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Carcinoma with medullary signs in mammary glands in cats: morphohistological study and immunohistochemical analysis

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Abstract

Breast carcinoma in cats is a serious oncological problem, especially considering that such tumors can cause metastasis to the lymph nodes and other organs. This article considers five cases of carcinoma with medullary signs in cats, of which two have metastases in regional lymph nodes. The morphohistological characteristics of tumors and the results of an immunohistochemical study using NCL and NPM1 markers are analyzed in detail.

Keywords: cats, mammary gland, carcinoma with medullary signs, morphohistological examination, immunohistochemical analysis, Nucleolin, NPM1, Nucleophosmin.

Introduction

Mammary gland tumors in cats account for about 15-20% of all registered tumors, which also occupy a significant place among all tumor diseases. This percentage may vary depending on the region, breed, and age of the animals studied (12).

Among the histotypes of mammary gland tumors in cats, the most common type of tumor is adenocarcinoma, which accounts for about 50-80% of all cases. But in clinical practice, there are cases of a rare type that accounts for about 15% of all adenocarcinomas – this is a carcinoma with medullary features (7).

Medullary breast carcinoma in cats is a tumor characterized by a specific morphological structure, with large, prominent tumor cells often exhibiting a high mitotic rate (6, 14). These cells are repeatedly enriched in lymphoid stroma, resulting in a typical medullary structure. Medullary carcinoma is of particular importance because of its aggressiveness and prognostically unfavorable course of the disease (8). Studies show that these tumors have a high degree of malignancy and can metastasize in the early stages. Another characteristic morphological principle is the presence of extensive necrotic zones (1, 3).

The use of biomarkers for immunohistochemical examination of breast tumors in veterinary medicine is a new, progressive diagnostic method that allows pathologists not only to clarify the diagnosis but also to assess tumor aggressiveness, which, in turn, helps develop individualized treatment approaches and improve treatment outcomes. To date, data on the use of NCL (Nucleolin) and NPM1 (Nucleophosmin) markers in the diagnosis of breast tumors in cats are quite limited. Studies focused on the mammary gland in cats often place great emphasis on traditional diagnostic methods and other markers of hormone receptors. Nevertheless, markers in veterinary oncology, and further research may reveal their potential for use in the diagnosis and prognosis of breast tumors in animals (13).

NCL and NPM1 markers are important molecules that are actively involved in various cellular processes. NCL is a multifunctional nuclear protein that plays a key role in gene regulation, ribonucleic acid (RNA) metabolism, and nuclear organization (5). This protein can promote tumor aggression and metastasis, as well as cross-tactical activation of pathways associated with cell survival. In the context of oncology, elevated NCL levels have been associated with tumor progression and poor prognosis in breast cancer patients (17, 20).

NPM1 also performs many functions in the cell, including involvement in nuclear transport, transcription regulation, and ribosomal biogenesis (10). It plays a role in DNA repair and in the behavior of cells under stress. The marker can also act as an oncogene, activating various signaling pathways and promoting tumor cell growth and survival. An increase in NPM1 concentration in tumor cells may be associated with tumor progression and poor clinical outcomes (11).

The purpose of this work is to present the morphohistological and immunohistochemical picture of breast carcinoma with medullary features in cats.

Material and Methods

Breast tumor samples surgically removed from cats by private veterinary clinics were sent to the Laboratory of Morphology and Pathology of the Faculty of Veterinary Medicine – StSAU (Stavropol, Russia) for histopathological diagnosis. To be sent to the aforementioned institution, a form of confidentiality and informed consent was required to conduct research on the submitted material. The samples were not collected specifically for this study; they were submitted by veterinary clinicians between September 2023 and December 2025, after surgery (n=5), as a therapeutic intervention. Thus, the study did not require additional ethical approval.

