A CASE OF MALIGNANT TRICHOEPITHELIOMA (MATRICAL CARCINOMA) IN A CAT: PATHOMORPHOLOGICAL AND IMMUNOHISTOCHEMICAL FINDINGS

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Summary


Malignant trichoepithelioma (MTE) or matrical carcinoma is a neoplastic change of matrical and inner root sheet cells. It is generally described in dogs, with no sex predisposition. In this case, a glassy skin mass was taken from a 8-year-old, female mixed breed cat. Histopathologically, it was presented with the typical matrical cells islands, central keratinisation and necrosis, hyalinated collagens, ghost cells and trichohyaline granules. Masson’s trichrome stain differentiated epithelial cells from connective tissue in the subcutis. Immunohistochemistry (ABC immunoperoxidase method) revealed the malignancy of matrical cells. The Ki67 marker was especially helpful to show the malignancy potential of cells. The Her2neu marker, one of epidermal growth factors, reacted to invasive cells located at the periphery of islands. Connexin 43 was useful to show loss of connection in malignant cells. Due to the less frequent occurrence and unusual localisation of MTE, the described case aimed at revealing microscopic and immunohistochemical findings by using potential markers of MTE tumours in the skin of cats.

Key words: cat, immunohistochemistry, malignant trichoepithelioma, pathomorphology

Malignant trichoepithelioma (MTE), also known as matrical carcinoma, originates from hair follicle matrical or inner root sheet (Goldschmidt & Hendrick, 2002). The tumour is a not frequently seen skin tumour and uncommon in cats. MTE is not identified in other species except in a mouse. It develops most commonly in dogs between 1 and 15 years of age in spite of being observed between 4 and 11 years of age in cats (Goldschmidt & Hendrick, 2002; Mulas et al., 2007). The occurrence of the tumour is documented in dogs although its clinical features are not mentioned, but it was suspected on the basis of ulcerative and large plaque-like appearance with rough edges (Gross et al., 2005). The tumour grows rapidly in the
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dermis and subcutaneous tissue and can metastasise to regional lymph nodes and lungs. It generally shows recurrence after operation. The skin appears “glassy” and ulceration is common. Its predilection sites are the dorsal trunk and the neck, thoracic region and tail (Goldschmidt & Hendrick, 2002). Microscopically, invasive anaplastic matrical cells or follicular infundibulum epithelial cells constitute trabecules, cord and nests of different sizes. Some cells can include eosinophilic intracytoplasmic trichohyaline granules. In some areas, distinct matrical keratinisation formation and necrotic cells which are composed of ghost or shadow cells in larger islands can be found (Goldschmidt & Hendrick, 2002).

In terms of immunohistochemistry, nuclear positivities are encountered using Ki67, PCNA, p21 and p27 in epithelial cells of malignant hair follicles of dogs (Inoue et al., 2006; Souza et al., 2008). β-catenin and pancytokeratin expressions in nuclei and cytoplasms of hair follicle epithelial cells are reported as benign counterpart in a cat (Tavasoli et al., 2013). Another reports positive reactions obtained by using different types of cytokeratins in epithelial cells of hair follicles in a mouse. In the same study, some positive reactions are also obtained by using profilaggrin and involucrin on the inner surface of squamous epithelium of hair follicles (Martin de las Mulas et al., 2008).

In this case, a circumscripct large tumour located at the subcutis of left gluteal region was described as malignant trichoepithelioma. The presentation was deemed valuable when compared to benign counterparts because of the rare occurrence in cats and the unusual localisation. There are no reports about connexin-43, Her2-neu and CEA expressions in malignant trichoepithelioma except for Ki67 expression. Ki67 is a powerful marker of malignant activity in the nuclei of all types of anaplastic cells (Hazan et al., 2002; Iqbal et al., 2002). It was believed that the immunohistochemical features of the case will throw a different light on skin tumors.

