Short Communication

OSTEOPONTIN IN MIXED BENIGN AND MALIGNANT MAMMARY TUMORS OF BITCHES. IMMUNOHISTOCHEMICAL STUDY

(OSTEOPONTINA EM TUMORES MAMÁRIOS MISTOS BENIGNOS E MALIGNOS DE CADELAS. ESTUDO IMUNO-HISTOQUÍMICO)

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SUMMARY

Osteopontin (OPN) is an extracellular phosphorylated glycoprotein, which is important in a large number of physiological and pathological processes. It is considered a marker for breast cancer prognosis, since its levels are elevated in tumors and plasma of human patients with metastatic breast cancer. There is no information in the literature on plasma levels of OPN in mammary tumors in dogs. This study aimed to investigate the immunoreactivity of epithelial cells in mammary gland neoplasms of bitches using anti-osteopontin antibodies. We selected 10 cases of benign canine mammary mixed tumors and 10 malignant, that means, carcinoma in mixed tumor. The technique used was immunohistochemistry with antigen unmasking by heat, inhibition of endogenous peroxidase and use of avidin biotin peroxidase complex (ABC). Detection was performed with DAB revelation. The number of immunolabeled cells was counted under a light microscope. It was found that, while benign tumors presented reactivity slightly higher than the malignant, the mean number of marked cells in each group was not significantly different. We conclude that, contrary to what occurs in humans, OPN in canine mixed mammary tumors can not be considered a marker of tumor behavior. The reason for this difference becomes a subject to be further explored.

KEY-WORDS: Osteopontin, neoplasia. Mixed mammary tumor. Dog. Immunohistochemistry.

RESUMO

A osteopontina (OPN) é uma glicoproteína fosforilada extracelular com importância em um grande número de eventos fisiológicos e patológicos. Seus níveis são elevados em tumores e no plasma de pacientes humanos com câncer de mama metastático, sendo considerada um marcador de prognóstico. Não há informações na literatura sobre os níveis plasmáticos de OPN em cães ou em neoplasias mamárias nessa espécie. Este trabalho teve como objetivo pesquisar a imunorreatividade em células de origem epitelial em neoplasias da glândula mamária de cadelas utilizando-se o anticorpo anti-osteopontina. Foram selecionados 10 casos de tumores mistos mamários caninos benignos e 10 malignos, ou seja, do tipo carcinoma em tumor misto. A técnica utilizada foi imuno-histoquímica, com desmascaramento de antígenos pelo calor, inibição de peroxidase endógena e emprego do complexo Avidina Biotina Peroxidase (ABC). A revelação da reação foi feita com DAB. A observação foi em microscopia de luz, com a contagem do número de células imunomarcadas. Verificou-se que, embora os tumores benignos apresentassem uma reatividade discretamente mais elevada que os malignos, as médias das células imunomarcadas de cada grupo não apresentaram diferenças significativas. Conclui-se que, ao contrário do que se verifica em humanos, a OPN, em tumores caninos mistos mamários, não pode ser considerada um marcador de comportamento tumoral. O motivo para essa diferença torna-se assunto a ser explorado.

PALAVRAS-CHAVE: Osteopontina. Neoplasia. Tumor misto mamário. Cão. Imuno-histoquímica.

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INTRODUCTION

Mammary gland tumor is the most common type of neoplasia in bitches. Approximately 50% of the canine mammary tumors are described as malignant (GILBERTSON et al. 1983, MOULTON, 1990, SCHAFER et al., 1998).

The extracellular matrix plays an important role in the development and progression of the tumor. It acts as a reservoir for many growth factors, cytokines and signaling molecules, which can regulate the behavior of malignant cells (COOK et al., 2006).

Osteopontin (OPN) is an extracellular phosphorylated glycoprotein, which is part of several physiological and pathological events (DENHARDT et al., 2001). Its levels are high in tumors and plasma of human patients with metastatic breast cancer, and it is known that osteopontin is related to tumor aggressiveness and shortening the survival time of these patients (SAMANT et al., 2007). In addition, OPN is produced in cells of a wide variety of human tumors and has been considered as a potential prognostic factor for metastatic breast cancer (NATASHA et al., 2006).

