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A Comparative Review of Mixed Mammary Tumors in Mammals

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Abstract

Mixed tumors are characterized by the histological identification of two or more cell types. Commonly, a mixture of epithelial and myoepithelial cells is included in abundant stroma, which can consist of myxoid, chondroid or bony matrices. Spontaneously arising mixed tumors are rare lesions in the human breast but are common in human salivary glands and canine mammary glands. Subtle histopathological characteristics and overlapping attributes of malignant lesions with other benign lesions can lead to a diagnostic challenge. Mixed tumors can present as benign or malignant. While malignant mixed tumors are quite rare in the human breast they have a poor prognosis. Benign mixed mammary tumors occur more frequently in female dogs than in humans and are usually associated with a good prognosis. This review will provide a comprehensive overview of mixed mammary tumors, across various mammalian species.

Keywords Carcinosarcomas · Carcinomas in benign mixed tumors · Epithelial element · Mesenchymal element · Metaplastic breast cancers · Mixed mammary tumors

Abbreviations

BMT	benign mixed mammary tumor
CS	carcinosarcoma
CK	cytokeratin
CBMT	carcinoma in benign mixed tumor
CMT	canine mammary tumor
CMMT	canine mixed mammary tumor
E-cadherin	epithelial cadherin
ECM	extracellular matrix
EMT	epithelial mesenchymal transition
ER	estrogen receptor
H & E	haematoxylin and eosin
HER1	human epidermal growth factor receptor 1
HER2	human epidermal growth factor receptor 2
HMGI-C	high mobility group protein isoform C
HMGI (Y)	high mobility group protein with two isomers—I and Y
MBC	metaplastic breast cancer / metaplastic breast carcinoma
NST	invasive ductal carcinoma of no special type
p63	tumor protein *p63*
PAB	pleomorphic adenoma of the breast
PIK3CA	phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha gene
PR	progesterone receptor
SMA	smooth muscle actin
WHO	The World Health Organization

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Introduction

A biphasic tumor is a neoplastic tissue that is characterized by two cellular components that exhibit malignant features [1]. In humans, mixed tumors are generally metaplastic carcinomas with matrix production [2]. The term metaplastic derives from the Greek for "changed in form," and is used to describe cells that appear to have transformed into a different tissue type [3]. A mixed tumor has both epithelial and mesenchymal elements and if either of these elements are missing, the tumor cannot be designated a "true" mixed tumor [4].

Mixed tumors are found most commonly in the salivary glands, but they can be found in most parts of the body. They are rarely found in the human breast but are common in canine mammary glands [5]. In the normal human mammary gland, epithelial cells constitute the duct lining. These epithelial cells are the most susceptible to malignant tumor development [6]. In contrast, mesenchymal cells are multipotent cells that can differentiate into a wide range of cell types such as fibroblasts, chondrocytes (cartilage cells), osteoblasts (bone cells) and adipocytes (fat cells) [7]. The objective of this review is to compare the general aspects of mixed mammary tumors in different mammalian species.

Mixed Tumors

The term 'mixed tumor' was described in 1874 by Minssen as a tumor type that has both epithelial and stromal origins [8]. In 1966, Eneroth cataloged the numerous other names for mixed tumors such as 'epithelial mixed tumor', 'complex adenoma', 'pleomorphic adenoma and adenocarcinoma', 'pleomorphic sialoadenoma' and 'epithelioma remanie' [4]. 'Mixed tumor' is still the most frequently used term, not because of its histogenesis but due to its histological description [4].

In humans, cases of mixed tumors have been reported in different organs including the salivary gland [9–11], mammary gland [11, 12], uterus [13, 14], cervix [15], vagina [16], ovary [17], peritoneum [18], gallbladder [19], skin [20], lung [21], esophagus [22], colon [23], larynx, palate, paranasal sinuses, nasal septum [11] and pancreas [24]. Whereas in dogs, mixed tumors have been reported in the mammary gland [25, 26], thyroid gland [27], lung [28], salivary gland [29], ceruminous glands [30] and eyelid [31]. Only a few cases of mixed tumors have been described in cats and they were localized to the mammary gland [32, 33], pancreas [34], mandibular salivary gland [35], biliary system [36] and lung [37]. Spontaneously arising mixed tumors have also been observed in the mammary gland, uterus and skin of rats [38].