The following breeds participated in the study: Abyssinian cat (1/5), Scottish Fold (1/5), Siamese (1/5), and cross-breed (2/5). There were 5 females, and the average age of the cats was 9.6 years (range 8-12 years). The samples were fixed in 10% buffered formalin solution, processed, cut

at 3 μm, and stained with hematoxylin-eosin (H&E). Histopathological examination was performed under light microscopy by a veterinary pathologist. The expression of NCL and NPM1 markers in selected samples was also evaluated according to the protocols. For this study, tissue sections were de-waxed with xylene and hydrated using a series of decreasing ethanol concentrations (100°, 95°, 70°). Then, pretreatment was performed using 3% peroxide blocking, and thermal extraction of the antigen was performed in a double boiler (Centek, China), placing histological glass slides in 0.01 M citrate buffer at pH 6.0 for 30 minutes in a special glass slide staining unit (Biovitrum, Russia). The primary antibodies were applied to the samples and left in a humid chamber at 4°C overnight. In the morning, the HiDef DetectionTM Amplifier (Mouse and Rabbit) detection system (CELL MARQUE, the Netherlands) and peroxide streptavidin were added. Visualization of expert data was performed using the DAB Substrate Kit (CELL MARQUE, the Netherlands) for 3-5 minutes, with constant monitoring under a microscope. The samples were then stained with Mayer's hematoxylin. The positive control was performed according to the manufacturer's instructions; for the negative control, the primary antibody was replaced with PBS in samples of similar tissues, which were run in parallel with samples containing the primary antibody.

When assessing immunostaining, the preparations were analyzed using a four-point semi-quantitative scale to assess the intensity of staining of the nuclei and cytoplasm (3+ (strong), 2+ (moderate), 1+ (weak), 0 (no staining)) and to estimate the percentage of positively stained cells (0 — no staining, 1 — less than 10% of cells, 2 — 11-50% of cells, 3 — more than 50% of cells). The pathologist determined representative areas containing an average of 150 cells (range 72-180), and the percentage of positive cells was estimated from an average of 10 fields at x400. The cells were sampled randomly, excluding stromal cells and artifacts. The staining intensity and the percentage of reactivity were recorded as the average values obtained from ten fields at high magnification.

Results

Macroscopically, nodular neoplastic growth was observed in the samples. The tumors were well-defined and soft in consistency with a uniform gray and moist incision surface, nevetheless, bleeding and necrosis were present in some cases.

Microscopically, the tumor samples showed solid growth (more than 75% of the tumor), with characteristic areas of necrosis, a clearly delineated tumor edge, and diffuse infiltration of the lymphoplasmocytic stroma. The cellular landscape of tumors was characterized by hard sheets of large, pleomorphic cells with indistinct borders, exhibiting a syncytial growth pattern and pronounced mitotic activity. In some areas, tumor cells with abundant cytoplasm and pleomorphic, vesicular nuclei containing one or more nucleoli, as well as a large number of mitoses and giant, multinucleated cells, were found. A characteristic feature of this tumor was dense lymphocytic infiltration of the tumor stroma (Fig.1).

In 2/5 of the cats, metastases were found in the lymph nodes. Macroscopically, the lymph nodes were enlarged, dense, and inelastic. There was an uneven surface on one lymph node, with visible hyperemia in some areas. An incision of the lymph nodes revealed a dense white substance with no clear boundaries between the cortical and cerebral substances.

Microscopically, the metastatic foci were more than 2.0 mm in diameter and replaced most of the lymph node architecture (Fig. 2). The tumor cells had a rounded or oval shape, varying degrees of atypia, pronounced cellular polymorphism, and large, hyperchromic nuclei with irregular contours. Upon visualization, the cytoplasm was extensive, and vacuole degradation was observed in some cells. The formation of so-called "pseudo-layered" structures was also observed, where cells are arranged in several layers with neoplastic changes.

The structure of the cortical and paracortical zones of the lymphoid tissue has been changed to a tumor-like one. Areas of destruction and necrosis were found, suggesting prior infiltration of blood vessels. Lymphocytic and plasmocytic infiltration is observed in the area of tumor metastases, which indicates the body's reaction to the tumor.

Microscopically, the metastatic cells exhibited the same morphological features as the primary tumors, confirming their histogenetic relationship. These signs suggest a progressive metastatic process with the presence of necrotic changes in the lymph node, which confirms the aggressiveness of medullary breast cancer in the cats under study.

During immunohistochemical examination of tumor cells, nuclear NCL expression was observed and scored on an intensity scale. According to our observations, high expression of the NCL marker was observed in 2/5 of the samples, 2/5 showed a moderate reaction, and 1/5 showed a weak reaction. An estimate of the percentage of positively stained cells is shown in Table No. 1.

