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Review Article

Canine Mammary Mixed Tumours: A Review

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Mammary mixed tumours are the most frequent neoplasias in female dogs. In humans, mixed tumours are frequently found in the salivary glands and are known as pleomorphic adenomas. In addition to their histomorphologic similarities, mixed tumours and pleomorphic adenomas have the potential to become malignant and give rise to carcinomas in mixed tumours and carcinomas ex-pleomorphic adenoma, respectively. The factors associated with malignant transformation are still poorly known in the case of canine mixed tumours. However, this form of neoplasia tends to be associated with a better prognosis than other malignant histological types. This paper discusses the main features associated with female canine mammary mixed tumours.

1. Introduction

Mammary tumours are the most frequent neoplasia in female dogs; therefore, these tumours represent a serious problem in veterinary medicine [1]. Mixed tumours are one of the most common tumour types in the female canine mammary glands. These tumours exhibit a complex histological pattern because they comprise elements from the epithelium and the mesenchyme and have the capacity to undergo malignant transformation, thereby giving rise mainly to carcinomas and less frequently carcinosarcomas and sarcomas in mixed tumours [2, 3].

Defining the origin of the several cellular elements involved in mixed tumours, as well as the factors contributing to malignant transformation is important in understanding the behaviour and evolution of this type of neoplasia. However, these components of mixed tumours still remain to be elucidate.

This paper discusses the main features associated with the clinical-epidemiological characteristics, histogenesis, malignant transformation, and comparative aspects of female canine mammary mixed tumours.

2. Definition/Morphology

Benign mixed tumours are characterised by the presence of benign epithelial elements (ductal and/or acinar and myoepithelial cells) and mesenchymal cells with cartilage and/or bone formation eventually combined with myxoid fibrous tissue [2] (Figure 1(a)).

The proliferating myoepithelial cells may exhibit a fusiform or stellate appearance, and these cells are often enveloped within an abundant extracellular matrix (myxoid matrix). The cartilage tissue is characterised by nodules or plaques of different sizes, including low or moderate numbers of chondrocytes and chondroblasts rarely exhibiting cellular morphological alterations. When bone tissue is involved, it comprises osteoid matrix-forming osteoclasts and mineralised bone. Certain cases also exhibit bone marrow, including haematopoietic and adipose tissue [4, 5].

A certain degree of pleomorphism and atypia is generally found in these tumours; therefore, the differential diagnosis is often difficult, especially regarding carcinomas in benign mixed tumours. The use of special staining techniques in order to analyse the integrity of the basement membrane

