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Case Report

Histopathological and Immunophenotypical Analysis of Canine Mucinous Rectal Adenocarcinoma

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Abstract

Rectal adenocarcinomas are uncommon in dogs and usually present poor prognosis. The present work describes the morphological and immunophenotypical findings of a rectal mucinous adenocarcinoma in a bitch. Histological analysis revealed a malignant epithelial proliferation in a tubulopapillary pattern forming multiple intratubular cell layers. Moderate amount of PAS-positive amorphous eosinophilic content within neoplastic tubules and extruded into the stroma was observed. Immunohistochemical analysis revealed neoplastic cells considered positive for cytokeratin, Her-2, COX-2 and E-caderin and with low p53 expression. A high proliferation index was observed. Based on histological and immunophenotypical findings, the diagnosis of mucinous adenocarcinoma was established.

Key Words: Neoplasm, gastrointestinal tract, canine, immunohistochemistry.

Introduction

Intestinal neoplasms represent 0.18-0.3% of all reported canine tumours (6, 4). Intestinal neoplasms are more frequently diagnosed in male dogs with advanced age (4) and in German Shepherds and Collies (8). Rectal adenocarcinoma is a rare intestinal malignant neoplasm (1, 2, 4, 8), often associated with a poor prognosis due to frequent recurrences, regional metastases and peritoneal dissemination (1).

Intestinal adenocarcinomas are characterized by their tubular, tubulopapillary or solid pattern. The presence of mucus in at least 50% of the neoplasm enables the diagnosis of a mucinous subtype (4). Mucinous content may be confirmed through Periodic acid-Schiff, toluidine blue and alcian blue staining methods (10).

Immunohistochemistry markers of prognostic value such as COX-2 (7), p53 (11), E-cadherin (1), Ki-67 (5), Her-2 (9) are important in the characterization of human and canine intestinal tumors. Therefore, the aim of this work was to describe morphological and immunohistochemical findings of a canine mucinuos rectal adenocarcinoma.

Case report

An adult female mongrel dog of unknown age was admitted to a small animal clinic presenting melena and a pendulous nodule in the rectal region. After surgical removal, the tumor was sent to the Laboratory of Comparative Pathology for histological analysis. Samples were fixed in 10% neutral buffered formalin and

embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) and periodic acid-Schiff. Pan-cytokeratin (CK AE1AE3, Dako, 1:100), Her-2 (polyclonal, Dako, 1:200), Cox-2 (SP21, Neomarkers, 1:80), p53 (polyclonal, Covance, 1:80), E-cadherin (CDH1, Invitrogen, 1:60), Ki-67 (Clone MIB-1, Dako, 1:25) and α-smooth muscle actin (ASMA) (1 A 4, Dako, 1:100) were used as primary antibodies. Tissue sections were deparaffinized in xylene, subjected to heat-induced antigen retrieval with an antigen retrieval solution (DAKO) at pH 6.0, in a water bath at 98 °C for 20 min. Slides were incubated for 60 minutes at room temperature with CK AE1AE3, Cox-2, p53, E-cadherin, Ki-67 and ASMA antibodies and overnight at 4°C with the Her-2 antibody. Endogenous peroxidase activity block was performed with 3% hydrogen peroxidase in methanol. The reaction was revealed by polymer (ADVANCE HRP–ready to use–DakoCytomation). Diaminobenzidine was used as a chromogen and sections were counterstained with Mayer's hematoxylin. CK AE1AE3 evaluation was qualitative. Her-2, p53, Cox-2 and E-cadherin evaluation was semi-quantitative, according to Schuell et al. (2006) (9), McEntee et al. (2002) (7), Aresu et al. (2010) (1) and Tollenaar et al. (1998) (11), respectively. Cell proliferation index was obtained according to Dutra et al. (2008) (3).