NCL expression was mainly nuclear, in the form of brown granules that evenly filled the entire compartment, sometimes without clear visualization of the nucleoli, with alternating lower cytoplasmic staining intensity. Also, in some areas, positive results were observed in the nucleoli, with NCL expression moderate. NCL expression was particularly pronounced in tumor cell nuclei, indicating its active involvement in cell survival and proliferation.

An immunohistochemical study revealed that NPM1 expression was observed in the nuclear region of tumor cells. According to our data, high expression of the NPM1 marker was observed in 2/5 samples, 2/5 had a moderate reaction, and 1/5 had a weak reaction. An estimate of the percentage of positively stained cells is shown in Table No. 1.

When imaging the immunoreactive material, it was found that positive NPM1 staining was predominantly nuclear, often with pronounced nucleolar localization, and that the nucleoplasm showed strong and moderate dark brown staining. Anisonucleosis was pronounced, which demonstrated a condition from micronuclei to the irregular, rounded, or elongated shape of giant nucleoli. No expression of this protein was observed in the cytoplasm.

NPM1 expression was also high, with staining localized to the nucleoli of tumor cells. This protein is involved in translation and cell cycle regulation, which may indicate high tumor cellular activity.

According to the study's results, both biomarkers, NCL and NPM1, exhibit variable expression in breast tumor cells in cats with medullary cancer. In general, NCL showed higher nuclear expression in tumor cells, while NPM1 was also nuclear and showed more pronounced staining in the nucleoli. These data may be useful for further understanding the pathogenetic mechanisms of medullary cancer in cats and for evaluating the potential of these biomarkers as prognostic factors.

Discussion

Medullary breast carcinoma is a rare histological type that accounts for less than 5% of all invasive human breast cancers (9). Nevertheless, this tumor histotype may exhibit distinctive histological and genomic features associated with aggressiveness, but as a rule, this type of cancer shows a more favorable prognosis compared to other subtypes and especially other forms of breast tumors. Numerous studies have shown a positive correlation between the presence of extensive lymphocytic infiltrate inside the tumor and its surrounding area and patient survival (2, 4).

Nevertheless, breast tumors in cats, especially if it is a carcinoma with medullary signs and low differentiation, often have an aggressive course. Metastasis to lymph nodes also indicates a more unfavorable prognosis, as this indicates the spread of cancer cells beyond the primary tumor (15).

Immunohistochemical studies using NCL and NPM1 markers can provide valuable insights into tumor biology. The biomarker NCL (Nucleolin) is a nuclear protein that plays an important role in cell proliferation and metabolism. Its expression is often elevated in tumor cells and can serve as a marker of neoplasia. In the context of mammary gland tumors in cats, high NCL levels may indicate increased tumor proliferative activity, which correlates with aggressiveness and a poor prognosis (18, 19).

The NPM1 marker (Nucleophosmin 1) is another nuclear protein involved in cell division and the regulation of apoptosis. NPM1 expression in tumor cells can serve as a marker of poor prognosis,

as it is associated with genome instability, an aggressive tumor course, and a predisposition to metastasis (16, 21).

Thus, the results of this study show that medullary carcinoma in cats can be aggressive, especially in cases with metastases. High expression of NCL is associated with tumor cell growth and survival, whereas NPM1 may play a role in angiogenesis and metastasis dissemination, making these markers potentially useful for prognostic assessment and the selection of treatment strategies. Immunohistochemical analysis, which showed increased expression of NCL and NPM1, can serve as an important diagnostic and prognostic tool in the assessment of these tumors, underscoring the need for further research in this area.

Conflict of Interest

The authors declare no competing interests.

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Tables

Table 1. Expression rates of NCL and NPM1 biomarkers in immunohistochemical (IHC) diagnostics in each case.

Breed	Intensity of staining NCL	Percentage of positively stained NCL cells	Intensity of staining NPM1	Percentage of positively stained NPM1 cells
	3+ (strong)	3 (more than 50%)	3+ (strong)	3 (more than 50%)
	2+ (moderate)	2 (11–50%)	3+ (strong)	3 (more than 50%)
	3+ (strong)	3 (more than 50%)	2+ (moderate)	2 (11–50%)
	1+ (weak)	1 (less than 10%)	1+ (weak)	1 (less than 10%)
	2+ (moderate)	2 (11–50%)	2+ (moderate)	2 (11–50%)