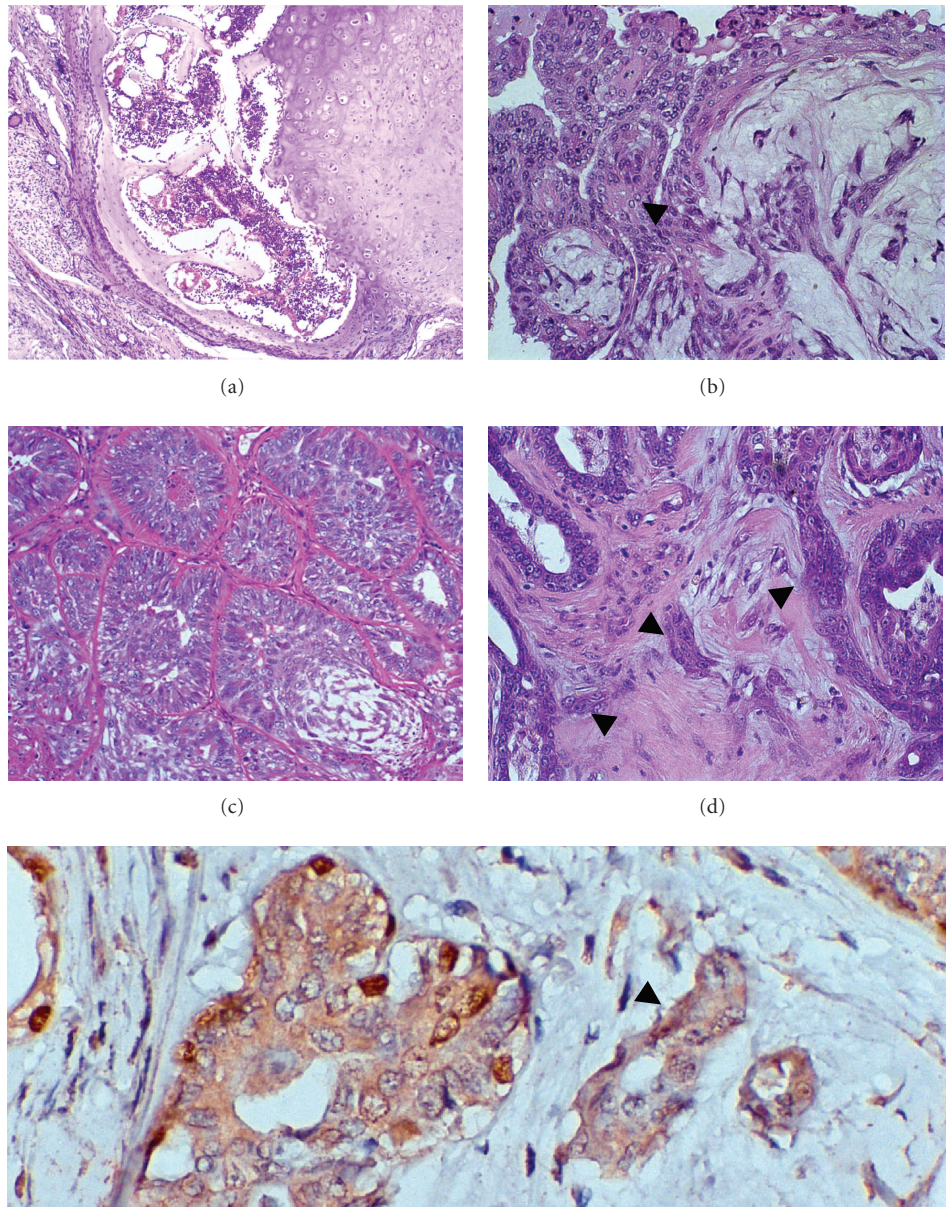

Figure 1: (a) Benign mixed tumor in canine mammary gland presenting chondroid and myeloid metaplasia. HE, 10x. (b) Ductal *in situ* carcinoma in benign mixed tumor in canine mammary gland presenting myoepithelial cells producing myxoid matrix. HE, 40x. (c) Carcinoma in benign mixed tumor in canine mammary gland presenting *in situ* carcinomatous areas and myoepithelial cell proliferation producing myxoid matrix. HE, 20x. (d) Carcinoma in benign mixed tumor in canine mammary gland presenting invasive areas in the adjacent stroma (arrow). HE, 40x. (e) Carcinoma in benign mixed tumor in canine mammary gland presenting absence of myoepithelial cells confirmed through negative p63 expression (arrow) in stromal invasive areas. Immunohistochemical stain with Mayer's haematoxylin counterstain, 60x.

allows for a better decision on the benign or malignant nature of this type of tumour [6–10].

According to the developing system of classification of carcinomas in mixed tumours, these carcinomas are characterised by a focal or nodular development of malignancy within a primarily benign mixed tumour [2].

Initially, the term "malignant mixed tumour" was applied to carcinomas arising in the context of benign mixed tumours. However, several authors used this same term for mixed tumours in which one or both (epithelial or mesenchymal) components were malignant [11, 12]. The term carcinosarcoma was used as synonym of malignant mixed tumour, even in cases without malignant transformation of one of the two cellular components [11].

In the classification scheme proposed by Misdorp et al. (1999), the expression "malignant mixed tumour" was

excluded and replaced by "carcinoma in mixed tumour," which is histologically different from carcinosarcoma. Carcinosarcoma refers to a neoplasia that exhibits the concomitant malignancy of both the epithelial and mesenchymal components and a more aggressive behaviour than the former [2, 13].

