10.22059/ijvm.2019.288591.1005019

Iranian Journal of Veterinary Medicine
Volume 14- Issue 03

Original Article
Online ISSN : 2252-0554

Canine Mammary Gland Cancer Stem Cell and its Potential Role in Malignant Biologic Behavior

Havva Marzban, Farhang Sasani*

Department of Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Abstract

BACKGROUND: Canine mammary gland cancers are the most prevalent malignancies in dogs. There are different challenges regarding management of these cancers in dogs and human, one hypothesis is related to small cellular subset of tumor mass called cancer stem cell. These cells are therapy resistant and cause metastasis and relapse even after primary successful treatment. The well-identified phenotypes for detecting this population are ALDH1+/CD44+/CD24-/Low biomarkers.

OBJECTIVES: A study to evaluate existence of cancer stem cells in canine malignant mammary glands tumor and assess effects of these cells on clinicopathological parameters of tumors were designed.

METHODS: In this study forty cases of canine mammary glands tumors were collected. All cases were tested via H&E and then Immunohistochemistry (IHC) methods. All samples were evaluated immunohistochemically for common markers of these tumor-initiating cells. Monoclonal antibodies against ALDH1, CD44 and CD24 were used. Some tumor aggressiveness-related parameters, including lymphovascular invasion, tumor grades and histotypes were assessed.

RESULTS: The present study revealed that 17.5% of cases were enriched with cancer stem cells and all of them were diagnosed as grade II and III ($P \leq 0.05$). Other findings showed all cancer stem cell-positive cases were significantly lymphovascular invasion positive ($P \leq 0.05$). The most common histotypes in this research were tubular, tubulopapillary and intraductal carcinomas.

CONCLUSIONS: Our results illustrated that cancer stem cells can be considered as reliable prognostic factors to predict severity of malignant behavior of canine malignant mammary glands cancer, which is comparable with human breast cancer.

KEYWORDS: Cancer, Canine, Malignant tumor, Mammary Glands, Stem Cells

Correspondence
Farhang Sasani, Department of Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Tel: +98 021-61117061, Fax: +98 (21) 66933222, Email: fsasani@ut.ac.ir
Received: 2019-09-14
Accepted: 2019-11-04

How to Cite This Article
Marzban , H., & Sasani , F. (2020). Canine Mammary Gland Cancer Stem Cell and its Potential Role in Malignant Biologic Behavior. Iranian Journal of Veterinary Medicine, 14(3), 329-342.

Introduction

The incremental evidence shows breast cancer has the highest rate of incidence in both dog (more than 180 cases per 100,000) and human (over 2 million new cases) and accounts for one of the main influential causes of death in women and intact female dogs (Pang *et al*., 2011; Winters *et al*, 2017; Bray *et al*., 2018). However ,along with progressive development in molecular pathology and different therapeutic strategies, there is increasing concern over recurrence, metastasis and high range of cancer-related deaths (Case *et al*., 2017). A small heterogeneous population of tumor mass with mostly similar properties of normal stem cells, including self-renewal capacity and differentiation to various cellular lineages has received meticulous attention as a main cause of tumor recurrence (Rogez *et al*., 2018). These so-called cancer stem cells (CSCs) are adequately equipped to overcome conventional cancer therapies in comparison to other differentiated cancer cells (Madjd *et al*., 2012; Rybicka *et al*., 2016). Different researches identified widely variable ranges of CSCs from 2% in breast cancers to 27% in melanoma (Im *et al*., 2015; Parmiani, 2016). Different approaches are applied to detect and characterize these infrequent cells, including immunohistochemical staining (IHC), flow cytometry, and functional assay (Rybicka *et al*., 2016).

The well-accepted stem cell biomarkers for human breast cancer (HBC) and canine mammary glands cancer (CMGC), including ALDH1, CD44 and CD24, are used to detect these tumor initiating cells as a well-characterized phenotype named ALDH1+/CD44+/CD24$^{-/Low}$ (Lee *et al*., 2011; Gavhane *et al*., 2016; Rybicka *et al*., 2016). Besides being therapy resistant, CSCs are able to create different biologic behaviors in cases with apparently similar histopathology but different metastatic potentials and patient outcomes. So, choosing an accurate therapeutic regimen based on specific molecular features of individual's cancer for both dog and human becomes challenging (Case *et al*., 2017).

In managing CMGC, veterinary oncologists come across challenges closely comparable to HBC. These spontaneously occurring tumors, in addition to clinical and molecular biologic similarities to HBC provide an opportunity to achieve the true meaning of public health with regard to both human and animal patients, with two-directional way of exchanging the latest therapeutic strategies from medicine to veterinary oncology and transferring new molecular discoveries and vice versa (Pang *et al*., 2011; Case *et al*., 2017; Nguyen *et al*., 2018; Marconato *et al*., 2019). Furthermore, in an attempt to discover CSCs potential interactions, the role of comparative oncology becomes increasingly apparent, in that it is possible to investigate probable correlations between these relapse-inducing cells and clinicopathologic parameters in the most relevant animal cases that have crucially significant priorities over experimental methods such as in vitro research (Queiroga *et al*., 2011; Rybicka *et al*., 2016; Nguyen *et al*., 2018).