A 8-year-old female mixed breed cat was submitted to the Department of Surgery, Ankara University, Faculty of Veterinary Medicine with complaints of excessive mass under the skin of the left gluteal region. After clinical examination, the mass was removed surgically and sent to Department of Pathology for diagnosis. After macroscopical examination, tissue samples were taken from lesions for histopathology and fixed in 10% formalin. Then, they were processed routinely and embedded in paraffin. Sections were cut at 5 μm thickness from paraffin blocks and stained with haematoxylin-eosin (H&E) and Masson’s trichrome methods. After histochemical stainings, indirect immunoperoxidase method (ABC-P) was applied using connexin 43 (Cx43), carcinoembryogenic antigen (CEA), Her2 neu, and Ki67 markers. To this end, after deparaffinization and dehydration, the peroxidase activity was blocked. Trypsinisation was performed by using 0.1% trypsin solution. Non-specific proteins were blocked with protein blocking sera ( Peroxidase Detection System, Novocastra, RE7110-K, Leica Biosystems). The sections were incubated with primary antibodies (polyclonal rabbit anti connexin 43, monoclonal mouse CEA, monoclonal mouse Her2neu, monoclonal rabbit Ki67– Table 1). Then, biotinylated link antibody and horse radish peroxidase (HRP) antibodies were applied, respectively ( Peroxidase Detection System, RE7110-K, Novocastra, Leica Biosystems). Control sections were treated with PBS instead of primary antibodies. As chromogen, 3-ami-
no-9-ethylcarbazole (AEC) (Santa Cruz Biotechnology Inc.) was selected. Counterstaining were performed with Gill’s haematoxylin. Sections were mounted with glycergel and examined on light microscope (Leica Microsystems, DM4000B).

The mass covered with skin weighed 20 g and its size was 5×4×1 cm. Its cut surface was homogeneous and of white colour (Fig. 1). Histopathologically, anaplastic hair follicle epithelium, having large and hypochromatic nuclei was observed. Prominent nucleoli, some of them with hyperchromatic nuclei, constituted islands such as cords and trabecules. There were keratohyalin formation and necrosis composed of cellular debris at the centre of large islands (Fig. 2 and 3). In addition, ghost or shadow cells were observed towards the centre of necrotic debris (Fig. 4). Eosinophilic trichohyaline granules were also visible in the cytoplasm of anaplastic cells (Fig. 5).

After Masson’s trichrome staining, malignant epithelial cell islands were differentiated from the stroma and the dermal connective tissue (Fig. 6). Immunohistochemically, the Ki67 and Her2neu positive reactions exhibited granular or diffuse patterns of brownish-red color localised in

**Table 1. Panel of used antibodies**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Features</th>
<th>Clone</th>
<th>Trade</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki67</td>
<td>Monoclonal rabbit</td>
<td>Clone SP6</td>
<td>Labvision</td>
<td>1:50</td>
</tr>
<tr>
<td>Her2neu</td>
<td>Monoclonal mouse</td>
<td>c-ErbB-2 oncoprotein</td>
<td>Novocastra</td>
<td>1:40</td>
</tr>
<tr>
<td>CEA</td>
<td>Monoclonal mouse</td>
<td>COL-1</td>
<td>Neomarkers</td>
<td>1:100</td>
</tr>
<tr>
<td>Cx43</td>
<td>Polyclonal rabbit</td>
<td>GJA-1</td>
<td>Abcam</td>
<td>1:100</td>
</tr>
</tbody>
</table>

**Fig. 1.** Macroscopic appearance of the mass.

**Fig. 2.** MTE cells (arrow) and central necrosis (asterix). Bar=55 µm, H&E.

**Fig. 3.** MTE cells (arrows). Bar=40 µm, H&E.
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cytoplasms of epithelial cells. However, CEA positive reactions were observed in Fig. 6. MTE islands differentiating connective tissue (arrows). Bar=40 µm, Masson’s trichrome stain.

cytoplasms and nuclei. Localisation of Cx43 positivities in cytoplasms and membranes of anaplastic epithelial cells was observed. Positive reactions were obtained by Ki67 in all anaplastic epithelial cells and by Her2neu – in invasive highly malignant cells (Fig. 7 and 8). However, all anaplastic matrical cells and hair follicle epithelia reacted slightly with Cx43 and CEA with (Fig. 9 and 10).

Malignant trichoepitheliomas (MTE) are rarely seen skin tumours in cats (Goldschmidt & Hendrick, 2002). In dogs, most affected are animals of late middle age. Although there is no defined sex predilection, a breed predisposition exists in Basset Hounds, Bull Mastiffs, Irish Setters, Poodles, English Spaniels, and Golden Retrievers. Tumours can develop on the trunk in dogs, but are encountered on the head, tail, and extremities in cats. The tumour originates from the hair follicle (infundibulum, isthmus, and inferior portions) (Weiss & Frese, 1974; Villalobos & Finlay, 2011). The mass in the subcutis in our 8-year-old mixed breed cat was localised in left gluteal region. For cats, this localisation is different from others reported in the literature. Metastasis is not common, possible sites are the regional lymph nodes and the lungs. Surgical excision is considered as the treatment of choice (Goldschmidt & Hendrick, 2002; Villalobos & Finlay, 2011). In the present case, the health condition of the patient was followed but we were not informed about any recurrence after the surgical excision. On the other hand, MTE generally develop exophytically and grow as intradermal masses. The masses appear as multiple grey-white foci of keratinous matrix. For many of them, the skin is generally ulcerated (Goldschmidt & Hend-
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rick, 2002; Villalobos & Finlay, 2011). In this case, the general appearance agreed to features reported in literature, but it had no ulcerative skin lesions.