Macroscopic analysis pointed to 1.0 x 0.5 x 0.5 cm nodule with a blackened mucosa and firm consistency. The cut surface presented a solid mass with black and white areas. Morphologically, a poorly delimited neoplastic proliferation of epithelial origin in a tubulopapillary arrangement with multiple intralobular layers was observed (Fig. 1). Tumor cells were characterized as pleomorphic and presented microvillus in their apical membrane, occasionally vacuolated abundant cytoplasm, large nucleus with fragmented chromatin and prominent nucleoli. Moderate quantity of a PAS-positive amorphous extracellular material was observed in the lumen of tubular structures (Fig. 2). Cytoplasmic vacuoles were also positive for PAS. Mitotic index was considered high, with a median of three mitoses per field (40x). A diffuse mononuclear lymphohistiocytic infiltrate was associated with hemorrhagic areas. Immunohistochemical analyses showed neoplastic cell reactivity for CK AE1AE3 (Fig. 3) and Her-2 with cytoplasmic membrane positivity in at least 30% of cells (Fig. 4). COX-2 expression in neoplastic cells was evaluated as strong and diffuse (Fig. 5). p53 showed low reactivity, only in 1% of cells. E-cadherin evaluation was absent in predominantly invasive areas and strong in over 60% of *in situ* areas (Fig. 6). Cell proliferation index was high with 38% of nuclear positivity for Ki-67 staining (Fig. 7).

Discussion

Primary tumors and metastases of mucinous adenocarcinomas are characterized by the formation of

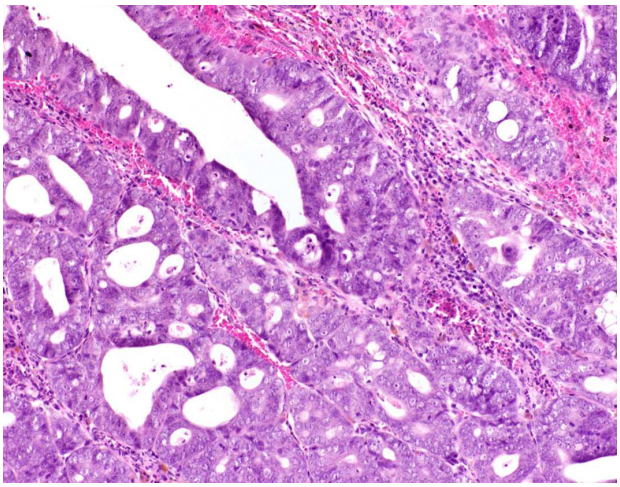

Figure 1. Epithelial cell proliferation in tubulopapillary pattern. Hematoxylin & Eosin, 20x.

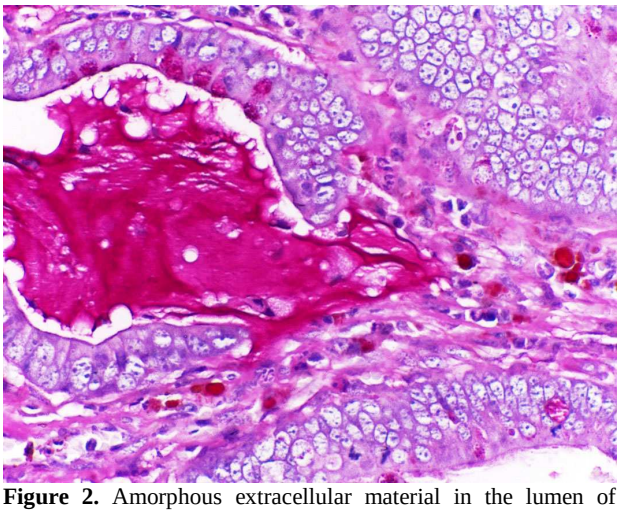

Figure 2. Amorphous extracellular material in the lumen of tubular structures. PAS-diastase staining, 60x**.**

