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EXPRESSION OF E- CADHERINE IN CANINE MAMMARY TUMORS

Phd. Nikola Karabolovski
Phd. Natasa Pejcinovska
Phd. Pance Dameski
Phd. Petar Dodovski
Phd. Talija Hristovska
Phd. Mimi Ristevski
Dvm. Igor Zdraveski
Dvm. Aleksandar Avramov
Dvm. Ivana Crnec
Veterinary faculty - Bitola

ABSTRACT

Reduction of expression of E-cadherin in canine mammary tumors has been correlated with invasive behavior, lymph node metastases and shorter survival period after tumor removal. The aim of this study was to examine the expression of E-cadherin in canine benign and malignat mammary tumors with different grade of malignancy. 38 canine mammary tumors were studied. In general, the percentage of positive stained cells was bigger in benign neoplasia and carcinomas with grade of malignancy The expression of E-Cadherin decreased with growth of the grade of malignancy. Among the groups of tumors of different grade of malignancy, statistical significant loss of expression of E-Cadherin was detected between benign lesions and carcinomas grade of malignancy II ($p< 0,01$), and between benign lesions and carcinomas III grade of malignancy ($p< 0,01$).

Key words: E-cadherin; invasion; mammary tumors; dog.

INTRODUCTION

Canine mammary tumors are the most common neoplasia in female dogs (MOE, 2001.).

Around 50% of all canine mammary tumors are diagnosed malignant, which give metastases in the distant organs (i.e. lungs, bones, liver etc.)

(HAMPE and MISDORP, 1974.; MACEWEN and WITHROW, 1996.). Distant metastases are the main cause for morbidity and mortality in these animals (PANTEL and BRAKENHOFF, 2004.). Malignant epithelial cells to give a metastases- to detach from the primary tumor, and to invade local tissue or to enter into blood or lymph vessels they must undergo molecular changes in the expression of certain proteins, particularly in those included in obtaining of intercellular connections and tissue stability such are cadherins (TSAI et all 2013.). This process is known as epithelial-mesenchymal transition (EMT) and is active during embryogenesis, wound healing and metastases in malignant tumors (KALLURI et all., 2009.).

Cadherins are calcium-dependent adhesion molecules mediating specific cell to cell adhesion (NOLLET et all 1999.). The best known member of cadherins- E-cadherin plays a crucial role in the process of EMT, when its expression is reduced (KALLURI, 2009.;THIERY et all 2009.). The vast majority of studies pointing out that the downregulated expression of E-cadherin in canine mammary tumors is related with high grade of tumor malignancy, invasion and distant metastases as well as shorter period of survival in affected bitches (RESTUCCI et all., 1997,; GAMA et all., 2008,; REIS et all., 2003,; YOSHIDA and all 2014). There are several studies demonstrating that E-cadherin reduced expression enhances tumor progression and invasion in several human cancers including breast cancer (VLEMINCKX and all., 1991, VOS et all., 1997.).

The goal of this study was to examine the expression of E-cadherin in canine mammary tumors with different grade of malignancy.

MATERIALS AND METHODS

In this study we used 38 canine mammary tumors –from the archive of department of pathology at Veterinary faculty-Bitola. The specimens were from pure and mixed breeds and the mean age of bitches were 10 years (from 5.5 to 15 years). The specimens were fixed in 10% neutral buffered formalin. After dehydration and embedding in paraffin wax, two sections were cut from each block. One section was stained with haematoxylin and eosin (HE) and the second section was used for E-cadherin immunohistochemistry (IHC).

The tumors were classified independently by two pathologists from HE-stained sections according to diagnostic criteria of the new proposed histological classification of canine mammary tumors from 2011 (GOLDSHMIDT et all., 2011.).

E-cadherin IHC

IHC assay was performed on 2µm sections of paraffin-embedded tissue samples. The sections were dewaxed in xylene and rehydrated through a series of graded alcohol solutions. Antigen retrieval was carried out by microwave treatment with ethylenediaminetetraacetic acid (EDTA) buffer ph 9 (Dakocytomation, code S2367) for 20min. Endogenous peroxidase activity was blocked by incubating the slides for 5 min at room temperature in Dako REAL peroxidase blocking solution (DakoCytomation, Code S1700). Sections were incubated with primary antibody for 30min using the monoclonal mouse anti –human E-cadherin (DakoCytomation No. M3612) diluted 1:100. This was followed by incubation for 30min with a ready- to - use secondary antibody (Dako REAL Envision/Horseradish Peroxidase, Rabbit/Mouse0 and with the substratDako REAL Diaminobenzidine +Chromogen for a further 10min. Rineses were done with DakoCytomation Wash Buffer between each step.