The aim of this study was to detect osteopontin as a marker in breast cells with mixed malignant and benign tumors, using the technique of immunohistochemistry, in order to establish a relationship between the expression of this glycoprotein and tumor behavior. Therefore, we selected 20 cases of canine mammary tumors, which showed areas with morphology compatible with mixed mammary tumors. Two experimental groups were formed with 10 animals each, where group B consisted of animals with mixed benign mammary tumor and group M, mixed malignant mammary tumor. Fragments of all selected cases were fixed in 10% formalin, buffered with phosphate (pH 7.2), followed by a routine process of paraffin embedding to obtain the histological section that were stained with hematoxylin and eosin.

The paraffin embedded sections were deparaffinized and hydrated to proceed with the immunohistochemistry technique. The method used was the avidin-biotin peroxidase (*Kit* DAKO LSAB, ref.. K0690ABC), developed by Hsu et al. (1981) with slight modification for the antibody anti-OPN anti-OPN (O 7264 – Sigma) diluted 1:200. The antigen retrieval was performed using a water bath at 92°C, during 40 minutes, with TRIS-EDTA (pH 9.0, 10mM Tris Base, 1mM EDTA) buffer. Detection was performed with chromogen diaminobenzidine revelation (DAB - DAKO ref. K3466). The markings were considered positive when the cytoplasm of neoplastic epithelial cells was clearly marked, even if minimally stained.

Different microscopic fields chosen randomly were analyzed in 100 cells. Two observers performed the counts, and the average of the two counts was the final mean for each neoplasia, as a percent of marked cells. Means were compared by Test t at 5%.

In most of the histological sections, the marked cells were easily identified in both benign (Figure 1) and malignant (Figure 2) tumors, so that the counting was done without difficulty. The differences between the mean counts of the marked cells in both groups, the percentage, were not significant and were, respectively, 76.5 ± 11.31 and M, 71.1 ± 8.17 for Groups B and M (Table 1).

Tuck et al. (1997) suggested that OPN may be a marker for aggressive breast cancer tumor and its high level in the primary tumor may predict the future appearance of metastasis. These data contrast with our findings, since no metastases were found among the analyzed tumors and benign tumors had slightly higher immunoreactivity compared to malignant tumors. Therefore, the high levels of this glycoprotein in mixed mammary tumors in bitches do not appear to be related with the malignant characteristics of the tumor.

It is observed that the antibody anti-OPN marked mammary epithelial cells, epithelial cells from epidermis, fibroblasts, chondroblasts, myoepithelial cells and some inflammatory cells such as macrophages, which appeared occasionally in some tumors. The marking of fibroblasts can be explained by recent studies suggesting that OPN may have a more focused role on the development of fibrous tissue (MAZZALI et al., 2002). Moreover, it is one of the most abundant proteins expressed by macrophages and a potent chemotactic stimulus for these (SCATENA et al., 2009).

It is concluded that in bitches, OPN can not be considered as a prognostic tumor marker, unlike human breast tumors where its high expression can be correlated with greater malignancy. The reason for this behavior is a subject for further research.

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Table 1 - Percentage of epithelial and breast cells of bitches, marked by immunohistochemistry with antibody anti-osteopontin in mixed benign and mixed malignant tumors.

	Benign mixed tumors	**Malignant mixed tumors**
Sequence	% Marked cells	% Marked cells
1	61	76
2	80	77
3	73	77
4	60	78
5	78	63
6	90	66
7	66	56
8	88	82
9	90	69
10	67	79
Means	**76.5 ± 11.31**	**71.1 ± 8.17**