Since the main issues addressed in oncology research are first finding prognostic factors involved in metastasis cascades and then targeting them combined with conventional methods, this research intends to evaluate prognostic capability of CSCs in CMGC as in HBC via IHC staining based on CD44, CD24, ALDH1 and to determine the probable associations between CSCs and clinicopathological features. Based on similar features in this regard, HBC treatment protocols can be used

in dog patients and all advances gained from researches in animal mammary gland cancer can be utilized in new preclinical and clinical designs for further progression. To our knowledge, the present study is the first Iranian regarding CSCs existence in CMGC.

Materials and Methods

This experiment was designated as a cross-sectional study which was extended for one year. A total of 40 samples were taken from referred mammary gland masses to veterinary hospitals from canines that underwent surgery during 2018-2019 and were diagnosed as CMGC by pathologist.

- Tumor histotypes

Based on Goldschmit classification (2011) just epithelial malignancies were included.

- Clinical data

All clinical data, including patient ages, tumor laterality and surgical procedures were obtained from patient records and pathology reports were extracted.

- Tumor grade

All slides were rechecked in order to regrade tumor samples, based on Nottingham grading score system (Cassali et al., 2017) which categorized all breast malignancies to three grades.

- Lymphovascular invasion

To determine whether lympho-vascular invasion (LVI) is present or not, the H&E stained slides were examined to investigate presencee of tumors cells around or inside the vessels which were reported as positive or negative cases.

- IHC

Procedure

Paraffin embedded blocks were selected based on proper criteria for IHC staining, as follows: the largest volume of tissue, the least necrosis and the most invasive part of tumor. After cutting to 3 µm, all slides were deparaffinized by xylene, rehydrated through ethanol and antigen retrieval was done by putting immersed slides on Tris buffer in microwave. Following that, peroxidation process was performed. Then, slides were incubated by monoclonal primary antibodies to assess CSCs presence at room temperature for 30-60 min, ALDH1 with CAT number (Sc-166362) (Mouse monoclonal antibody (H-8), dilution: 1:50-1:500), CD44 with CAT number (NBP1-47386) (CD44 antibody (8E2F3), dilution: 1:200-1:1000) and CD24 with CAT number (Sc-19651) (Rat monoclonal antibody (M1/69), dilution: 1:50-1:500) were used. Then washing 3 times in Tris buffer, each time for 5 min was done.

After completing the exposure to secondary antibody and HRP polymer of MACH 1 Universal HRP-polymer, Biocare Medical Co. (Pacheco, California, USA) at room temperature for 1 hr, incubation at room temperature for 30-60 min, then washing 3 times, each time 5 min in Tris buffer were done. Amplification of antigen- antibody links with betazoid DAB chromogen was done. Then samples were counterstained by hematoxylin and immersed in alcohol for dehydration and xylene for clearing, finally they were mounted.

IHC evaluation

According to modified Alred Scoring System (Qureshi *et al*., 2010), semiquantitative analysis of epithelial tumoral cells immunoreactivity at invasive parts with cutoff ≥ 1% was made. Intensity and proportion rate of CD44 and CD24 as membranous markers and ALDH1 as a cytoplasmic antigen with this cut point were estimated. The main focus of the experiments was to detect CSCs-like cells, so the widely accepted phenotype,

ALDH+/CD44+/CD24-/Low, was used to call cases as positive and the remaining phenotypes were called negative. Positive control was done based on Santa Cruz (Dallas, USA) and Novus Biological (Colorado, USA) Company' protocols to validate antibodies specificities. According to each protocol, human gall bladder tissue, formalin-fixed, paraffin-embedded mouse blood smear and paraffin-embedded human lung cancer tissue are control positives for ALDH-1, CD24 and CD44, respectively, and normal mammary gland tissue accounts for negative control.

Statistical analysis

Since this study was a pilot one, exclusively descriptive data were generated for variables, including patient age, surgical methods, tumor laterality, tumor histology, and statistical analysis was performed only for tumor grade, CSCs and LVI status. SPSS software was used for data analysis and correlation between quantitative and qualitative parameters was assessed by t-test and Chi Square test, respectively. It should be noted that $P \leq 0.05$ was considered significant.

Results

Clinicopathological results

In the present study, 40 cases of canine mammary gland cancers were examined with the mean age of 8.61 ± 2.41 years (ranging from 5 to 14 years). Age data was not available in four cases. Tumor laterality was divided into the left and right side with 60.7% and 39.2%, respectively. Surgical methods were operated in 28 cases including: simple regional mastectomy (53.5%), unilateral mastectomy (28.7%) and lumpectomy (17.8%). Cancerous mammary glands were diagnosed in 78.6% of cases as single, while only 21.4% of cases had cancerous mass in two or more mammary glands. Involvement of regional mammary glands was categorized as thoracic, abdominal or both with 21.4%, 75.0% and 3.6%, respectively. Tumor grades as I, II and III were observed in 42.5%, 47.5% and 10% of cases, respectively. More details with regard to clinicopathologic characteristics of samples were shown in Table 2 and Figure 1.

