Relationship Between the Immunodetection of Alpha-Smooth Muscle Actin and the Aggressiveness of Mammary Papillar Tumors in Female Dog

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Summary

Papillary carcinoma is a mammary neoplasia of women and female dogs characterized by papillary fibrovascular projections lined by epithelial cells. Evaluation on the biology of these tumors can be done by immunohistochemistry through detection of alpha-smooth muscle actin protein in the papillary myoepithelium, which lacks such a molecule during malignant proliferations. Thus, this study aimed at determining the malignancy degree of papillary mammary tumors of female dogs by immunohistochemistry. Twenty samples of mammary neoplastic tissues collected from female dogs treated in the Veterinary Hospital at FCAV were evaluated by Hematoxylin and Eosin staining (H&E) and tumor cells were immunolabelled with monoclonal antibody to alpha-smooth muscle actin (α-SMA). Five out of 20 cases showed positive immunolabeling greater than 10% of the total immunolabeling. The remaining fourteen cases presented immunostaining lesser than 10% showing decrease or absence of α-SMA labeling in the myoepithelium of the papilla tumors. All those cases in which immunostained cell was over 10% of the neoplasm (5 immunostains of 20 total cases) were classified as benign whereas those below 10% of immunostained in the slid were considered as malignant. Therefore, immunohistochemistry played an essential role in differentiating benign and malignant papillary tumors of bitches as already described for female. Tumor classification by conventional methods, such as H&E staining, can lead to erroneous interpretations on the real biological behavior of the papillary mammary tumor.

quantification of the immunostained cells, 10 large-magnification fields (40 x obj. lens) were randomly chosen, where the counts of immunolabellated cells were measured in an area of 0.19625 µm^2. Counting criteria were those used by [3], who used the semi-quantitative evaluation method. Thus, samples containing less than 10% of immunodetected cells were considered as negative and those containing more than 10% were interpreted as positive.

Results

Regarding the number of cells positive for α-SMA, five of the 20 samples had MC labeling greater than 10% of the total (Figures 1A and 1B). In the remaining samples, α-SMA detection was less than 10% or absent in the papillary formations of the mammary tumor (Figures 1C and 1D). Samples with α-SMA positivity greater than 10% were classified as benign

papillary tumors, while those with markings below 10% were considered malignant papillary tumors (Table 1).

Discussion

Data generated in this study showed that the immunodetection of MC played an important role on the aggressiveness classification of the papillary mammary tumor of female dogs. There are currently few reports evaluating this neoplasia in female dogs. However, in the latest consensus on diagnosis, prognosis and treatment of mammary gland tumors in female dogs, it was proposed that papillary tumors in this species should also be differentiated between benign or malignant behaviors by techniques such as immunohistochemistry [1]