[a] NCL - Nucleolin, NPM1 - Nucleophosmin

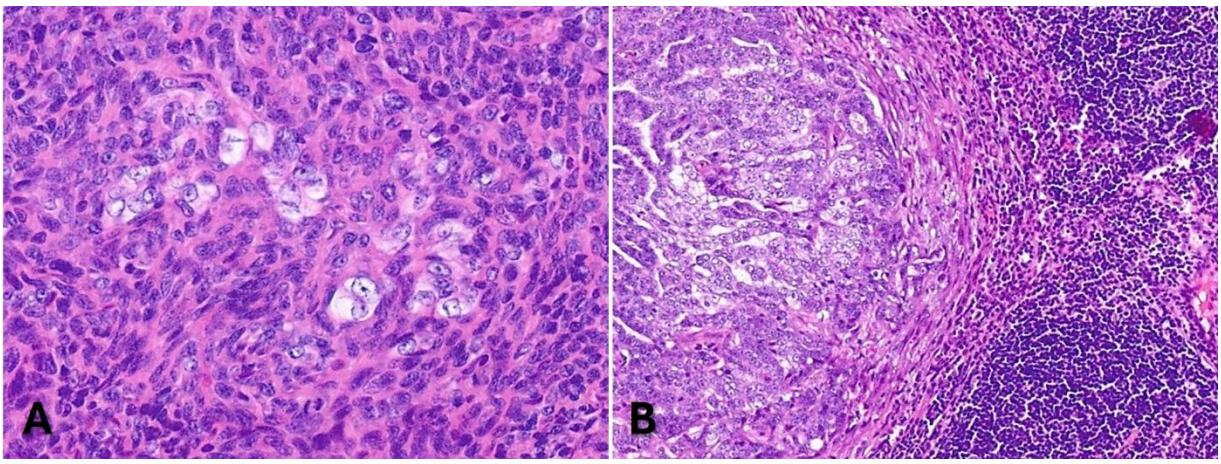

Figure 1. Micrographs of histopathological lesions of the mammary glands in cats. A. Medullary carcinoma (G3). In the photo, 60x: Syncytial growth of tumor cells, rounded cells with vesicular nuclei. B. Inguinal lymph node. In the photo, 40x: a macrometastasis of medullary carcinoma is detected.

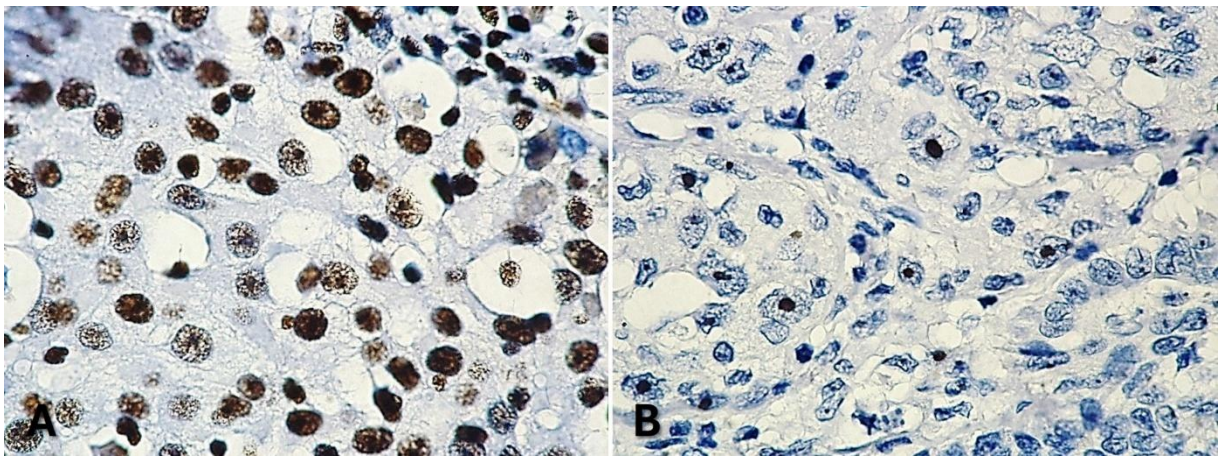

Figure 2. Micrographs of histopathological lesions of the mammary glands in cats. A. Medullary carcinoma (G3). In the photo 60x: Positive immunoexpression of antibodies to nucleolin. B. Medullary cancer (G3). In the photo 60x: Positive immunoexpression of antibodies to nucleophosmin