Table 1. Description of clinicopathologic characteristics

Mean age (range)		8.61 ± 2.41
(n=36)		
Age group	<8	11 (30.5%)
	8-11	20 (55.5%)
	>11	5 (13.8%)
Histologic grade (n=40)	I	17 (42.50%)
	II	19 (47.50%)
	III	4 (10%)
Tumor laterality (n= 28)	Left	17 (60.7%)
	Right	11 (39.2%)
Surgical options (n=28)	Lumpectomy	5 (17.8%)
	Simple or regional mastectomy	15 (53.5%)
	Unilateral mastectomy	8 (28.7%)
Involved mammary glands (n=28)	Single	22 (78.6%)
	Two or more	6 (21.4%)

Mean age (range)		8.61 ± 2.41
Regional frequency (n= 28)	Mammary glands thoracic region	6 (21.4%)
	Mammary glands abdominal region	21 (75.0%)
	Both regions involved	1 (3.6%)
Tumor histotypes	Tubular carcinoma	13 (32.5%)
	Tubulopapillary carcinoma	8 (20%)
	Intraductal carcinoma	6 (15%)
	Complex carcinoma	4 (10%)
	Solid carcinoma	3 (7.5%)
	Ductal carcinoma	2 (5%)
	Anaplastic carcinoma	1 (2.5%)
	Inflammatory carcinoma	1 (2.5%)
	Adenosquamous carcinoma	1 (2.5%)
	Cribriform carcinoma	1 (2.5%)

Table 2. IHC results of CSCs markers

CSC markers	Positive	Negative
ALDH1	n=32 (80%)	n=8 (20%)
CD44	n=7 (17.5%)	n=33 (82.5%)
CD24	n=7 (17.5%)	n=33 (82.5%)
CSC status	Present	Absent
	n=7 (17.5%)	n=33 (82.5%)
Tumor grade	CSC positive	P-value
Grade I	n=0 (0%)	0.008
Grade II	n=5 (71.4%)	0.008
Grade III	n=2 (28.5%)	0.008

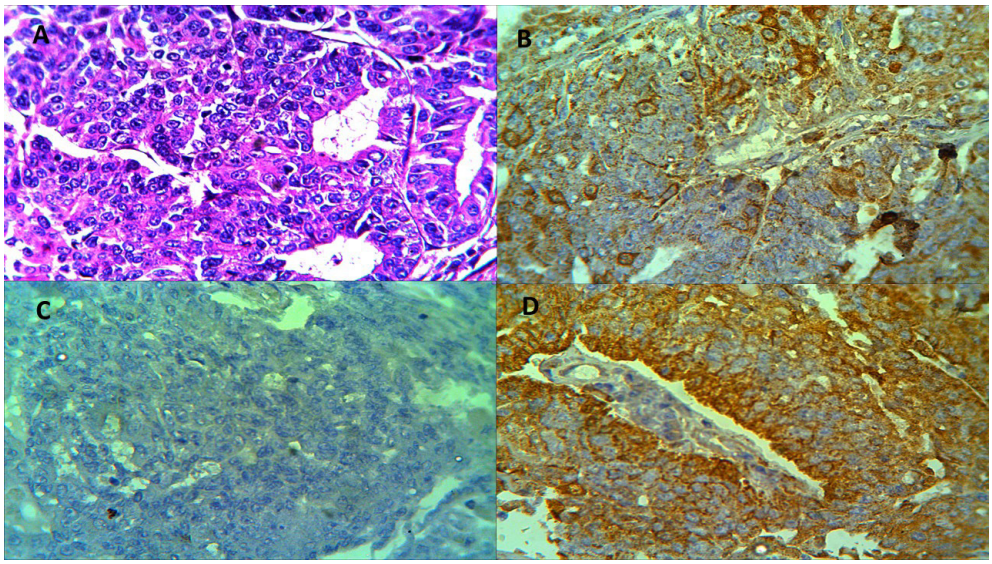

Figure 1.

Evaluation of LVI in all cases showed that 72.5% of cases are positive (n=29), while only 27.5% of cases were negative (n=11). Tumor classification based on aggressiveness highlighted that grade II had the highest percentage of LVI positivity (55.1% grade II), followed by grade I (31.0%) and grade III (13.7%).

IHC results:

CSCs markers

Among 40 cases of this study ALDH1+ was observed in 80% of them, and ALDH1-, CD44+, CD44-, CD24+ and CD24- were as follows: 20%, 17.5%, 82.5%, 17.5% and 82.5%, respectively. More details regarding IHC results are provided in Table 2 and Figures (B, C and D). In addition, 15% of cases were positive for both ALDH1 and CD44, from which 83.3% and 16.6% were grade II and III, respectively. Surprisingly, all grade I cases only showed ALDH1 positivity.