Mixed tumors can be benign or malignant. The former are identified by the presence of benign epithelial components and mesenchymal cells with cartilage and/or bone development, combined with myxoid fibrous tissue [39]. The latter consists of epithelial and stromal components, both of which are malignant [39–42]. Very little is known about the processes which underlie the pathophysiology of mixed tumors. The various forms and types of mixed mammary tumors in different mammals will be described in this review.

Mixed Mammary Tumors in Humans

In humans, mixed tumors are more common in the salivary gland than in the breast. However, mixed tumors in dogs are found most frequently in the mammary gland [43]. In humans, mixed mammary tumors can be benign or malignant.

Benign Mixed Mammary Tumors in Humans

Benign mixed tumors of the breast were first described by Lecéne in 1906 [44]. They are also known as "pleomorphic adenomas of breast" (PAB) [11]. This tumor type has a wide histomorphologic diversity but an excellent prognosis [45].

Less than 100 cases of PAB have been reported (see Table 1) [11, 44, 46–92]. PAB is seen more commonly in females than in males (10:1 ratio) [11] and can be found in individuals of all ages. However, it is most common in the second to seventh decades. In their study, Sato et al., stated that there is a tendency for the tumors to occur in the right breast (R: L = 3:2). PAB are more likely to be located in the sub-areolar region, which indicates that the tumor is likely to have arisen from the large duct [79].

PAB is often asymptomatic and is usually only recognized through imaging carried out for other reasons. PAB mainly affects women aged 23 to 73 years of age and typically appears as a single palpable lump [45, 82]. This tumor type can vary in size between 0.6 cm and 17 cm in diameter, but most were around 2 cm in diameter [69].

Microscopically, PAB is a mixture of epithelial and myoepithelial cells in *abundant* stroma, which may consist of myxoid, chondroid or osseous matrices. These tumor cells can form lobules and ducts. Epithelial cells vary from cuboidal to columnar with bland cytological nuclear attributes and low mitotic activity, which can be organized as tubular structures, islands, cords or sheets and can display apocrine differentiation. The surrounding myoepithelial cells are polygonal, plasmacytoid, fusiform or stellate, with small nuclei and cytoplasm that varies between clear and eosinophilic. The myoepithelial cells are either combined with the epithelial cells or distributed throughout the stroma, where the stroma may range from loose to myxoid, to chondroid or osseous [11].

Eighty cases of PAB have been described (see Table 1). Table 1 lists the clinicopathological characteristics of cases reported in the literature. All cases were confirmed as PAB. There were 75 (93.75%) women compared to only 5 men, indicating a female: male ratio of 15:1. Patients' ages ranged from 19 to 85 years. Myxoid matrix was present in 46 (57.5%) of cases, chondroid matrix in 51 (63.75%) of cases and osteoid stroma in 23 (28.75%) of cases. No histopathological descriptions were available for 14 (17.5%) of these cases.

The PAB and pleomorphic adenomas of the salivary gland have similar immunohistochemical characteristics. PABs are positive for ER and negative for PR [71, 77, 79]. PAB is positive for cytokeratin 7 (CK7) in the ductal epithelial cells, positive for S100 and smooth muscle actin (SMA) in myoepithelial cells [92]. The spindle and satellite myoepithelial cells nuclei are positive for HMGI-C and HMGI (Y) proteins, suggesting a

Table 1 The reported cases of mammary pleomorphic adenoma in humans (1906–2016)

