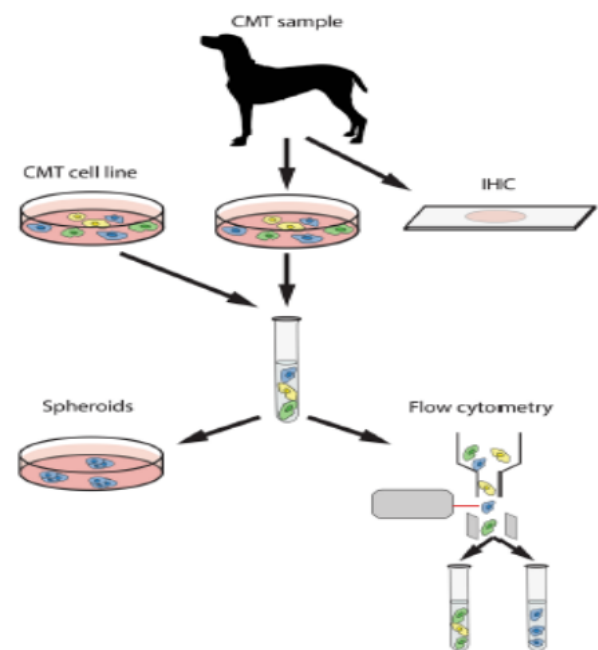


Figure 2 :Methods for isolation of CSCs(45).

fine-needle aspiration cytology is a gold standard test in the diagnosis of mammary tumors in dogs compared with histopathological one the aspirated specimens are spread onto the glass slides and dried then stained with May–Grunwald–Giemsa stain ,three aspirations must be performed using needle with a 22-gauge attached to a syringe before surgically excising the mammary glands for histopathological examination (52,53and 54) figure 3and 4.

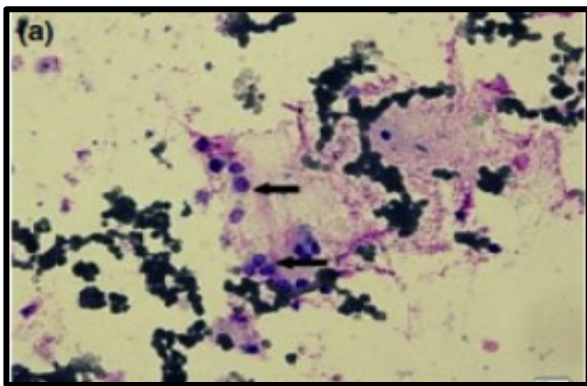


Figure 3 : Benign tumor ,fine-needle aspirations, epithelial cells stain blue(black arrow),May–Grunwald–Giemsa stain, bar20 lm.(52).

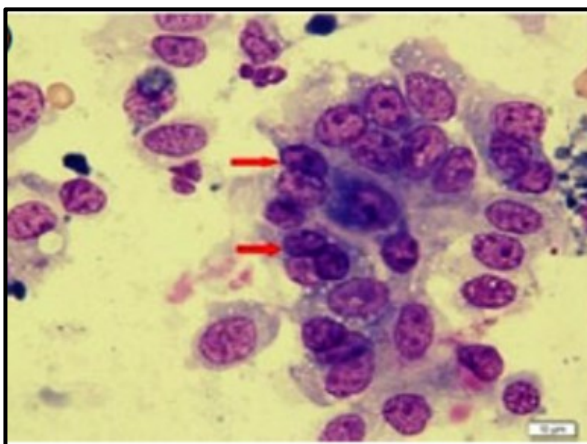


Figure 4 :Malignant tumor fine needle aspiration(red arrow), May–Grunwald–Giemsa stain ,bar 10 lm. (52) .

Immunohistochemistry (IHC) is another technique used for diagnosis , quantification, tumor pathological classification and identification of cancer stem cell, immunohistochemistry used in expression of CD44 and CD24 in 5- μ m sections (55and56). A study by Magalhaes et al.,2013 about 130 canine mammary tumors samples were expressed for CD24 and CD44 , the CD24- / CD44+was detected in lymph node metastases with higher-grade while the CD24+ type was more identified in lower grade , other groups revealed Similar results which show a higher recurrences of CD24- / CD44+cells with weak prognosis,CD24 has surface protein like mucin it increase proliferation and motility of cancer cells as well as metastasis (57, 58 and 59).Results might be often underestimated due to uneven localization of putative cells expression of CD44 within tissue is non-homogenous and difficult in serial sectioning of samples, Finally some consideration must be taken when analyzing data like sample type as well as isolation technique 60, 61 ,62and63) Figure 5.