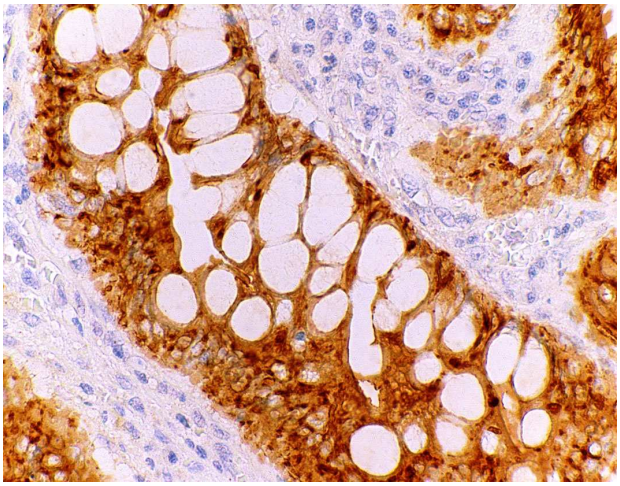

Figure 3. Positive staining of neoplastic epithelial cells for CK AE1/AE3. Immunohistochemical stain with Mayer's hematoxylin counterstain, 60x.

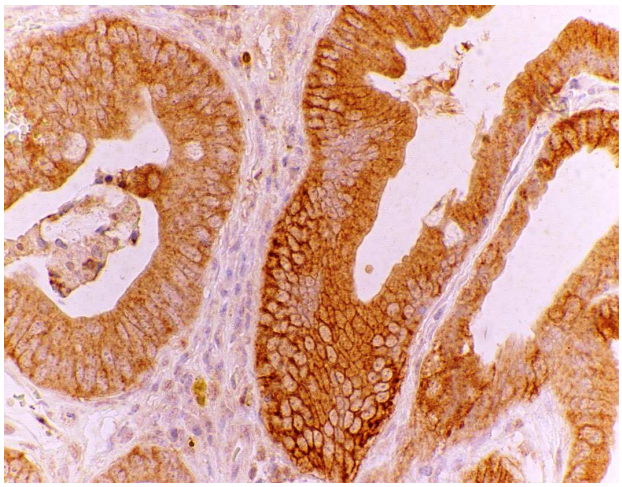

Figure 4. Positive staining of neoplastic epithelial cells for Her-2 with cytoplasmic membrane positivity in at least 30% of cells. Immunohistochemical stain with Mayer's hematoxylin counterstain, 60x

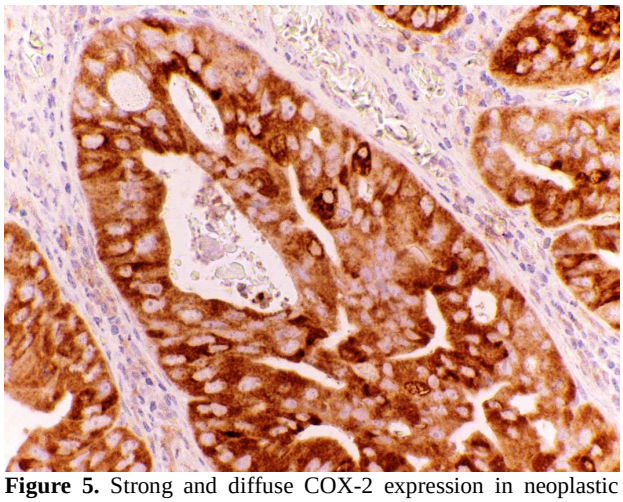

Figure 5. Strong and diffuse COX-2 expression in neoplastic cells. Immunohistochemical stain with Mayer's hematoxylin counterstain, 60x.