HE staining is the most commonly used technique in the routine diagnosis and classification of
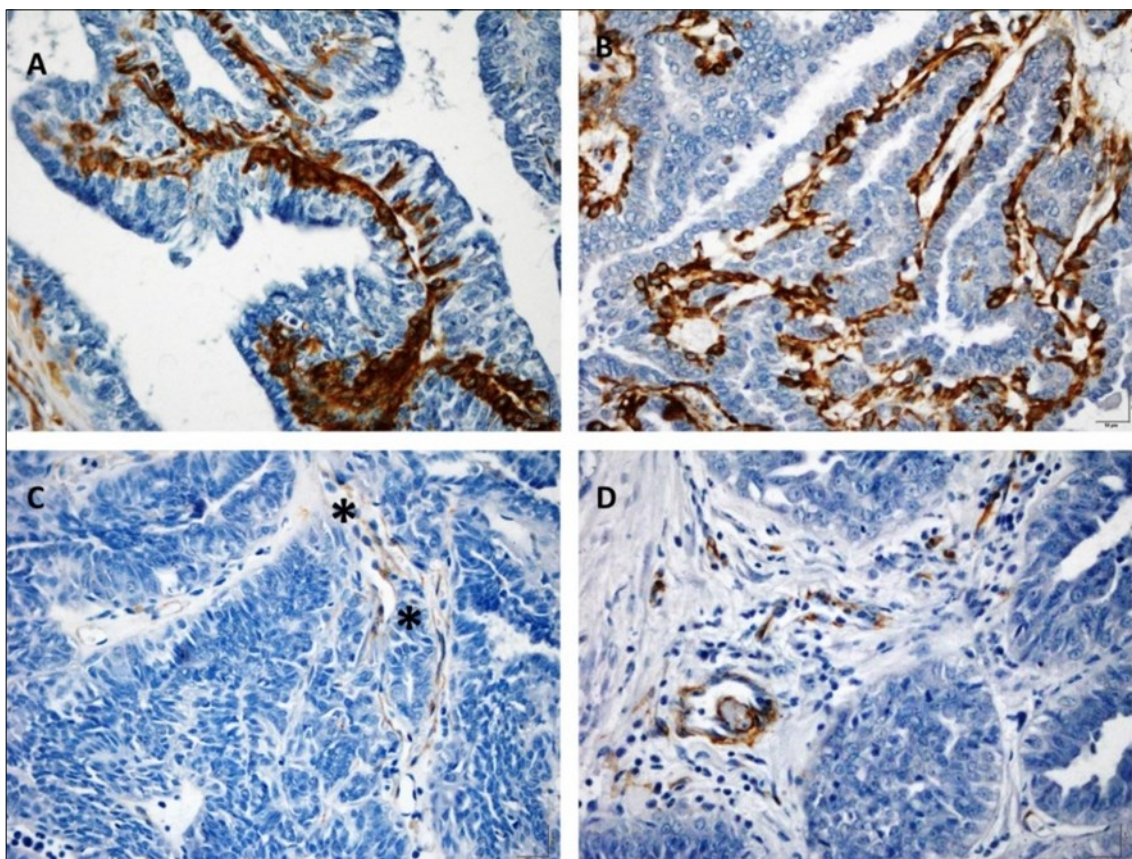
Figure 1. Photomicrographs of papillary tumors in mammary gland of female dogs. (A) Papillary tumor with intense positive marking for the α-SMA within the papillae (bar = 10 µm). (B) Positive to moderate positive papillary tumor for α-SMA (bar = 10 µm). Papillary tumors with reduced (C) or scarce immunolabeling (*) (D) for the same antibody (bar = 10 µm). All samples were classified by HE analysis as papillary carcinoma. Complex of Peroxidase bound polymers.

Table 1. Percentage of immunostained cells with α-SMA in mammary papillary tumors in female dogs. Five cases were detected in which the immunostaining was greater than 10% of the tumor and 15 cases where the detection of immunostained cells was less than 10%, according to CHU et al. (2012)[3].

Samples	HE	IHC (%)	Result of IHQ	Final classification
1	PC	46,51	> 10%	Benign
2	PC	7,47	< 10%	Malignant
3	PC	74,39	> 10%	Benign
4	PC	13,06	> 10%	Benign
5	PC	0,82	> 10%	Malignant
6	PC	2,82	> 10%	Malignant
7	PC	8,38	< 10%	Malignant
8	PC	2,01	> 10%	Malignant
9	PC	0,97	> 10%	Malignant
10	PC	4,29	> 10%	Malignant
11	PC	29,08	> 10%	Benign
12	PC	8,9	< 10%	Malignant
13	PC	5,92	< 10%	Malignant
14	PC	1,49	> 10%	Malignant
15	PC	3,56	< 10%	Malignant
16	PC	4,68	> 10%	Malignant
17	PC	0,44	> 10%	Malignant
18	PC	11,09	> 10%	Benign
19	PC	16,8	< 10%	Malignant
20	PC	9,05	> 10%	Malignant

Caption: HE (hematoxylin and eosin); IHC (immunohistochemistry); PC (papillary carcinoma);

mammary neoplasia of female dogs, nevertheless this method is not suitable to accurately identify the proportion of MC present in papillary tumors, especially for those cases in which the sample is very small or was not completely forwarded to the lab for analysis [4,910,6]. In the present study, the HE-based microscopic analysis was fundamental for distinguishing the histological tumor pattern (papillary), for determination of the mitotic index and invasion capacity to adjacent tissue, among other aspects. However, this method was not sufficient to show the biological behavior of the tumor, depending on the presence or absence of MC. In the present study, 25% of the samples (5/20) were classified as malignant in HE analysis, but by immunohistochemistry they were benign, since they showed a high percentage of α-SMA antibody positive cells.