In carcinomas in benign mixed tumours, the carcinomatous proliferation might exhibit *in situ* or infiltrative growth, which is suggested by the loss of the continuity of the myoepithelial and basement layers associated with invasion of the stroma by neoplastic cells. In this case the previously benign lesion might eventually be fully replaced by carcinomatous tissue [3]. Thus, the phenotypic assessment of myoepithelial cells is important in the differential diagnosis between these types of lesions (Figures 1(b), 1(c), and 1(d)).

3. Clinical-Epidemiological Characteristics

The data on the frequency of benign mixed tumours are difficult to compare, due to divergences among the various classification systems that have been suggested over time [11, 12]. According to the literature, mixed tumours represent 50% to 66% of canine mammary neoplasias [14]. These tumours usually appear in animals 6 to 10 years old, most frequently in females, although they can also affect males [14–16]. Mixed tumours are thought to occur independently from breed [17]. However, Mulligan (1949) found high incidence in the breeds of Cocker Spaniel, Fox Terrier, and Boston Terrier. They more commonly affect the caudal (inguinal, caudal abdominal, and cranial abdominal) glands and occasionally the cranial (caudal and cranial thoracic) glands [14, 17, 18].

Current surveys of cases assessed based on the latest veterinary classification system showed that 40% to 50% of benign tumours are mixed tumours [1, 19]. Data regarding age are scarce, but several studies have reported that benign mixed tumours affect mostly young animals between 3 and 9 years old [20].

Carcinomas in mixed tumours represent 10% to 40% of the total number of diagnosed carcinomas [1, 19, 21]. Recent surveys report carcinomas in mixed tumours as the most frequent histological type, representing 20% to 32% of all mammary malignant tumours [1, 22].

Histological characterisation of mammary tumours might be considered an independent prognostic factor. Carcinomas in mixed tumours are associated with an average survival time that is 2- to 3-fold higher than that of other canine mammary carcinomas. Thus, animals presenting with this histological type exhibit a favourable prognosis when compared to animals presenting other types of carcinomas. As such it might be considered a protective factor against the risk of canine mammary tumours associated death [22, 23].

One explanation for the better prognosis associated with mixed tumours is related to the expansive growth pattern of these tumours, exhibiting little lymphatic invasion and a low metastatic index [23, 24]. The size of the carcinomatous area included within a canine mammary mixed tumour might also be a factor that affects prognosis [22].

An early and complete surgical excision followed by a histopathological diagnosis is recommended in the treatment of all canine mammary tumours. Surgical delay might result in larger tumours and make their removal more difficult [25]. Moreover, the epithelial component of mixed tumours might exhibit a malignant transformation, thereby giving rise to a carcinoma. As a result, worsening of the clinical progression and consequently prognosis of this disease may occur. Although surgery is able to successfully treat most cases, the identification of cases requiring alternative therapies is mandatory [26].

The biological behaviour of carcinomas in mixed tumours may vary in accordance to the histological type of the malignant epithelial component of the tumour. Because these tumours are inserted within a benign lesion, these neoplasias are expected to be associated with a better prognosis and the affected animals to exhibit longer survival rates.

4. Malignant Transformation

Factors determining the malignant transformation of benign mixed tumours have been the focus of some studies [9, 10]. There are few studies on the malignant progression of canine neoplasias [27]. However, in the 1970s, Moulton et al. hypothesised that if mixed tumours had sufficient time to grow, they would undergo malignant transformation. Later, Genelhu et al. (2007) and Bertagnolli et al. (2009) observed molecular alterations that might contribute to the malignant transformation of benign mixed tumours, such as a loss of p63, ΔNp63, and E-cadherin and β-catenin expression [10, 20].

A recent study showed that the overexpression of the epidermal growth factor receptor (EGFR) by malignant epithelial cells might occur early in the carcinogenesis of mixed tumours. Moreover, alterations in the expression of this molecule may play a crucial role in the process of malignant transformation in the epithelial component of this histological type [27].