Further analysis illustrated that 17.5% of cases were enriched with cancer stem cell, in which 71.4% and 28.5% of them were diagnosed as grade II and III, respectively ($P \leq 0.05$). Interestingly, despite high occurrence of grade I, we could not detect any CSCs positive cells.

Association between tumor histology types and cancer-initiating cells was demonstrated in Table 3. Furthermore, all positive CSCs cases accounted for LVI positive (7 of 29; 24.1%). More details regarding these correlations are provided in Table 4. Also, associations between tumor histology and LVI status are summarized in Table 5.

Table 3. Frequency of histology types enriched with CSCs

Tumor histology	Frequency of CSC+
Tubulopapillary carcinoma	2
Tubular carcinoma	1
Solid carcinoma	1
Anaplastic carcinoma	1
Ductal carcinoma	1
Inflammatory carcinoma	1
Total number of CSC +	7

Table 4. Correlation between LVI status and CSCs existence

	CSCs-positive	CSCs-negative	P-value
LVI-positive	n=7 (100%)	n=22 (55%)	0.025
LVI-negative	n=0 (0%)	n=11 (27.5%)	0.025
Total number	n=7	n=33	

Table 5. Description of Tumor histology and LVI status

Tumor histology	Frequency/percentage (Total number =40)	LVI positive	LVI negative
Tubular carcinoma	13 (32.5%)	n=9 (69.23%)	n=4 (30.76%)
Tubulopapillary carcinoma	8 (20%)	n=7 (87.5%)	n=1 (12.5%)
Intraductal carcinoma	6 (15%)	n=3 (50%)	n=3 (50%)
Complex carcinoma	4 (10%)	n=2 (50%)	n=2 (50%)
Solid carcinoma	3 (7.5%)	n=2 (66.6%)	n=1 (33.3%)
Ductal carcinoma	2 (5%)	n=2 (100%)	n=0 (0%)
Anaplastic carcinoma	1 (2.5%)	n=1 (100%)	n=0 (0%)
Inflammatory carcinoma	1 (2.5%)	n=1 (100%)	n=0 (0%)
Adenosquamous carcinoma	1 (2.5%)	n=1 (100%)	n=0 (0%)
Cribriform carcinoma	1 (2.5%)	n=1 (100%)	n=0 (0%)
Total number or percentage	100%	n=29	n=11

Discussion

This study was designed to determine the presence of CSCs in canine malignant mammary gland tumors, and to evaluate CSCs associations with clinical and biological features of these malignancies. Based on the literature, despite possessing a rare population, CSCs are suspected for their probable roles in tumor aggressiveness, therapy resistance and recurrence (Queiroga *et al*., 2011; Aleskandarany *et al*., 2015; Rogez *et al*., 2018). In medical oncology, CSCs have been postulated as an independent prognostic factor in different solid tumors such as HBC (Mohamed *et al*., 2018). The final goal of most recent studies is achieving the precise and detectable factor to predict cancer fate in both human and animal patients (Horimoto *et al*., 2016; Case *et al*., 2017; Flynn et al., 2019). In an attempt to pursue this goal, we proposed the prognostic role for CSCs in CMGC. In the current work, 17.5% of tumor specimens contained CSCs that was the most interesting result in accordance with findings in HBC which demonstrated an average range from 2% to almost 27% (Camerlingo *et al*., 2014; Im *et al*., 2015; Parmiani, 2016).

High frequency of CD44+/CD24-/Low/ALDH1+ has significant impacts on patient outcome, since these populations are associated with poor prognostic variables which cause aggressive behavior and high metastatic potential (Magalhães *et al*., 2013; Rogez *et al*., 2018).

To support the significance of these cells, early studies have found circulated cancer cells enriched with CD44+/CD24- population in bone marrow of patients with metastatic breast cancers, which enhances the value of this phenotype as an appropriate prognostic factor (Marsden *et al*., 2012; Horimoto *et al*., 2016; Salvador *et al*., 2019).

Another important finding was that the whole CD44+/CD24- populations were ALDH1 positive, which is in contrast to the results of other studies that showed less than 1% coverage between CD44+/

CD24- cells and ALDH1+ (Horimoto *et al*., 2016). This discrepancy is related to different study designs; the latter was carried out on breast cancer cell lines.

On the other hand, aggressive behavior is exacerbated by increased number of expressed markers by cancer cells (Lee *et al*., 2011; Horimoto *et al*., 2016; Strati *et al*., 2019). In agreement with this hypothesis, we could demonstrate all CSCs-positive cases are expressed by both CD44+/CD24- and ALDH1+. Additionally, they all belonged to grade II and III, in contrast to grade I cases that were CSCs-negative and expressed only ALDH1. On the other side, our results also illustrated significant correlation between CSCs and tumor grade ($P\leq0.05$). These results differ from Rabinovich *et al*. (2018) finding that depicted ALDH1-positivity in high percentage of grade III tumors. Horimoto *et al*. (2016) regarding these discrepancies showed that these markers should be used together in an attempt to detect more population of CSCs. Since in this study we showed CSCs have a significant association with grade ($P\leq$ 0.008), our result highlights the importance of this metastasis-initiating population.