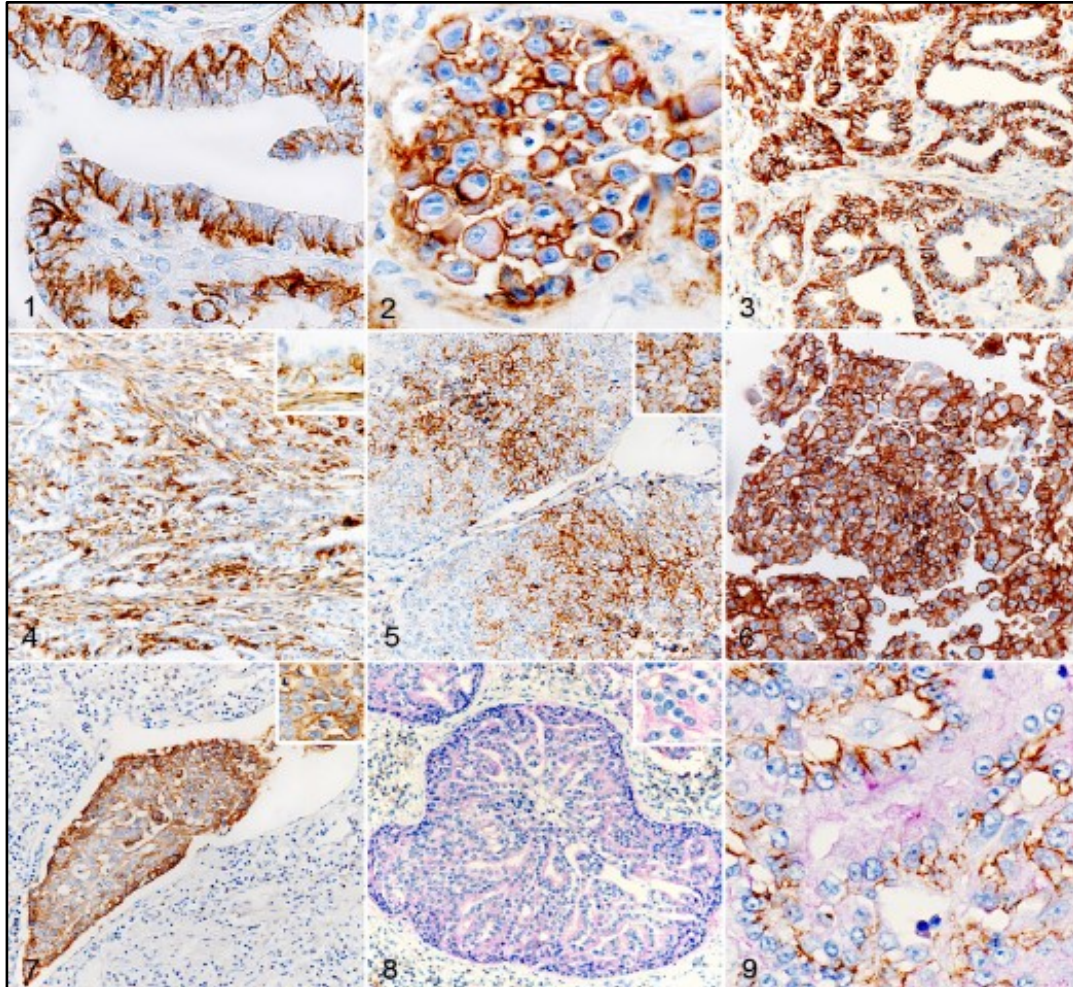


Figure 5: Mammary anaplastic carcinoma ,CD44\CD24 Immunohistochemistry.CD44 which appear brown in color and localized on the all cells membrane , poorly stained cytoplasm with CD24- :1- simple tubular carcinoma,2- Anaplastic carcinoma ,3- Tubular simple carcinoma ,4- complex tubular carcinoma,5- Closely filled cells with Solid carcinoma,6- Anaplastic carcinoma,7- Anaplastic tumor,8- Solid carcinoma,9- Solid carcinoma with Luminal cells. higher magnification.(2).

Conclusions

mammary tumors in dogs is the most common tumors ,CSCs Represents a small population of cells in malignancies that possess self-regeneration ability and differentiated into a variety of cell types. stem cell tumors resist chemo- and radiotherapy and have same human surface biomarkers .canine stem cells play a major role in the histogenesis of mammary tumors, especially in the production of the mixed tumors. CSC have been previously discovered in

many human cancers but its recently identified in canine tumors for this reason dog is a good model for the study of human mammary cancer and clinical experiments. Cancer stem cells can be identified, characterized and diagnosed by several methods including biomarkers and immune histochemical techniques.

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