Number of cases	Author (reference)	Metaplastic tissue type (number of cases)	Patient age	Sex
1	Reid-Nicholson et al. [11]	Myxoid matrix (1)	59	F
2	Lecéne [44]	Chondroid matrix (2); Osteoid matrix (2)	25	F
2	Nadal [46]	N/A	54	F
			44	F
2	D' Allianes [47]	N/A	19	F
			NA	F
1	Gioia [48]	N/A	42	M
1	Nabert [49]	N/A	38	F
1	Poluektov and Shestakova [50]	N/A	63	F
9	Smith and Taylor [51]	Chondroid matrix (9); Osteoid matrix (6)	23–77 (Median age 55)	F
1	Paikova [52]	N/A	39	F
1	Kermarec et al. [53]	NA	68	F
1	William and Leach [54]	Chondroid matrix (1); Osteoid matrix (1); Myxoid matrix (1)	72	F
3	Jakimowicz and Gramata [55]	N/A	57	F
1	Sheth et al. [56]	Chondroid matrix (1); Osteoid matrix (1); Myxoid matrix (1)	78	F
1	Medina and Uehlinger [57]	NA	78	F
3	Makek and Von Hochstetter [58]	Chondroid matrix (2); Osteoid matrix (2); Myxoid matrix (2)	35	2 F
			60	1 M
			78	
1	McClure et al. [59]	Myxoid matrix	46	F
1	Van der Walt and Rohlova [60]	Chondroid matrix (1); Osteoid matrix (1); Myxoid matrix (1)	67	F
1	Spagnolo and Shilkin [61]	Chondroid matrix (1); Osteoid matrix (1); Myxoid matrix (1)	46	F
1	Zafrani et al. [62]	N/A	66	F
1	Cuadros et al. [63]	Chondroid matrix (1); Myxoid matrix (1)	65	F
1	Segen et al. [64]	Chondroid matrix (1); Myxoid matrix (1)	75	F
1	Willen et al. [65]	Chondroid matrix (1); Myxoid matrix (1)	76	F
1	Kjell et al. [66]	Chondroid matrix (1); Myxoid matrix (1)	61	F
1	Balance et al. [67]	Chondroid matrix (1); Osteoid matrix (1); Myxoid matrix (1)	77	F
6	Moran et al. [68]	Chondroid matrix (6); Myxoid matrix (6)	37, 58, 61,76, 81 and 85	F
2	Chen et al. [69]	Chondroid matrix (2); Myxoid matrix (2)	58–75	F
1	Nevado et al. [70]	Chondroid matrix (1); Myxoid matrix (1)	84	F
10	Diaz et al. [71]	Chondroid matrix (6); Osteoid matrix (4); Myxoid matrix (10)	50–67 (median age 65)	F 9 M 1
1	Simha et al. [72]	Chondroid matrix (1); Myxoid matrix (1)	65	M
1	Agnantis et al. [73]	Chondroid matrix (1); Osteoid matrix (1); Myxoid matrix (1)	62	F
1	Narita and Matsuda [74]	Chondroid matrix (1); Osteoid matrix (1); Myxoid matrix (1)	70	F
1	Moshinaga et al. [75]	Chondroid matrix (1); Myxoid matrix (1)	74	F
1	Parham and Evans [76]	Chondroid matrix (1)	NA	F
1	Ficks [77]	Chondroid matrix (1); Osteoid matrix (1); Myxoid matrix (1)	43	F
1	Kumar et al. [78]	Myxoid matrix (1)	47	F
1	Sato et al. [79]	Chondroid matrix (1); Myxoid matrix (1)	55	F
1	Molland et al. [80]	Chondroid matrix (1); Myxoid matrix (1)	24	M
1	Dominicis et al. [81]	Chondroid matrix (1); Myxoid matrix (1)	59	F
1	Iyengar et al. [82]	Myxoid matrix (1)	72	F
1	Shum et al. [83]	Chondroid matrix (1); Myxoid matrix (1)	85	F
1	Mizukami et al. [84]	Chondroid matrix (1); Myxoid matrix (1)	76	F
1	Djakovic et al. [85]	Chondroid matrix (1); Osteoid matrix (1); Myxoid matrix (1)	NA	F
1	Leekha et al. [86]	Chondroid matrix (1); Myxoid matrix (1)	60	F
1	Khamechian et al. [87]	Chondroid matrix (1); Myxoid matrix (1)	61	F
1	Gupta et al. [88]	Chondroid matrix (1); Myxoid matrix (1)	NA	F
1	Kelten et al. [89]	Chondroid matrix (1); Osteoid matrix (1)	57	F
			78	F
1	Ginter et al. [90]	Chondroid matrix (1); Myxoid matrix (1)	42	F
1	Radu et al. [91]	N/A	60	F
2	Han et al. [92]	Chondroid matrix (2); Myxoid matrix (2)	28	F
			47	F

N/A, not available

histogenesis similar to that of pleomorphic adenoma of the salivary glands [79].