In this research in order to evaluate LVI as a prognostic characteristic, it is interesting to note that all seven CSCs cell- rich samples were LVI positive ($P\leq$ 0.025). LVI status can predict patient outcome even in cases which are diagnosed lymph node-negative both clinically and histopathologically, but their tumor recurs after some years despite primary successful treatment (Aleskandarany *et al*., 2015; Bartosh *et al*., 2016). One of the possible explanations might be the separation of a fraction of tumor mass called circulating CSCs, that express CD44+/CD24-/ALDH1+ markers which, as a potential source of originating metastatic colonies are proliferatively quiescent (Mohamed *et al*., 2018; Strati *et al*., 2019). Furthermore, circulating CSCs act as dormant cancer cells and they are generated before the first evolution of clinically evident tumors. So they could escape from routine therapies and they are able to find their favorable niches. After many years or a long time, these cells can reactivate themselves and make metastatic tumors, all of these features lead to therapy failure (Marsden *et al*., 2012; Payne *et al*., 2012; Bartosh *et al*., 2016; Fu *et al*., 2017; Flynn *et al*., 2019).

There was a significant association between tumor grade and LVI ($P\leq0.05$). Also, tumor grade II and III had a higher percentage of vascular invasion compared to grade I, which is supported by Aleskandarany *et al*. (2015) results, presenting that there is a strong correlation between these two parameters and the least occurrence of LVI positivity in grade 1 tumors. Other studies provided corresponding results regarding strong prognostic efficacy of histologic grading to predict metastatic relapse (Case *et al*., 2017; Rakha *et al*., 2018).

The most common histotypes observed in this study were tubular carcinoma, tubulopapillary carcinoma and intraductal carcinoma (Figure. A), respectively, which is in agreement with several studies (Rasotto *et al*., 2012; Patel *et al*., 2019).

One cohort study confirmed the accurate value of different CMGC histotypes in predicting patient outcome, while three common histotypes in this study do not have prognostic value compared to other tumor types such as comedocarcinoma and adenosquamous carcinoma (Canadas *et al*., 2018). In present work only one case was

diagnosed as adenosquamous carcinoma. Since we did not follow this patient, there was not any related information regarding survival.

According to Rogez *et al*. (2018) CD44+/CD24- markers are more commonly expressed in tubular carcinoma than solid carcinoma. In our research we could find CSCs in both histotypes.

Gavhane *et al*. (2016) showed ALDH1 positivity in different tumor histotypes including 2 simple carcinomas and 12 complex carcinomas. But in our study, none of the complex carcinomas were enriched with these biomarkers.

The results of this study indicate that mean age of all cases was 8.61 ± 2.41, completely in accordance to Patel *et al*. (2019).

Another clinically relevant finding was regarding the age of dogs affected by cancer. The highest percentage of patients in the present research were 8-11 years old, which seems to be comparable to Winters *et al*. (2017) findings in HBC and additionally according to Queiroga *et al*. (2011) categorization in which group 8-11 years were similar to 51-68 years in human.

Returning to the hypothesis proposed in the introduction, this study was designated to determine CSCs existence in canine mammary gland carcinomas and to assess probable correlations between these rare population and clinicopathologic criteria. According to the finding regarding significant associations between CSCs and some short survival variables, it is now possible to consider CSCs as a reliable prognostic parameter for CMGC. One of the more remarkable results to emerge from this study is that patterns of correlations in canine tumor samples were in close agreement with CSCs in HBC.

Despite a limited sample size in this work, it was a promising step to achieve better understanding of biologic behavior of CMGC. Additionally, the exact significance of comparative oncology is further clarified and taking advantage of the latest molecular and therapeutic progresses reciprocally between human and animal patients has become more possible. The key strength of this study is the pilot research with respect to incidence rate of CSCs in CMGC and it can serve as a base for future studies with larger sample size and survival evaluation for answering many questions which have been shown in in this work.

Acknowledgments

Sincere thanks to our collaborators who provided the samples used in this research and to the editor and the referees for their exceptionally helpful comments during the review process. We grateful to Mr. Reza Aqa Ebrahim Samani for his technical support.

Conflict of interest

The authors declared that there is no conflict of interest.

References

Aleskandarany, M.A., Sonbul, S.N., Mukherjee, A., Rakha, E.A. (2015) Molecular mechanisms underlying lymphovascular invasion in invasive breast cancer. Pathobiology, 82, 113–123. https://doi.org/10.1159/000433583. PMID: 26330352

Bartosh, T.J., Ullah, M., Zeitouni, S., Beaver, J, Prockop, D.J. (2016) Cancer cells enter dormancy after cannibalizing mesenchymal stem/stromal cells (MSCs). PNAS 6447–6456. https://doi.org/10.1073/pnas.1612290113. PMID: 27698134.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., Jemal, A. (2018) Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J

Clin, 68, 394–424. https://doi.org/10.3322/caac.21492. PMID: 30207593.