The key morphological characteristic for the differential diagnosis of carcinomas in canine mixed tumours is the presence of areas of invasion or microinvasion within benign mixed tumours [3]. The *sine qua none* condition required to establish stromal invasion is the rupture of the basement membrane and the myoepithelial cell layer surrounding the carcinoma *in situ* [28]. However, in some cases, the visualisation of this area with standard stains, such as hematoxylin-eosin, is extremely difficult. Thus, the use of special stains, such as periodic acid Schiff (PAS) stain, and of antibodies identifying the proteins expressed in the myoepithelial cells, such as p63 (Figure 1(e)), smooth muscle alpha actin, high-molecular-weight cytokeratins, maspin, and calponin, may aid in the identification of invasion foci in mixed mammary tumour of dogs [7–10, 20, 29].

Myoepithelial cells surround the epithelial structure in premalignant lesions and carcinomas *in situ* and serve as a barrier [30] hindering the progression of *in situ* carcinomas into invasive carcinomas [31]. It is believed that this suppressive ability of the myoepithelial cells depends on

their full differentiation and that changes in their molecular expression pattern might result in cell function changes. Undifferentiated myoepithelial cells might promote tumour progression [31]. Bertagnolli et al. (2009) observed *in vivo* that carcinomas evolving within canine mixed tumours exhibited decreased p63 expression, which suggests a loss of myoepithelial cells in this area, thereby favouring the invasive and progressive characteristics of these tumours [10]. However, the mechanisms leading to the interruption of this layer are still poorly known (Man et al. 2003). Studies on human breast neoplasias have shown a reduced expression of the oestrogen receptors and of tumour-suppressive proteins, such as maspin, WT-1, and p63, by the epithelial cells close to areas exhibiting a loss of myoepithelial cells, thereby contributing to the aggressiveness and invasiveness of the tumour [28, 30, 32].

In canine mammary tumours, certain components of the extracellular matrix also seem to participate in the process of malignant transformation. Some authors have reported an accumulation of proteoglycans and chondroitin sulphate in both the stroma around the tumour cells and the matrix produced by proliferating myoepithelial cells [33]. Versican, a type of sulphated proteoglycan, is highly expressed by proliferating fusiform cells and myxoid areas of mixed tumours [34]. Erdélyi et al. (2005) showed that the *in vivo* accumulation of versican in the myxoid matrix is associated with the early differentiation of tissue into cartilage [34]. Moreover, the overexpression of this molecule was observed in the invasive areas of malignant tumours, including carcinomas in mixed tumours, indicating the participation of this proteoglycan in the invasion by tumour cells [35].

5. Histogenesis

The origin of the several components of mixed tumours is a subject of long-standing controversy and is not yet fully understood. In the 1940s, Allen (1940) reported 4 cases of canine mammary mixed tumours and, based on their morphological characteristics, this author suggested that the cartilage present in this type of neoplasia is probably derived from adult epithelial cells [36]. Other authors [37, 38] found evidence indicating that cartilage and bone are derived from the stromal connective tissue.

The hypothesis supported by the greatest amount of evidence states that the mesenchymal components originate from myoepithelial cells. The first evidence was based on the analysis of cartilage- and bone-forming cells with the use of histochemical and physical methods, as well as by electron microscopy [39–44].

Immunohistochemical tools enable the definition of the molecular changes in the myoepithelial cells assumed to be involved in cartilage formation and indicate a progressive transition of the myoepithelial cells into mesenchymal cells during cartilage formation. A reduction in the expression of myoepithelium typical markers, such as cytokeratins, p63, smooth muscle alpha actin, and maspin, was observed [6–10] in myoepithelial cells. In addition, the mesenchymal phenotype was confirmed by the presence of vimentin and S-100 [6, 7, 45].