The sections were counterstained with hematoxylin and mounted.

Section from canine perineal gland was used as positive control.

Evaluation of immunohistochemical staining for E-cadherin was executed by examining five randomly selected areas in the tissue section under 200x magnification. The percentage of negative or faint stained luminal epithelial cell was estimated and graded from 0 to 3. Zero point meant good expression, 1 point represented weak expression in 5-20% of the tumor cells, 2 points 20-50% of the tumor cells have lost expression and 3 point meant that >50% of the tumor cells lost expression.

Statistical analysis.

Data collected in the survey were statistically analyzed using computer program STATISTICA, statsoft. (2011.), Version 10. www.statsoft.com

To test the statistical significance of differences among samples parametic and non parametic tests of significance were used. Statistical hypothesis were tested at the level of significance of $P=0.05$ i.e the difference between samples was considered to be significant if $P<0.05$. The Kolmogorov-Smirnov test was used to check whether the data were normally distributed. Single Factor analysis was performed using analysis of variance Kruskal-Wallis ANOVA.

RESULTS

Histological examination yielded 8 benign tumors (2 complex adenomas, 2 benign mixed adenomas, 2 benign hyperplasias, 1 tubulopapilary adenoma, 1 simple adenoma), 8 carcinomas grade of malignancy I (5 tubulopapilary

carcinomas, 3 complex carcinomas), 11 carcinomas grade of malignancy II (6 complex carcinomas, 3 tubular carcinomas, 1 papilary carcinoma), carcinomas grade of malignancy III (4 solid carcinomas, 3 anaplastic carcinomas, 2 comedo carcinomas, 2 tubulopapilary carcinomas). SLIKI!!!!

Positive immunostaining for E-cadherin was evident on the membrane of luminal epithelial cells . In general, the percentage of positive stained cells was bigger in benign neoplasia and carcinomas with grade of malignancy I (Photo 1).

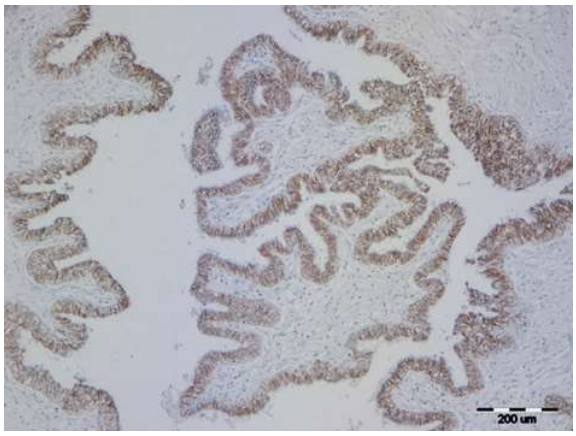

Photo 1. Adenoma, positive membrane E-cadherine staining, IHC x200.

The expression of E-Cadherin decreased with growth of the grade of malignancy(Photo 2 and 3).

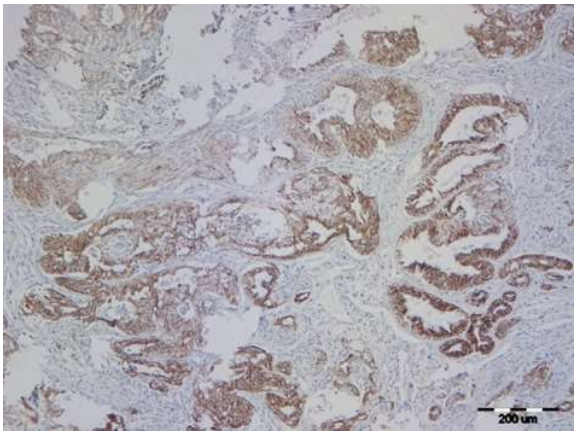

Photo 2. Decreased expression of E-cadherine in mammary carcinoma, grade of malignancy II, IHC x200.

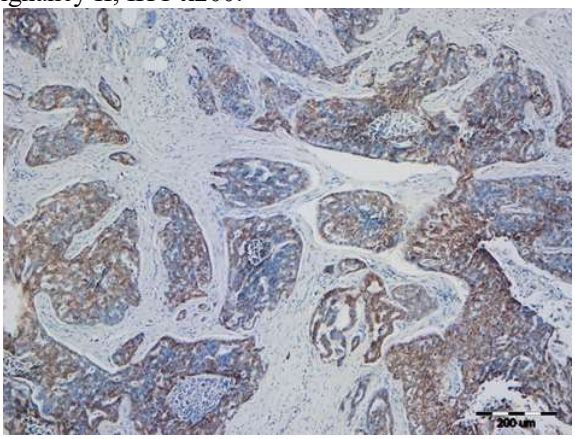

Photo 3. Weak expression of E-cadherine in mammary carcinoma grade of malignancy III, IHCx200.