Camerlingo, R., Ferraro, G.A., Francesco, F.D., Romano, M., Nicoletti, G., Bonito, M.D., Rinaldo, M., D'andrea, F., Pirozzi, G. (2014) The role of CD44+ /CD24-/low biomarker for screening, diagnosis and monitoring of breast cancer. Oncol Rep, 31, 1127-1132. https://doi.org/10.3892/or.2013.2943. PMID: 24366074.

Canadas, A., Franc, M., Pereira, C., Vilac, R., Vilhena, H., Tinoco, F., et al. (2018) Canine mammary tumors: comparison of classification and grading methods in a survival study. Vet Pathol, 1-12. https://doi.org/10.1177/0300985818806968. PMID: 30381007.

Case, A., Brisson1, B.K., Durham, A.C., Rosen, S., Monslow, J., Buza, E., et al. (2017) Identification of prognostic collagen signatures and potential therapeutic stromal targets in canine mammary gland carcinoma. PLOS ONE, 12(7), 1-19. https://doi.org/10.1371/journal.pone.0180448. PMID: 28683102.

Cassali G. D.1, Karine A. Damasceno2, Angélica C. Bertagnolli3, Alessandra Estrela-Lima4, Gleidice E. Lavalle5, Giovana W. Di Santis6, Andrigo B. De Nardi7, Cristina G Fernandes8, Bruno Cogliati9, Renata Sobral10, Fernanda V. Amorim da Costa11, Enio Ferreira1, Breno S. Salgado12, Cecilia B. Campos13, Mario J. M. H. D'Assis4, Laís P. Silva4, Marília C. A. Machado4, Bruna F. Firmo7, Fernanda C. Nunes1,3, Karen Y. R. Nakagaki1 (2017). Consensus regarding the diagnosis, prognosis and treatment of canine Mammary Tumors: Benign Mixed Tumors, Carcinomas in Mixed Tumors and carcinosarcomas. Braz J Vet Pathol, 2017, 10(3), 87 – 99. https://doi.org/10.24070/bjvp.1983-0246.v10i3p87-99

Flynn, A.L.B., Schiemann, W.P. (2019) Autophagy in breast cancer metastatic dormancy: tumor suppressing or tumor promoting functions? J Cancer Metastasis Treat, 5(43), 1-12. https://doi.org/10.20517/2394-4722.2019.13. PMID: 31431926.

Fu, Q., Huang, T., Wang, X., Lu, C., Liu, F., Yang, G., et al. (2017) Association of elevated reactive oxygen species and hyperthermia induced radiosensitivity in cancer stem-like cells. Oncotarget, 8(60), 101560-101571.https://doi.org/10.18632/oncotarget.21678. PMID: 29254186.

Gavhane, D.S., Singh, A., Sood, N.K., Gupta, K. (2016) Expression of stem cell biomarker aldehyde dehydrogenase 1 (ALDH1) in canine mammary tumor. Indian J Anim Sci, 86 (3), 285–287.

Goldschmidt, M., Pen˜a, l., Rasotto, R., Zappulli, V. (2011) Classification and grading of canine mammary tumors. Vet Pathol, 48(1), 117-131. https://doi.org/10.1177/0300985810393258. PMID: 21266722.

Horimoto, Y., Arakawa, A., Sasahara, N., Tanabe, M., Sai, S., Himuro, T., et al. (2016) Combination of cancer stem cell markers CD44 and CD24 is superior to ALDH1 as a prognostic indicator in breast cancer patients with distant metastases. PLOS ONE, 11(10), 1-11. https://doi.org/10.1371/journal.pone.0165253. PMID: 27768764.

Im, K.S., Jang, Y.G., Shin, J.I., Kim, N.H., Lim, H.Y., Lee, S.M., et al. (2015) CD44+/CD24– cancer stem cells are associated with higher grade of canine mammary carcinomas. Vet Pathol, 52(6), 1041-1044. https://doi.org/10.1177/0300985815593121. PMID: 26173453.

Lee, H.E., Kim, J.H., Kim, Y.J., Choi, S.Y., Kim, S.W., Kang, E., et al. (2011) An increase in cancer stem cell population after primary systemic therapy is a poor prognostic factor in breast cancer. Br J Cancer, 104, 1730 – 1738. https:// doi.org/10.1038/bjc.2011.159. PMID: 21559013.

Madjd, Z., Ramezani, B., Molanae, S., Asadi-Lari, M. (2012) High expression of stem cell marker ALDH1 is associated with reduced BRCA1 in invasive breast carcinomas. Asian Pacific J Cancer Prev, 13, 2973-2978.https://doi.org/10.7314/apjcp.2012.13.6.2973. PMID: 22938492.