Malignant Mixed Mammary Tumors (Human Metaplastic Breast Cancers)

Metaplastic breast cancer (MBC) was identified in 1973 by Huvos and colleagues [93]. This tumor type is aggressive and often results in metastasis [93]. The pathogenesis of MBC is not well understood, but it has been suggested that the tumor develops from an adenocarcinoma undergoing metaplasia to non-epithelial stromal cancer [12]. MBCs often overexpress epidermal growth factor receptor (HER1) [12, 94]. MBCs are mostly triple negative tumors (lack of ER, PR and HER2 expression). One study showed some MBCs are not triple-negative because they express ER and PR and others express

HER2 [95]. Lim and colleagues showed that triple-negative features were found in 77% of MBCs [95]. Patients with metaplastic carcinomas often do not benefit from a classical adjuvant chemotherapy regimen [96].

MBC represents 0.25–1% of breast cancer cases diagnosed annually [97]. CS of the breast accounts for 0.08–0.2% of all breast cancers [98]. MBC usually affects women over 50 years of age [99]. Metaplastic squamous cell carcinoma of the breast is also an extremely rare breast cancer, comprising less than 0.1% of all breast cancers [100].

In 1989 a comprehensive study of MBCs was undertaken by Wargotz and Norris [101]. They categorized MBCs into five subtypes which include spindle-cell carcinoma, squamous cell carcinoma, matrix-producing carcinoma, carcinosarcoma (CS) and osteoclastic giant cell [101–105] (see Table 2). The World Health Organisation (WHO) in 2011 subdivides MBC into two types: (1) the epithelial sub-group without stromal elements that includes squamous cell carcinoma, fibromatosis-like metaplastic carcinoma, low grade adenosquamous and spindle cell carcinomas and (2) the mixed sub-group with mesenchymal components, which includes carcinoma with chondroid and/or osseous metaplasia and CS [106–110] (see Table 2). The term "carcinosarcoma" is formally reserved for tumors where the demarcation between epithelial and mesenchymal elements is distinct [98]. The 5-year survival rate of breast CS is 49%, worse than other MBCs [111]. The Wartgotz and Norris's classifications of MBCs continue to be used within the WHO classifications (see Table 2).

Clinical imaging features of MBC can resemble invasive breast carcinoma or nonmalignant lesions. Imaging can demonstrate an uneven or circumscribed palpable lump with spiculated portions on a mammogram and an ultrasound shows a solid asymmetrical mass or combined cystic mass. MBCs can resemble benign lumps with regular, round/ ovoid masses on a mammogram and circumscribed hypoechoic solid masses on magnetic resonance imaging. MBC presents frequently as a rapidly growing mass that is often larger (i.e. >2 cm) than the more common types of breast cancer [112, 113].

Histologically, MBC is a poorly differentiated tumor that contains ductal carcinoma cells combined with spindle, squamous, chondroid and/or osseous components. The spindle cell subtype is characterized by cohesive sheets of spindle cells. The spindle cell element usually resembles a low-grade sarcoma or granulation tissue, which may be difficult to distinguish microscopically [114]. The squamous cell carcinoma subtype is classified by the presence of polygonal cells, eosinophilic cytoplasm and possible 'keratin pearl' development [114]. Carcinosarcomas (CSs) consist both malignant epithelium and malignant stroma [114]. The matrix-producing subtype consists of an obvious carcinoma with chondroid and/or osseous stromal matrix without a spindle cell element [115]. MBC less commonly displays axillary nodal metastases than invasive breast cancer, regardless of the larger tumor size. The incidence of axillary lymph node involvement ranges from 6% to 26% [116].

Lien and colleagues conducted transcriptional profiling using microarrays to clarify the genetic expression profiles of MBCs and their differences from breast ductal carcinoma using four MBCs and 34 ductal carcinomas [117]. This study established that there are unique gene expression patterns for metaplastic carcinomas and adenocarcinomas of the breast. In MBCs, eighty-seven genes were overexpressed, and 121 genes were underexpressed [117]. Many of the 87 upregulated genes were associated with the production of extracellular matrix (ECM), such as genes associated with ECM synthesis, remodelling and adhesion/motility/migration and with skeletal development and/or chondro-ossification [117]. On the other hand, genes coding for proteins associated with maintaining epithelial structures, such as the cytoskeleton, cell–cell adhesion molecules and tight junctions, were downregulated [117]. In MBCs, the overexpression of ECM-related genes and the underexpression of epithelial-related genes (particularly in mixed mesenchymal and epithelial MBCs) could be the reason for the sarcomatous alteration together with ECM development, when compared with adenocarcinomas of breast [117].