Antibodies to detect myoepithelial tissue into mammary tumors samples have already been utilized for diagnostic purposes in humans, and the alpha smooth muscle antibody is one of those recommended for detection of MC in papillary tumors of both women and bitches [1,2,4,6,9]

The results from a study with tubulo-papillary carcinomas in female dogs demonstrated that there is an overestimation on the malignancy of neoplasia diagnosed by HE technique [3]. These authors reported that by using p63 and calponin antibodies nine tumors that had been classified as malignant by HE method were actually a benign process. Our data also supports the idea of an overestimation of malignancy when using HE staining to evaluate tumor samples. By using this criteria set up by [3], in which benign tumors had a positive mark greater than 10% of the entire tumor extension whereas malignant tumors should have positive MC below 10% of the total neoplastic cells, we

showed that five out of 20 predetermined malignant process by HE technique were in fact benign neoplasia with preservation of MC.

There are many specific antibodies available to detect myoepithelium in papillary tumors such as smooth muscle myosin heavy chain, CD10, calponin, maspine, p63, cytokeratins 5, 14 and 17, and Wilms-1 tumor. For some authors the target tissue should be tested with at least two of these markers to assure the prognosis. In the present study, we opted to use the smooth muscle alpha-actin protein alone because it was demonstrated to be sufficient to identify MC in tumors of humans [4,10] and it was possible to observe this aspect in dogs.

Conclusion this study, the immunohistochemical technique was essential for the differentiation between benign and malignant papillary neoplasia in bitches, as it had already been established for papillary tumors in women. Histological classification of papillary mammary tumors based on HE staining alone can bring erroneous interpretations of the true biological behavior of this tumor type and increases the diagnoses of malignancy, in turn, changing the directions of the treatments.

Conflict of Interest

The authors declare that there were no conflicts of interest.

References

1. Cassali GD, Lavalle GE, Ferreira E, Estrela-Lima A, De Nardi AB, et al (2013). Consensus for the Diagnosis, Prognosis and Treatment of Canine Mammary Tumors - 2013. Brazilian Journal of Veterinary Pathology, 7, 38-69.

2. Calderaro J, Espie M., Duclos J, Giachetti S, Wehrer D (2009). Breast Intracystic Papillary Carcinoma: An Update. Breast Journal, 15, 6, 639-44.

3. Chu PY, Yeh CM, Yeh KT., Liu CH (2012). Tubulopapillary carcinoma-a frequent over-diagnosed entity in canine mammary gland tumors. Tawain Veterinary Journal, 38, 2, 67-74.

4. Destexhe ED, Lespagnard L, Egeyter MD., Eymann RH, Coignoul F (1993). Immunohistochemical identification of myoepithelial, epithelial, and connective tissue cells in canine mammary tumors. Veterinary Pathology, 30, 2, 146-154.

5. Misdorp, W.; Else, R. W.; Hellmén, E.; Lipscomb, T. P. Histological classification of mammary tumors of the dog and the cat. In: WHO international histological classification of tumors of domestic animals. Washington: Armed Forces Institute of Pathology, 1999. v. 8, p. 3-59. (Second Series).

6. Pal SK, Lau SK, Kruper L, Nwoye U, Garberoglio C, Gupta RK, Paz B, Vora L, Guzman E, Artinyan A, Somlo G (2010). Papillary carcinoma of the breast: an overview. Breast Cancer Research Treatment, 122, 3, 637–645.

7. Stefanou D, Batistatou A., Nonni A, Arkoumani E, Agnantis NJ (2004). p63 expression in benign and malignant breast lesions. Histology and Histopathology, 19, 2, 465-71.

8. Stolnicu S (2005). Morphologic and immunohistochemical criteria for the diagnosis of papillary intracystic carcinoma. Romanian Journal of Morphology and Embryology, 46, 1, 17-21.

9. Ueng SH, Mezzetti T, Tavassoli FA (2009). Papillary neoplasms of the breast - a review. Archives of Pathology & Laboratory Medicine, 133, 6, 893-907.

10. Zhang RR, Man YG, Vang R, Saenger JS, Barner R, Wheeler DT, Liang CY, Vinh TN, Bratthauer GL (2003). A subset of morphologically distinct mammary myoepithelial cells lacks corresponding immunophenotypic markers. Breast Cancer Research, 5, 5, R151-6.