Atypical Vimentin Expression in a Feline Salivary Gland Adenocarcinoma with Widespread Metastases

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ABSTRACT. We report herein a feline salivary gland adenocarcinoma with widespread metastases to draining lymph nodes, liver and lung, as well as an unusual metastasis to the spleen. Histologically, the primary salivary gland tumor consisted of low columnar to polygonal epithelial cells forming tubules and trabeculae. The spleen was infiltrated with sheets of poorly differentiated large round cells. Interestingly, morphologic change in epithelial cells was accompanied with the acquisition of vimentin intermediate filaments, a feature particularly evident in the splenic metastasis. This study highlights the role of epithelial cell plasticity during carcinogenesis and metastasis.

Key words: adenocarcinoma, epithelial-mesenchymal transition, feline, metastases, vimentin.

A 13-year-old neutered male domestic shorthair cat was presented at the National Veterinary School of Alfort (France) for a recent onset of anorexia and marked dyspnea. The cat also had a right submandibular mass noticed one month earlier. Chest radiographs were performed and revealed generalized miliary pulmonary opacifications. Due to the cat’s poor condition and the likelihood of underlying neoplasia, euthanasia was elected.

A complete necropsy was performed. In place of the right mandibular salivary gland, which was no more macroscopically visible, we found a round, 2 cm in diameter, firm and infiltrative mass. This neoplasm was lobulated, white, with areas of liquefactive necrosis. It contrasted with the left mandibular salivary gland which appeared lobulated, tan with a smooth surface. Metastases were observed in both retropharyngeal lymph nodes and in the right cervical lymph node. These lymph nodes were enlarged and measured approximately 1.5 cm in diameter. They were diffusely white with increased firmness and included some areas of liquefactive necrosis. Metastases also involved all lobes of both pulmonary and hepatic parenchyma as milary, white, firm and umbilicated nodules, measuring between 1 and 5 mm. Although few similar metastatic nodules were found in the spleen, major finding for this organ was a diffuse marked hypertrophy (10 × 4.1.5 cm) with a pale and firm parenchyma. Other lymphoid organs were not grossly modified, including lymph nodes draining the lungs, the liver and the spleen.

Tissue samples from all lesional organs were fixed in 10% neutral-formalin and routinely processed. Periodic Acid Schiff reaction (PAS), Schmorl and Toluidine Blue staining, and immunostaining were performed on paraffin-embedded sections of the salivary gland neoplasm and the spleen. Primary antibodies used were monoclonal mouse anti-B lymphocyte antigen 36 kD (BLA 36, 1:50, clone A27.42, AbCys, France), monoclonal mouse anti-cytokeratin (1:50, clone AE1/AE3, Dako, Denmark), polyclonal rabbit anti-CD3 (1:200, clone A0452, Dako), monoclonal mouse anti-CD79a (1:100, clone HM57, Dako), monoclonal mouse anti-DC-LAMP (CD 208) (1:100, clone 104G4, AbCys), monoclonal mouse anti-Myeloid/Histiocyte Antigen (1:50, clone MAC 387, Dako), monoclonal mouse anti-Smooth Muscle Actin (SMA, 1:100, clone 1A4, Dako), monoclonal mouse anti-vimentin (1:100, clone V9, Dako). A streptavidin-biotin detection method with diaminobenzidine as chromogen (Ventana DAB detection kit, Ventana Medical Systems, Inc., Illkirch, France) was used to reveal primary antibodies.