When bony or cartilaginous elements are detected in any tumor, the microscopical diagnosis becomes challenging because these ectopic elements could be wrongly diagnosed as a breast osteosarcoma [117]. Therefore, it is important that breast tumors with prominent sarcomatous elements are analyzed immunohistochemically [118]. Marian et al. examined a case of carcinosarcoma and demonstrated focal expression of actin, CD10 and p63 and overexpression of CK5/6, CK17 and CK34βE12. The localization of these proteins verified the myoepithelial origin of this tumor [119]. Moreover, the expression of these markers suggested that MBCs are part of

Table 2 Wartgotz and Norris classification and the WHO classification of MBCs

Wartgotz and Norris classification (1989)	WHO classifications (2011)
1 Squamous cell carcinoma	1 Squamous cell carcinoma
	2 Low-grade adenosquamous carcinoma
2 Spindle cell carcinoma	3 Spindle cell carcinoma
	4 Fibromatosis-like metaplastic carcinoma
	5 Myoepithelial carcinoma
3 Matrix producing MBC	6 Carcinoma with mesenchymal differentiation (chondroid and osteoid)
4 Carcinosarcoma	7 Carcinosarcoma
5 Osteoclastic giant cell	

the 'basal-like subtype' of breast tumors [119]. This research group also demonstrated that mesenchymal elements of this tumor type were positive for osteopontin, vimentin, CD56, and HER1 and lacked ER, PR and HER2 expression. The sarcomatous tumor cells were also negative for synaptophysin, S100 and CD34. MBCs are strongly positive for vimentin and focally positive for claudins and E-cadherin [120]. Cimino-Mathews et al. demonstrated that MBCs were positive for Sox10, indicating that these subtypes of tumors might have a myoepithelial origin [121].

Treatment of MBC is like for invasive ductal carcinoma of no special type (NST). The standard treatment can include surgery (lumpectomy or mastectomy), radiation, adjuvant chemotherapy, hormone treatment and targeted therapy. In some cases, several types of therapy are used together to accomplish the most effective results [112, 122, 123]. The 5-year disease-free rate for MBC is around 40%, with an overall survival rate that varies from 49% to 68% [123]. Comparing patients with MBC and NST, MBC has a worse 5-year disease free survival rate of 39–56% versus 60.3–77% [124].

Mixed Mammary Tumors in Non-Human Mammals

Mixed Mammary Tumors in Dogs

More mixed tumors have been described in dogs than in any other domestic animals [25, 26]. The mammary gland of female dogs is the most common site for these lesions [25]. Canine mixed mammary tumors (CMMTs) have complicated morphology, developing epithelial, mixed and mesenchymal tumors [125–127] (Fig. 1).

CMMTs appear in female dogs from 6 to 10 years of age [43]. Based on the literature, mixed tumors constitute 50% to 66% of all canine mammary tumors [43]. Approximately 40% to 50% of benign canine mammary tumors are mixed tumors [43]. Malignant mixed mammary tumors represent 20% to 32% of all malignant mammary tumors [43]. Mulligan observed the high occurrence of mixed mammary tumors in the breeds of Fox Terrier, Cocker Spaniel, and Boston Terrier [128]. The sizes of CMMTs can vary from a few millimetres to 20 cm [129]. They have a firm and generally cartilaginous or bony consistency [129]. Metastases from malignant CMMTs have been detected in the lymph nodes, lung, liver, kidney and occasionally in other organs [129].

There are three types of CMMTs: benign mixed mammary tumors (BMTs), carcinoma in benign mixed tumors (CBMTs) and CSs. These are characterized by the presence of benign or malignant epithelial and mesenchymal components based on their histological subtypes.