Magalhães, G.M., Terra, E.M., Vasconcelos, R.O., Bandarra, M.D.B., Moreira, P.R.R., Rosolem, M.C., et al. (2013) Immunodetection of cells with a CD44+/CD24- phenotype in canine mammary neoplasms. BMC Vet Res, 9, 1-6. https://doi.org/10.1186/1746-6148-9-205. PMID: 24119896.

Marconato, L., Facchinetti, A., Zanardello, C., Rossi, E., Vidotto, R., Capello, K. (2019) Detection and prognostic relevance of circulating

and disseminated tumour cell in dogs with metastatic mammary carcinoma: a pilot study. Cancers, 11(163), 1-14. https:// doi.org/10.3390/cancers11020163

Marsden, C.G., Wright, M.J., Carrier, L., Moroz, K., Pochampally, R., Rowan, B.G. (2012) "A novel in vivo model for the study of human breast cancer metastasis using primary breast tumor-initiating cells from patient biopsies". BMC Cancer, 12:10. https://doi.org/10.1186/1471-2407-12-10. PMID: 22233382.

Mohamed, S.Y., Mohamed Kaf, R., Mostafa Ahmed, M., Elwan, A., Ashour, H.R., Ibrahim, A. (2018) The prognostic value of cancer stem cell markers (Notch1, ALDH1, and CD44) in primary colorectal carcinoma. J Gastrointest Cancer. https://doi.org/ 10.1007/s12029-018-0156-6.

Nguyen, F., Peña, L., Ibisch, C., Loussouarn, D., Gama, A., Rieder, N., et al. (2018) Canine invasive mammary carcinomas as models of human breast cancer. Part 1: natural history and prognostic factors. Breast Cancer Res Treat, 167, 635–648. https://doi.org/ 10.1007/s10549-017-4548-2. PMID: 29086231.

Pang, L.Y., Cervantes-Arias, A., Else, R.W., Argyle, D.J. (2011) Canine mammary cancer stem cells are radio- and chemo- resistant and exhibit an epithelial-mesenchymal transition phenotype. Cancers, 3, 1744-1762. https://doi.org/10.3390/cancers3021744. PMID: 24212780.

Parmiani, G., (2016) Melanoma cancer stem cells: markers and functions. Cancers, 8, 34. https://doi.org/10.3390/cancers8030034

Patel, M.P., Ghodasara, D.J., Raval, S.H., Joshi, B.P. (2019) Incidence, gross morphology, histopathology and immunohistochemistry of canine mammary tumors. Ind J Vet Sci Biotechnol, 14(4), 40-44. http://doi.org/10.21887

Payne, R.E., Hava, N.L., Page, K., Blighe, K., Ward, B., Slade, M., Brown, J., Guttery, D.S., Zaidi, S.A.A. , Stebbing, J., Jacob, J., Yagu¨e, E., Shaw, J.A., Coombes, R.C. (2012) The presence of disseminated tumour cells in the bone marrow is inversely related to circulating free DNA in plasma in breast cancer dormancy. Br J Cancer, 106, 375 – 382. http://doi.org/10.1038/bjc.2011.537. PMID: 22166803.

Queiroga, F.L., Raposo, T., Carvalho, M.I., Prada, J., Pires, I. (2011) Canine mammary tumours as a model to study human breast cancer: most recent findings. In Vivo, 25, 455-466. PMID: 21576423.

Qureshi, A., Pervez, S. (2010). Allred scoring for ER reporting and it's impact in clearly distinguishing ER negative from ER positive breast cancers.J Pak Med Assoc, 60(5), 350-353.

Rabinovich, I., Sebastião, A.P.M., Lima, R.S., Urban, C.d.A., Jr., E.S., Anselmi, K.F., Elifio-Esposito, S., Noronha, L.d., Moreno-Amaral, A.N. (2018) Cancer stem cell markers ALDH1 and CD44+/CD24– phenotype and their prognosis impact in invasive ductal carcinoma. Eur J Histochem, 62(3), 2943. http://doi.org/https://doi.org/10.4081/ejh.2018.2943

Rakha, E.A., Aleskandarany, M.A., Toss, M.S., Mongan, N., ElSayed, M.E., Green, A.R., Ellis, I.O., Dalton, L.W. (2018) Impact of breast cancer grade discordance on prediction of outcome. Histopathology,73(6), 904-915. http://doi.org/10.1111/his.13709. PMID: 29999192.

Rasotto, R., Zappulli, V., Castagnaro, M., Goldschmidt M.H. (2012) A retrospective study of those histopathologic parameters predictive of invasion of the lymphatic system by canine mammary carcinomas. Vet Pathol, 49(2), 330-340. http://doi.org/10.1177/0300985811409253. PMID: 21670194.

