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Original article

Molecular based subtyping of feline mammary carcinomas and clinicopathological characterization

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ABSTRACT

Molecular classification of feline mammary carcinomas (FMC) from which specific behavioral patterns may be estimated has potential applications in veterinary clinical practice and in comparative oncology. In this perspective, the main goal of this study was to characterize both the clinical and the pathological features of the different molecular phenotypes found in a population of FMC (n = 102), using the broadly accepted IHC-based classification established by St. Gallen International Expert Consensus panel.

The luminal B/HER2-negative subtype was the most common (29.4%, 30/102) followed by luminal B/HER2-positive subtype (19.6%, 20/102), triple negative basal-like (16.7%, 17/102), luminal A (14.7%, 15/102), triple negative normal-like (12.7%, 13/102) and finally, HER2-positive subtype (6.9%, 7/102). Luminal A subtype was significantly associated with smaller tumors ($p = 0.024$) and with well differentiated ones ($p < 0.001$), contrasting with the triple negative basal-like subtype, that was associated with larger and poorly differentiated tumors ($p < 0.001$), and with the presence of necrotic areas in the tumoral lesion ($p = 0.003$). In the survival analysis, cats with Luminal A subtype presented the highest survival time (mean OS = 943.6 days) and animals with triple negative basal-like subtype exhibited the lowest survival time (OS mean = 368.9 days). Moreover, two thirds (64%, 32/50) of the queens with multiple primary tumors showed different molecular subtypes in each carcinoma, revealing that all independent lesions should be analyzed in order to improve the clinical management of animals.

Finally, the similarities between the subtypes of feline mammary tumors and human breast cancer, reveal that feline can be a valuable model for comparative studies.

Introduction

Feline mammary carcinomas have a deserved reputation of highly malignant behavior with most tumor types being considered similarly as bad news as far as prognosis is considered [1]. However, the considerable variety of histological types and clinical outcomes up rise the concern that there may be some variations that need to be exploited as therapeutic approach may vary from surgical excision and nothing else to combinations of this with chemotherapy. The gain in discriminating between various types of prognosis may be not just beneficial for the female cats and their owners but also for the characterization of an animal population, readily available, that has not been conveniently exploited in translational studies in cancerology. It has long been proven that feline mammary tumors have much more similarities to human mammary tumors, than rodent tumor models [1–3], with some studies suggesting a viral etiology to mammary tumors in both species [4–8] and some emphasizing the zoonotic potential of feline mammary tumor virus [4,9] (Retroviridae).

It is generally accepted that early detection and more effective treatments are key factors that explain longer survival times in human cases of mammary carcinoma [10]. More effective treatments have benefited from advances in tumor classification evolving from systems based upon molecular and immunophenotypic markers. The first molecular classification for breast cancer was proposed by Perou and colleagues (2000), which divided

Abbreviations: FMC, feline mammary carcinomas; IHC, immunohistochemistry; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor-2; CK, cytokeratins; OR, odds ratio; OS, overall survival; DFS, disease free survival; Luminal B/HER2−, Luminal B/HER2-negative; Luminal B/HER2+, Luminal B/HER2-positive; TN, triple negative.

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breast tumors in four groups (luminal-like, basal-like, HER2+ and normal-like), according to their gene expression analysis [11]. The prohibitive cost of this multigenic assay, led to the development of alternatives, such as the evaluation of biomarkers using immunohistochemistry (IHC) analysis, which was suggested as a surrogate for gene expression profiling [12,13]. Recently, the St. Gallen International Expert Consensus panel proposed an IHC-based classification that establishes six biologically distinct breast cancer subtypes: luminal A, luminal B/HER2-negative, luminal B/HER2-positive, HER2-positive (non-luminal), triple negative basal-like and triple negative normal-like [14].

The luminal A is the most common subtype and the one with better prognosis. It is characterized by the overexpression of estrogen and/or progesterone receptor (ER and/or PR), HER2-negative status and low Ki-67 index [14,15]. Luminal B breast tumors display a more aggressive behavior than luminal A and are divided in two subtypes: the luminal B/HER2-negative which show positive staining for ER and/or PR, negative expression for HER2 and high Ki-67 index levels, and the luminal B/HER2-positive subtype that shows ER and/or PR and HER2 overexpression [14,16]. Patients with luminal tumors usually benefit from endocrine therapies. Within the non-luminal subtypes, the HER2-positive subtype is characterized by the HER2 overexpression in the absence of hormone receptors (ER and PR). This subtype is associated to a poor prognosis, but fortunately, specific anti-HER2 therapies have improved the survival rate of patients [15,17,18]. Finally, the triple negative tumors show the worst prognosis of all breast cancer subtypes and are characterized by the lack of ER, PR and HER2 expression. They are classified in basal-like and normal-like tumors, based upon the cytokeratin expression (CK 5/6, 14 and 17), that is positive in the basal-like tumors [19–22].