BMTs are usually encapsulated and composed of epithelial elements (i.e. ductal and/or acinar epithelial cells and myoepithelial cells) (Fig. 1a) within an obvious mesenchymal element, capable of producing myxoid, cartilaginous or osseous tissue to various extents [39]. In this neoplasm, the epithelial element can show low cellular atypia and a low mitotic index [39]. The ductal epithelial element can present different growth patterns and the gland lobules are often poorly preserved [130]. This epithelial element is usually compressed by the stromal element of the tumor [130]. The myoepithelial

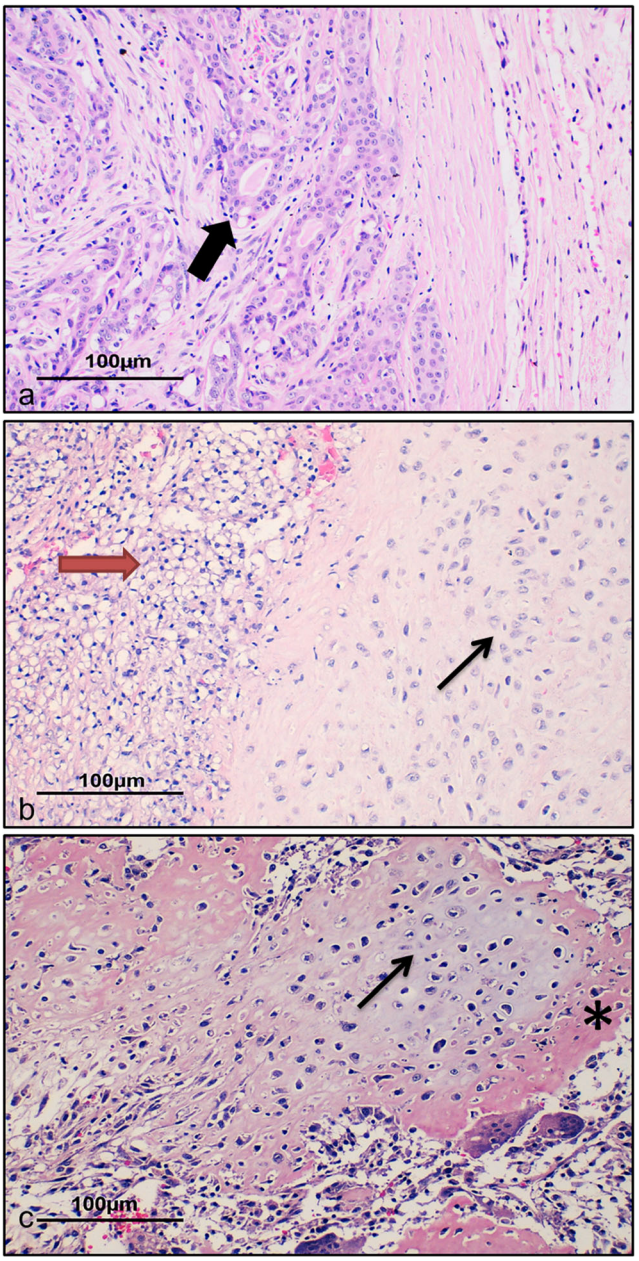

Fig. 1 Canine Mixed Mammary Tumors. **a** Photomicrograph of a benign mixed mammary tumor with clusters of cuboidal epithelial cells in tubular formation (thick black arrow). **b** Photomicrograph showing the carcinosarcoma with both carcinomatous (thick red arrow) and sarcomatous elements (thin black arrow). **c** A mixed mammary tumor with chondro/osteosarcomatous components, chondroid areas consist of pale blue matrix (thin black arrow) with pinkish bone matrix (asterisk) between the osteoblasts. H&E. Bars, 100 μm

cells display a fusiform morphology and are usually surrounded by abundant fibrillar myxoid matrix. The chondroid tissue consists of mature and immature chondrocytes. When osseous material is presented, it can consist of osteoid-producing osteoblasts, trabeculae and calcified bone [126].

In the classification suggested by Misdorp et al., the phrase "malignant mixed tumor" was discontinued and the carcinomas associated with BMTs started to be named carcinomas in benign mixed tumors (CMBTs) or complex carcinomas [130]. Microscopically, CMBTs consist of malignant epithelial and benign mesenchymal elements that can be chondroid, osteoid or adipose tissue [130]. The epithelial element is seen as foci or nodules of cuboidal to columnar epithelial cells with different pleomorphisms, cellular atypia and atypical mitoses. Carcinomatous proliferation can invade or even entirely alter the pre-existing benign tumors [130].