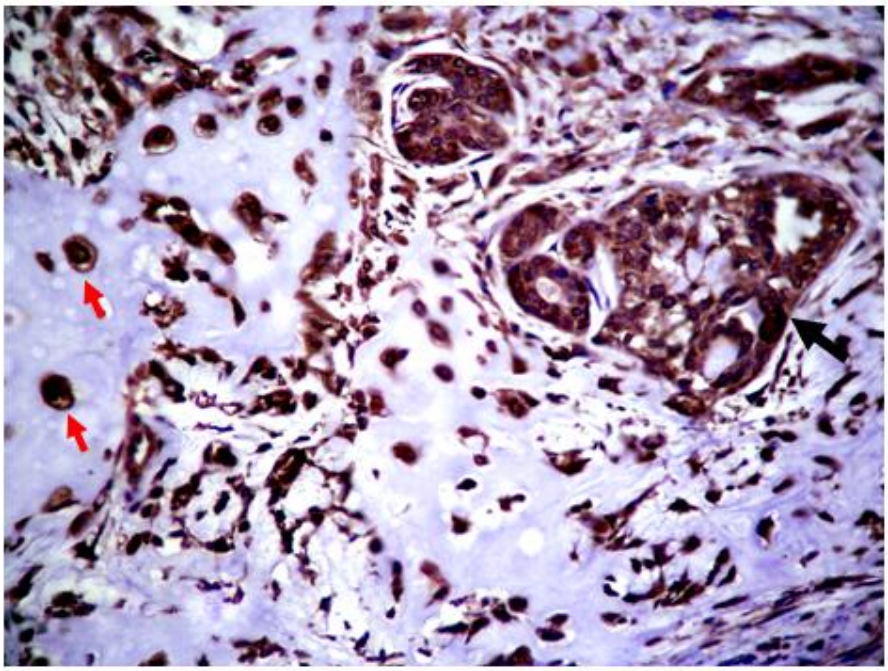

Figure 1 - Benign mixed mammary tumor in bitches. Immunohistochemical staining for osteopontin. Epithelial cells (large arrow) and chondrocytes (thin arrow) stained brown are observed. ABC, obj. 20x.

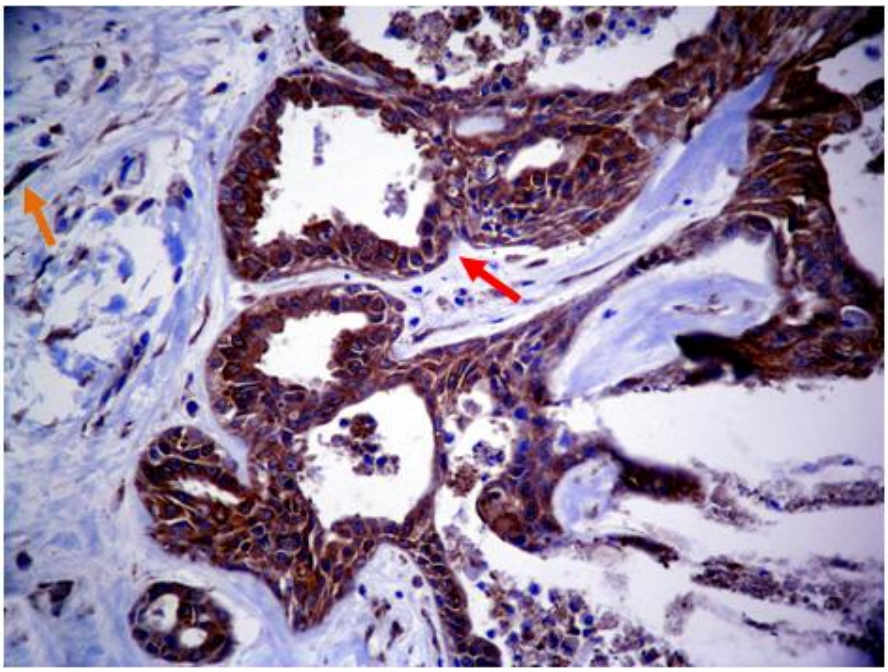

Figure 2 - Carcinoma in canine mixed mammary tumor. Immunohistochemical staining for osteopontin. Epithelial cells (red arrow) and fibroblasts (thin arrow) stained brown are observed. ABC, obj. 20x.

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