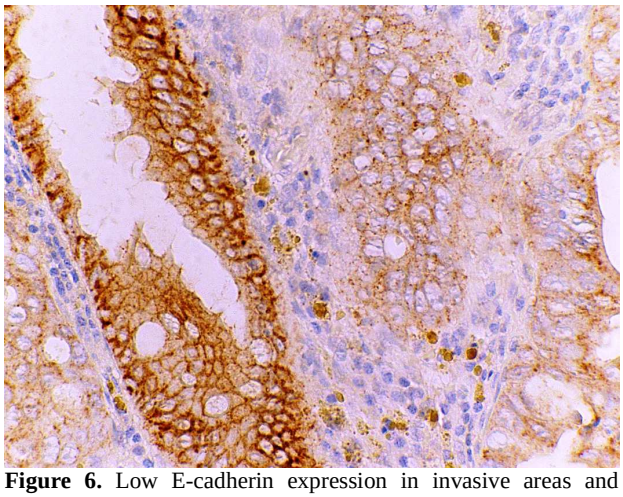

Figure 6. Low E-cadherin expression in invasive areas and strong in over 60% of *in situ* areas. Immunohistochemical stain with Mayer's hematoxylin counterstain, 60x.

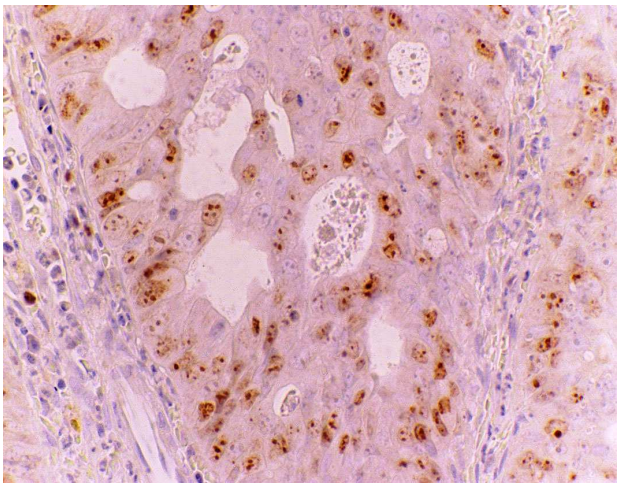

Figure 7. High cell proliferation index with 38% of nuclear positivity for Ki-67 staining. Immunohistochemical stain with Mayer's hematoxylin counterstain, 60x.

mucin lakes surrounded by malignant epithelium (4). Intracellular mucin can rarely be observed (10). In this paper, though both forms were described, mucin was predominantly extracellular and presented chromatic affinity for PAS. Occasional detached neoplastic cells surrounded by mucinous material were found. In addition, early diagnosis may have been responsible for a favorable prognosis, since distant metastases were not observed.

Epithelial histogenesis was confirmed immunohistochemically through CKAE1/AE3 positivity. Some molecules, such as growth factor receptors, appear to be very important in the development and progression of numerous epithelial neoplasms (9). In this report, the neoplasm presented strong Her-2 positivity (score 3), contrary to previous findings in colorectal tumors in humans in which membranar Her-2 expression is absent in most cases (9). In addition, intense reactivity for Cox-2 was observed in this as in another canine study (7). This molecule is probably related with the development of this tumor in dogs (7) and COX-2 selective inhibitors may be a therapeutic possibility for COX-2 positive cases. In this report, ASMA immunostaining allowed the visualization of neoplastic epithelial cells infiltrating the lamina propria and submucous layers, confirming the aggressiveness of this neoplasm. E-cadherin expression loss was mainly observed in invasive areas. A previous report ratifies this finding, indicating that epithelial cell junction loss contributes towards an increase in invasive capacity (1).

Based on literature findings, the prognostic value of p53 is controversial. In this report, despite the aggressive characteristics of the neoplasm, an intense reaction for p53 was not observed. Tollenaar et al. (1998) did not detect significant evidence of the prognostic value of this molecule. Ki-67 expression was observed as intense and diffuse in neoplastic cells. This characteristic is important to define the proliferative index and describe the biological behavior of the tumor. High indexes as the

observed in this case are generally related to low differentiation grade and poor prognosis (5).

The diagnosis of mucinous rectal adenocarcinoma was possible due to its histological characteristics. However, immunophenotypical characterization is extremely important in order to define the biological behavior and prognosis of this tumour. Studies containing a larger number of cases should be performed in order to understand the prognosis of this neoplasm.

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