Among the groups of tumors of different grade of malignancy, statistical significant loss of expression of E-Cadherin was detected between benign lesions and carcinomas grade of malignancy II ($p< 0,01$), and between benign lesions and carcinomas III grade of malignancy ($p< 0,01$). Slika

Tumor type/ grade	**Loss of E-cadherin**
Tubulopapilary adenoma	0
Lobular hyperplasia	0
Benign mixed tumor	0

Lobular hyperplasia	0
Complex adenoma	0
Complex adenoma	0
Simple adenoma	1
Benign mixed tumor	0
Tubulopapilary carcinoma I	1
Tubular carcinoma I	1
Tubulopapilary carcinoma I	2
Complex carcinoma I	1
Complex carcinoma I	2
Tubulopapilary carcinoma I	2
Tubulopapilary carcinoma I	1
Complex carcinoma I	1
Complex carcinoma II	2
Papilary carcinoma II	1
Tubular carcinoma II	2
Complex carcinoma II	2
Tubular carcinoma II	1
Tubulopapilary carcinoma II	2
Tubular carcinoma II	3
Complex carcinoma II	2
Complex carcinoma II	1
Complex carcinoma II	2
Complex carcinoma II	2
Comedo carcinoma III	3
Solid carcinoma III	3
Tubulopapilary carcinoma III	2
Tubulopapilary carcinoma III	2
Solid carcinoma III	3
Solid carcinoma III	3
Comedo carcinoma III	3
Anaplastic carcinoma III	3
Solid carcinoma III	3
Anaplastic carcinoma III	3

DISCUSSION

Reduced or lost expression of E-cadherin means poor prognosis in both human (SIITONEN et al., 1996,; ZSCHIESCHE et al., 1997,; YOSHIDA et al., 2001,; LIM et al., 2002.). and canine mammary tumors (MATOS et al., 2006,; RESTUCCI et al., 1997,; REIS et al., 2003.). The results of this study have shown that the expression of E-cadherin was significantly reduced or lost in canine mammary tumors with high grade of malignancy compared with normal tissue and benign tumors or dysplasia. Almost every benign tumor had good expression of E-cadherin. Similar results with the present findings have been demonstrated in the previous studies (RESTUCCI et all., 1997., MATOS et all., 2006,; REIS et al., 2003.).

In our study low expression of E-cadherin was found in solid carcinomas, these findings are in concordance with some previous studies made by MATOS et al. (2006.), but are less striking with that made by Restucci et al. (1997.), who detected almost zero expression in six canine solid carcinomas. In the present study the expression of E-cadherin was high in tubular and tubulopapillary carcinomas, this findings are not strange because of the fact that although neoplastic, tubular and papillary structures require certain degree of morphological organization, which mostly depends on adhesion molecules (cadherins) (TAKEICHI, 1991.).

In this study the expression of E- cadherin was decreased with increasing the grade of malignancy, that is in accordance with the previous study of RESTUCCI et al. (1997.) but not with the study made by MATOS et al. (2006.) where expression of E-cadherin was not decreased in a stepwise fashion with increases of the grade of malignancy. In addition, in human breast cancer, there are also some conflicting results in the literature. GAMALLO et al. (1993.), had found greater immunoreactivity in grade I carcinomas than in grade II and grade III.

In another study YOSHIDA et al. (2014), have detected reduced expression of E-cadherin in malignant canine mammary tumors and have also noticed correlation between reduced expression and shorter period of survival after tumor removal.

Unfortunately we were not able to make additional comparisons between the size of the tumors, possible metastases and expression an period of survival in affected animals.

In contrary, in neoplastic cells of inflammatory breast cancer, which is one of the most aggressive cancer, the expression of E-cadherin is consistently elevated(72).

CHARAFE-JAUFFRET et al. (2004), also found E-cadherin expression in this highly metastatic carcinoma. Further more they explained that the reduction of expression of this marker is a transient event that allows malignant cells to invade blood and lymph vessels and tissues, in circulation cancer cells reexpress E-cadherin, in that way they facilitate intercellular adhesion and enabling the formation of cohesive tumor emboli.

In conclusion, our study has shown correlation between reduction of expression of E-cadherin and the grade of malignancy in canine mammary tumors. Further studies should include follow-up examine of affected bitches after tumor removal, in that way to confirm the correlation between expression of this marker and total survival period.

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