Rogez, B., Pascal, Q., Bobillier, A., Machuron, F., Lagadec, C., Tierny, D., et al. (2018) CD44 and CD24 expression and prognostic significance in canine mammary tumors. Vet Pathol, 1-12. http://doi.org/10.1177/0300985818813653. PMID: 30558511.

Rybicka, A., Król, M. (2016) Identification and characterization of cancer stem cells in canine mammary tumors. Acta Vet Scand, 58(86) 1-7. http://doi.org/10.1186/s13028-016-0268-6. PMID: 27993142.

Salvador, F., Llorente, A., Gomis, R.R. (2019) From latency to overt bone metastasis in breast cancer: potential for treatment and prevention. J Pathol, 1-13. http://doi.org/10.1002/path.5292. PMID: 31095738.

Strati, A., Nikolaou, M., Georgoulias, V., Lianidou, E.S. (2019) Prognostic significance of TWIST1, CD24, CD44, and ALDH1 transcript

quantification in EpCAM-positive circulating tumor cells from early stage breast cancer patients. Cells, 8, 652. http://doi.org/ 10.3390/ cells8070652. PMID: 31261917.

Winters, S., Martin, C., Murphy, D., Shokar, N.K. (2017) Breast cancer epidemiology, prevention, and screening. Prog Mol Biol Transl Sci, 1-32. http://doi.org/10.1016/bs.pmbts.2017.07.002. PMID: 29096890.

10.22059/ijvm.2019.288591.1005019
Iranian Journal of Veterinary Medicine
Abstracts in Persian Language
Online ISSN *2252-0554*

مجله طب دامی ایران، ۱۳۹۹، دوره ۱۴، شماره ۳، ۳۲۹-۳۴۲

نقش سلول های بنیادی سرطان در معیارهای کلینیکوپاتولوژیک کارسینوماهای غدد پستانی سگ

حوا مرزبان، فرهنگ ساسانی*

گروه پاتولوژی دانشکده دامپزشکی دانشگاه تهران، تهران،ایران

(دریافت مقاله: ۲۳ شهریور ماه ۱۳۹۸، پذیرش نهایی: ۱۳ آبان ماه ۱۳۹۸)

چکیده

زمینه مطالعه: تومورهای بدخیم غدد پستانی سگ از شایع ترین تومورها در این گونه می‌باشند. در مدیریت این سرطان ها در سگ و انسان چالش های بسیاری وجود دارد. فرضیه ای در ارتباط با زیرگروه کوچکی از سلول های توموری به نام سلول های بنیادی سرطان وجود دارد. بنابر این فرضیه این سلول ها مقاوم به درمان بوده و حتی پس از یک درمان ابتدایی موفقیت آمیز مجدداً موجب متاستاز و عود می گردند. شناخته شده ترین فنوتیپ برای شناسایی این سلول ها ALDH1+/CD44+/CD24-/Low می باشد.

هدف: در این مطالعه حضور سلول های بنیادی سرطان و نقش آن ها در پارامترهای کلینیکوپاتولوژیک تومورهای بدخیم غدد پستانی سگ مورد مطالعه قرار گرفت.

روش کار: در این طرح ۴۰ نمونه از تومورهای بدخیم غدد پستانی سگ جمع آوری گردید. تمامی نمونه ها به روش معمولی هماتوکسیلین- ائوزین و سپس ایمونوهیستوشیمی رنگ آمیزی شدند و برای تعیین سلول های بنیادی سرطان از آنتی بادی هایی ضد ALDH1، **CD44** و **CD24** استفاده گردید. همچنین سایر پارامترهای مرتبط با تهاجم از جمله تهاجم لنفی عروقی، گرید و هیستوتایپ مورد مطالعه قرار گرفتند.

نتایج: مطالعه حاضر نشان داد که ۱۷/۵٪ از موارد شامل سلول های بنیادی سرطان بودند و تمامی این نمونه ها با در گرید دو و سه قرار گرفتند ($P \leq ۰/۰۵$). نتایج همچنین حاکی از آن بودند که تمامی نمونه های مثبت از نظر سلول بنیادی سرطان همچنین با دارای تهاجم لنفی عروقی بودند ($P \leq ۰/۰۵$). شایع ترین هیستوتایپ های مطالعه شده در این طرح شامل توبولار کارسینوما، توبولوپاپیلاری کارسینوما و اینتراداکتال کارسینوما بودند.

نتیجه گیری نهایی: تحقیق حاضر نشان داد که سلول های بنیادی سرطان در تعیین پیش آگهی بدخیمی تومورهای پستانی سگ می‌تواند نقش مهمی ایفا نماید که این نتایج با نتایج بدست آمده از مطالعات مشابه بر روی سرطان پستان انسان مطابقت داشت.

واژه‌های کلیدی:

تومورهای بدخیم، سرطان، سگ، سلول های بنیادی، غدد پستانی.

نویسندهٔ مسئول: فرهنگ ساسانی، گروه پاتولوژی دانشکده دامپزشکی دانشگاه تهران، تهران،ایران. ایمیل: *Fsasani@ut.ac.ir*