The microscopical MTE appearance can be diverse. It is occasionally multilobulated and non-circumscribed. Some of tumours may be in infiltrative forms by trichogenic differentiation and highly proliferative epithelial cells. At the centre, there is a trichogenesis characterised by matrical keratin deposition. Intracytoplasmic eosinophilic trichohyalin granules are found in peripheral epithelial cells (Goldschmidt & Mcmanus, 2000; Goldschmidt & Hendrick, 2002; Gross et al., 2005). The centre of matrical keratinisation may show calcification (Goldschmidt & Hendrick, 2002; Mulas et al., 2007). Apart from these, melanin pigmentation, foreign body

**Fig. 7.** Ki67 positivity in cytoplasms of MTE cells (arrows). Bar=40 µm, ABC-P.

**Fig. 8.** Her2neu positivity in cytoplasms of invasive MTE cells (arrows). Bar=40 µm, ABC-P.
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reaction or purulent inflammation due to secondary bacterial complication can be found (Weiss & Frese, 1974).

Malignant trichoepithelial cells are generally found as islands, chords or trabecules composed of basaloid cells with differentiation to internal and external root sheath. At the periphery, there are malignant cells with pleomorphic hyperchromatic nuclei and scanty eosinophilic cytoplasm and thickened basal lamina (Goldschmidt & Mcmanus, 2000; Goldschmidt & Hendrick, 2002; Villalobos & Finlay, 2011). These cells have usually moderate or high mitotic activity (Weiss & Fresse, 1974; Gross et al., 2005). On the other hand, a matrical differentiation is developed with participation of ghost or shadow cells at the centre of epithelial islands. Also they can include fibrotic or

Fig. 9. Slightly positive Cx43 cells (arrows). Bar=35 µm, ABC-P.

Fig. 10. Slightly positive CEA cells (arrows). Bar=50 µm, ABC-P.
mucinous stroma. In higher malignant forms, central cystic degeneration and large necrotic areas are found (Goldschmidt & Mcmanus, 2000; Villalobos & Finlay, 2011). In our case, similar results were obtained, but there were no melanin pigmentation, foreign body reaction, secondary bacterial complication and cystic degeneration.

There is little information about immunohistological features of trichoepithelioma. They were investigated in only few studies on hair follicle tumours with p21, p27, PCNA, Ki67 and cytokeratin antibodies (Inoue et al., 2006, Mulas et al., 2007, Souza et al., 2008). Mild or moderate reactions are obtained with markers. However, in this case, the carcinoembryogenic antigen (CEA), Her2neu (cerbB oncoprotein) and connexin 43 (Cx43) markers apart from Ki67 marker were used. In this context, useful results from Her2neu and Ki67 were obtained. Her2neu is one of epithelial growth factor receptors that is generally utilised for showing invasive cells especially in the breast, endometrium, gastric cancer prognosis (Tan & Yu, 2007; Santin et al., 2008). Up to now, it is demonstrated in cutaneous melanoma in terms of its expression in human skin (El-Sheikh et al., 2009). It is not used for detecting invasive cells and prognosis evaluation in skin tumours. In this case report, the Her2neu and Ki67 markers were preferred for showing malignancy and were found useful. The Cx43 marker is gap junction or transmembrane protein and normally plays a critical role in cellular adhesion and coordination (Cameron et al., 2003). It is reported that Cx43 has a tumour supressive role (Langlois et al., 2010). In the present case, there was a high malignancy due to loss of cell to cell adhesion between malignant cells. Hence, slight Cx43 positivity is interpreted as a result of abnormal proliferation. CEA is also a glycoprotein providing cellular adhesion. It is used to show malignancy especially in gastric, colonic, breast and lung adenocarcinoma (Thomas et al., 2008). The marker is a cell surface glycoprotein and is utilised to detect high anaplastic features of malignant skin tumours cells in of different origin (Heyderman et al., 1984; Thomas et al., 2008). In this patient, the marker was applied to MTE cells to show high anaplasia, however, less positive reactions were obtained when compared to other marker positivities. The slight positive reactions suggested that CEA is not as effective as Cx43 to indicate loss of cellular adhesion. Therefore, the Her2neu and Cx43 markers should be selected by pathologists to show malignancy of skin tumours apart Ki67 and other markers documented in the literature.

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