The assessment of the expression of proteins involved in chondrogenesis reinforced the initial evidence. Calponin [29], β II tubulin [46], versican and aggrecan [34, 35], collagens [34, 47, 48], 3B3(-) neoepitope [33], bone morphogenetic protein 6 and its receptors (BMP-6) [49–51], and chondromodulin-1 (ChM-1) [51] are expressed in myoepithelial proliferation areas and/or chondrocytes and seem to participate in cartilage formation. Molecules involved in cell-extracellular matrix adhesion, such as tenascin, fibronectin, and the neural cell adhesion molecule (NCAM), apparently contribute to the differentiation of the myoepithelium [52].

A further question thus arises; what might be the relationship between the epithelial and mesenchymal components of mixed tumours? A suggested hypothesis states that these components originate from stem cells with a high capability for divergence. This assumption is grounded on immunohistochemical studies [9, 34] and on the observation that the epithelial and mesenchymal components of mixed tumours are monoclonal [6, 53, 54].

Recently, Ferletta et al. (2011) found cells exhibiting stem-cell characteristics in a line developed from a benign mixed tumour. This finding might represent a step forward in studies of stem cells in canine mammary tumours [55].

6. Comparative Aspects

In humans, epithelial tumours associated with production of myxoid or osteochondroid matrix are uncommon in the breast and are associated with an uncertain prognosis. These tumours have been described as metaplastic carcinomas with matrix production. However, this pattern of neoplastic proliferation is frequent in human salivary glands, where the tumours are known as mixed tumours or pleomorphic adenomas [56].

In addition to the histological similarity, mammary mixed tumours in female dogs and human salivary pleomorphic adenomas exhibit other similar features. Both tumour types derive from exocrine glands exhibiting similar architecture, the age of onset when they appear is similar, and malignant epithelial transformation can occur and is mainly associated with rapid growth and recurrence [2, 20, 57–60].

The occurrence of carcinomas ex-pleomorphic adenomas in the salivary glands is infrequent in humans, but these tumours are usually aggressive and result in distant metastases, similar to observations of human mammary metaplastic carcinomas [59–61].

In carcinomas ex-pleomorphic adenomas of the humans, myoepithelial cells surrounding carcinomatous areas exhibit a reduction in the expression of smooth muscle alpha actin, calponin, cytokeratin 14, CD10, laminin, maspin, and p63 [20, 62]. A similar pattern of antigen expression involving cytokeratins, p63, vimentin, protein S-100, β-catenin, and E-cadherin was observed in canine mixed tumours, suggesting that myoepithelial cell proliferation plays an important role in the genesis of these tumours [10, 20, 45]. Another similar pattern of alteration observed in these tumours concerns gene p53 mutations and the accumulation of its protein product [59, 63–65].

Alterations in other proteins involved in the regulation of the cell cycle, such as p21 and c-myc [66], and growth factor receptors, such as HER-2 and EGFR [67–69], and a decrease of adhesion molecules, such as E-cadherin and β-catenin, and oestrogen and progesterone receptors [20, 69, 70], have also been observed in both human and canine tumours.

Current comparative studies suggest that the matrix-producing glandular tumours observed in canine and human mammary glands and human salivary glands exhibit the same tumourigenic characteristics. Defining the prognostic and predictive similarities among these tumours might provide better information on the clinical behaviour of these tumours and support the use of a spontaneous canine model in studies of human carcinomas.

7. Conclusions and Perspectives

Regarding clinical behaviour, mammary benign mixed tumours occur frequently in dogs and are usually associated with a good prognosis. However, divergences in nomenclature and histological classification over time make it difficult to analyse data on relapses, malignant transformation, and biology of these tumours. Studies focusing on clinical features, malignant transformation, histogenesis, and epithelial-mesenchymal interactions might provide new information required to elucidate the clinical and biological behaviour of this type of tumour in the veterinary medicine setting.

From a comparative perspective, canine mammary mixed tumours and human pleomorphic adenomas of the salivary gland exhibit morphological and molecular similarities, suggesting a similarity in the pathogenic mechanisms involved in malignant transformation and histogenesis.

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