Microscopically, the salivary gland was completely replaced by a partially encapsulated, lobulated, densely cellular tumor that infiltrated the surrounding connective tissue (Fig. 1). This neoplasm consisted of an epithelial cell proliferation organized in tubules lined with one to several cellular strata, or in small to large trabeculae with central comedo-type necrosis (Fig. 2). Tubules and trabeculae were surrounded by a collagenous walls. Cells had a low columnar to polygonal morphology and measured about 20–25 μm. Cytoplasm was moderately abundant, acidophilic and rarely contained a clear vacuole. Nucleus was vesicular with a large basophilic central nucleolus. Cellular atypia was marked, with pluriunucleation, loss of cellular polarity and loss of cellular cohesion. Mitotic rate was high reaching 3 mitosis per high power field (400 × magnification). Numerous lymphatic and venous vascular neoplastic emboli were noticed. Sections of both retropharyngeal and right cervical lymph nodes, and of pulmonary, hepatic and splenic nodules were also examined. All these organs, including lymph nodes, contained randomly distributed nodules. Similarly to the primary tumor, these nodules formed tubules and trabeculae. A collagenous stroma reaction and necrotic foci of variable size were associated with these neoformations. Cytologic features were similar to the...
lesion in the salivary gland. In addition to the rare nodules described above, the most prominent lesion of the spleen was a marked and diffuse infiltration of the red pulp by neoplastic round cells. These highly proliferating neoplastic round cells disrupted the white pulp which harbored only few remaining lymphoid areas (Fig. 3). These round cells measured 20 μm in diameter and were characterized by a vesicular nucleus with a large basophilic nucleolus and a moderately abundant, acidophilic, cytoplasm. Very rarely, cytoplasm contained a small clear vacuole. Numerous bi- or pluri-nucleated cells were present. PAS reaction performed on the salivary gland neoplasm and on the splenic round cell tumor revealed rare mucous intracytoplasmic vacuoles, which were stained in pink (Fig. 4). All neoplastic cells in the mandibular salivary gland were cytokeratin-positive, showing a strong cytoplasmic staining. Less than 50% of neoplastic cells were vimentin-positive in the center of the tumor, displaying also a strong cytoplasmic staining, and more than 90% were vimentin-positive in the periphery of the neoplasm, particularly at the front of invasion (Figs. 5, 6). Interestingly, neoplastic round cells in the spleen were all strongly positive for vimentin and mostly cytokeratin-positive (> 90%) (Figs. 7, 8). These neoplastic round cells were negative for CD3 (lymphocytes T marker), CD79a or BLA 36 (lymphocytes B markers), DC-LAMP (histiocytic marker) and MAC 387 (macrophagic marker) immunostaining and did not show cytoplasmic granules with Schnorl staining (for melanocytes) or Toluidine Blue staining (for mast cells). Moreover, in the salivary gland and the spleen, neoplastic cells were not labeled with SMA. Based on these findings, we concluded to a salivary gland adenocarcinoma with widespread metastases including a diffuse involvement of the splenic parenchyma.

Adenocarcinoma is the most common salivary gland tumor in cats, occurring preferentially in the mandibular gland (59%) and in the parotid gland (19%) [4, 5]. Its incidence is 8.4/100,000 [5]. According to the current classification, malignant salivary gland tumors of epithelial lineage that do not present specific features of differentiation are referred as adenocarcinoma [5]. The lesion reported here did not fit into other microscopic categories of carcinoma. Although we could detect rare mucous producing cells, there was neither foci of small intermediate cells and squamous cells, features of mucoepidermoid carcinoma, nor foci of acinic cell types, features of acinic cell carcinoma [3, 5]. This tumor was unusual in that most epithelial neoplastic cells strongly expressed vimentin. Pattern of vimentin expression was not restricted to trabecular peripheral cells, as seen in mucoepidermoid carcinoma [3]. Differential diagnosis could also include myoepithelial tumors, which express vimentin. However, presence of well formed tubules with a clearly delineated lumen [11] and lack of SMA immunostaining of neoplastic cells ruled out a myoepithelial lineage. In conclusion, although vimentin expression in this salivary gland tumor could be somehow misleading, morphological and immunohistochemical features of this tumor confirmed the diagnosis of adenocarcinoma. Of note, vimentin expression in feline malignant epithelial tumors has been previously reported in feline mammary carcinoma [2, 10].

The microscopic differential diagnosis for a round cell tumor in the spleen in cats includes lymphoid cell neoplasm, histiocytic and dendritic cell neoplasm, mast cell tumor or metastatic tumors tumor such as amelanotic melanoma or carcinoma. As neoplastic round cells were positive for cytokeratin and vimentin and negative for the other markers described above, we conclude to a metastatic adenocarcinoma and excluded other neoplasms.