In Veterinary Medicine, some investigation has been conducted to find biomarkers that could improve the prognosis accuracy in cats with mammary carcinomas [23–33]. This effort is important since feline malignant mammary tumors are very common in cats, representing the third most common tumor in this species. They are predominantly malignant (85%–95%) and clinically very aggressive [1,33,34]. Recently, three studies have used a panel of markers to immunophenotype feline mammary carcinomas (FMC) [35–37]. However, the small number of tumor samples and the use of different classifications led to contradictory conclusions. In this study, we aimed to overcome this difficulty clinically characterizing the different subtypes identified in a large population of female cats presenting mammary carcinomas (n = 102), using the IHC-based classification established by St. Gallen International Expert Consensus panel [14]. Significant statistical associations between cancer subtypes and 19 clinicopathological features were evaluated, and a univariate analysis of overall survival (OS) and disease free survival (DFS) was performed.

Table 1
Allred Score guidelines for ER and PR staining.

Score for percentage of positive tumor cells		Score for average intensity of staining	
Score	Interpretation	Score	Interpretation
0	No staining	0	None
1	<1%	1	Weak
2	1–10%	2	Average
3	10–33%	3	Strong
4	33–66%		
5	>66%		

Allred score (0–8) = The Σ of both scores.

Material and methods

Study population

A total of 102 female cats with mammary carcinoma were enrolled in this prospective study from September 2009 to January 2014. Animals were presented at the Teaching Hospital of the Faculty of Veterinary Medicine, University of Lisbon (FVM-ULisboa), and the owners gave permission to collect samples from their pets and to use the animal's clinical data. All mammary tumors were surgically obtained after mastectomy, except for 9 cases that were collected at necropsy. Tumor samples were collected in accordance with the EU Directive 2010/63/EU and research was approved by the Ethics Committee of the FVM-ULisboa.

The following clinical and pathological features were evaluated and recorded: age, breed, reproductive status at the time of the surgery (intact *versus* spayed), previous administration of progestogens for oestrus control, number, location and size of tumor lesions, treatment performed (none, surgery), extension of the surgery (lumpectomy, unilateral mastectomy or bilateral mastectomy), stage of the disease (TNM system) [1], disease free survival (DFS) and overall survival (OS).

Tumor histological classification

Mammary tumors were fixed in 10% buffered formalin during 24–48 hours and were processed for routine histological examination. All tumors were classified according to the World Health Organization (WHO) classification system [38]. The malignancy grade was scored from I to III using the Elston & Ellis scoring system [39] and the presence of necrotic areas within the lesions, lymphatic invasion by neoplastic cells, lymphocytic infiltration and cutaneous ulceration was recorded. In 93 cases, the regional lymph nodes were also collected and analyzed.

Immunohistochemistry

For each primary tumor, a specific area was selected (6 mm in diameter), avoiding the necrotic and the non tumoral areas, sections were obtained and immunohistochemical staining was performed for detection of the following proteins: ER, PR, feline homologue of HER2 (*f*HER2), CK 5/6 and Ki-67. IHC protocols and score interpretation were performed as previously described [32,36,40,41].

Classification of the staining results was made according to the Allred Score guidelines for interpretation of ER and PR staining (Table 1) and HER2 was interpreted according to the American Society of Clinical Oncology (ASCO) guidelines which criteria are summarized in Table 2 [12,42–44].

A tumor was considered positive for ER and PR when presenting a score >2 [32,41,43]; for *f*HER2 when achieving the score 2 + or 3+ [2,25,40]; and for CK 5/6 status, when revealing cytoplasmic and/or membrane labeling of 1% of the tumor cells [35,41,45]. For Ki-67, a

Table 2
*f*HER2 immunohistochemistry scoring criteria.

Score	Interpretation
0	No staining
1+	Weak, incomplete membrane staining in any proportion of tumor cells
2+	Complete membrane staining that is either no uniform or weak in intensity but with obvious circumferential distribution in at least 10% of cells
3+	Uniform intense membrane staining of at least 10% of invasive tumor cells