Over half of feline cases of salivary gland tumors have distant metastases at the time of diagnosis [4]. Involvement of lymph nodes is common. Broad metastases have also been reported in lung, liver, pancreas, heart, adrenal gland, diaphragm, body wall and bone [5]. In the present case, the splenic diffuse carcinomatous metastasis was unexpected, as well as its histomorphological and immunohistochemical features. Indeed, these splenic metastatic carcinomatous cells express simultaneously epithelial and mesenchymal markers. The simultaneous expression of epithelial and mesenchymal markers was also detected in the primary tumor and was most prominent at the front of invasion. This phenotypic change is reminiscent of the process of “Epithelial-Mesenchymal Transition” (EMT), of increasing interest in the comprehension of carcinogenesis in human medicine. Indeed, EMT includes loss of cell polarity and cohesion, increased motility and acquisition of a mesenchymal phenotype (expressed through downregulation of epithelial markers as cytokeratins and upregulation of mesenchymal markers as vimentin) and is thought to precede steps of invasion and metastasis [6]. Various stages of EMT can occur and vimentin positive neoplastic cells retaining expression of cytokeratins have been described [1, 6]. Our case could represent such an intermediate stage of EMT. Alternatively, vimentin/cytokeratin positive cells could also arise from myoepithelial histogenesis [9]. This possibility has been excluded in the present case, based on microscopic findings and immunostaining. Finally, one study suggested that vimentin/cytokeratin positive cells could be derived from progenitor cells with a bilinear (glandular and myoepithelial) differentiation potential [7]. Yet, this hypothesis requires further experimental support.

The acquisition of vimentin staining by neoplastic cells of epithelial origin is correlated with a higher metastases rate and lower survival time in cervical and breast carcinomas in humans [6]. Although no formal evidence exists, vimentin expression in feline mammary carcinoma has been proposed as indicative of malignancy [10]. In cats, the histopathological features of salivary gland tumors are considered as not helpful to provide a prognosis without any other complementary exam [4]. Whether the acquisition of a vimentin staining by neoplastic cells of epithelial origin is correlated with a higher metastases rate and a lower survival time in cats requires further investigation.
Fig. 1. Cat, right mandibular salivary gland. Salivary parenchyma is completely replaced by a nodular tumor, that is partially encapsulated, lobulated, densely cellular and infiltrated surrounding connective tissue. HES stain. Bar=250 μm.

Fig. 2. Cat, right mandibular salivary gland. Tubular to trabecular infiltrative adenocarcinoma. Epithelial cells have a low columnar to polygonal morphology and show marked atypia. HES stain. Bar=40 μm.

Fig. 3. Cat, spleen. Round cell tumor infiltrating the red pulp and disrupting the white pulp. Few neoplastic round cells contain small clear intracytoplasmic vacuoles. Bi- or multinucleated cells are numerous. Mitotic figures are frequent. HES stain. Bar = 40 μm.

Fig. 4. Cat, spleen. Few neoplastic round cells contain small intracytoplasmic vacuoles with mucinous material positive for PAS reaction (arrowheads). Bar=20 μm.

Fig. 5. Cat, periphery of the salivary gland neoplasm. Neoplastic cells are all strongly cytokeratin positive. Immunoperoxidase staining, hematoxylin counterstain. Bar=50 μm.

Fig. 6. Cat, periphery of the salivary gland neoplasm. Most of neoplastic cells are strongly vimentin positive. Immunoperoxidase staining, hematoxylin counterstain. Bar=50 μm.

Fig. 7. Cat, spleen. Most of neoplastic cells are strongly cytokeratin positive. Immunoperoxidase staining, hematoxylin counterstain. Bar=50 μm.

Fig. 8. Cat, spleen. Neoplastic cells are all strongly vimentin positive. Immunoperoxidase staining, hematoxylin counterstain. Bar=50 μm.
REFERENCES