CSs are rare tumors in canine mammary glands, compared with CBMTs and mimic those seen in humans. Presently, the word "carcinosarcoma" is applied to mixed tumors with both malignant epithelial and stromal mesenchymal elements [39] (Fig. 1b). These neoplasms could be well circumscribed and encapsulated, with a nodular shape or infiltrative edges [103]. The epithelial element of these tumors can exhibit adenomatous, mucinous, squamous or anaplastic growth patterns [130]. The mesenchymal element can also differ from fibrous tissue to cartilage to bone [39]. The metastasis in dogs is comparatively common compared to CBMTs.

Surgical removal is suggested in the therapy of all canine mammary tumors. Surgical delay can result in larger tumors and make their elimination more complicated [25]. CBMTs have a survival rate that is 2- to 3-times greater than that of other canine mammary carcinomas [43]. Therefore, animals presenting with this histological type have a good prognosis in comparison to animals with other forms of carcinomas [43]. CSs are aggressive tumors and have a poorer prognosis [131]. The average time between diagnosis and death for CS is 18 months [42].

Molecular Subtyping of CBMTs and CSs

CMTs can be classified into the same five molecular subtypes as human breast cancers. Immunohistochemical bio-markers have been suggested to help establish the molecular classification and describe these subtypes. These subtypes are classified into two hormone (ER and/or PR-positive subtypes: luminal A -like and luminal B -like) and three hormone receptor-negative subtypes (HER2-overexpressing, basal-like, and normal-like). Sassi at al. used a panel of antibodies specific for ER, PR, HER2, CK5/6 and CK14 on a series of 45 CMTs to characterize the molecular subtypes of human breast cancer and identified three tumor subtypes (luminal A-like, luminal B-like and basal-like) but there were no HER2-overexpressing or normal-like subtypes were present in their study [132]. Gama et al. used an immunohistochemical panel specific for (ER, HER2, CK5, p63 and P-cadherin) with a group of 102 CMTs and identified four tumor subtypes (luminal A-like, luminal B-like, basal-like and HER2-overexpressing) [133]. Saad et al. used a panel of antibodies specific for ER, PR, HER2, CK5/6, p63 and vimentin on a series of 101 CMMTs (including eight CMBTs and five CSs) to identify the molecular classification of CBMTs and CSs. They found, basal-like (31%), luminal B-like (38%), HER2-overexpressing (23%) and luminal A-like (8%) subtypes [125].

Mixed Mammary Tumors in Cats

Mammary tumors are the third most common type of cancer in cats, following cutaneous tumors and lymphoma [32, 134]. Benign tumors of the feline mammary gland are rare and account for only around 10% of these tumors [135]. Although the majority of feline mammary tumors are of epithelial origin, malignant changes may arise in mesenchymal tissues, causing the formation of mixed mammary tumors and sarcomas [136]. Feline mixed mammary tumors are less common than carcinomas [135].

CSs are a highly malignant mixed mammary tumor which contains a combined cell population with malignant proliferation of both mesenchymal and epithelial elements [137]. In cats, CSs are rare [138]. Only two cases have been reported, so it is impossible to know how prevalent CS is in cats. The age of both cats was 13 years old [32, 33].

Spontaneously Occurring Mixed Mammary Tumors in Rodents

Mixed mammary tumors do occur spontaneously, or they can be chemically induced in rodents. Usually both elements (epithelial and mesenchymal) are malignant [38]. Naturally-occurring mixed adenocarcinomas of the mouse mammary gland generally contain a minimum of two cell types. First, cuboidal or columnar epithelial cells form nests and/or glandular or papillary structures [139]. These cells may develop from either the mammary alveoli or from the ducts [139]. Second, the tumor elements that present may be of mesenchymal or myoepithelial origin [139]. Reham et al., (1989) examined three spontaneously arising and eight chemically-induced mixed mammary tumors in mice. The neoplastic glandular structures were surrounded by spindle-shaped cells that were markedly eosinophilic. These spindle-shaped cells initially appeared almost identical to fibroblasts and were considered to have a myoepithelial origin [139]. Rare strands of connective tissue were identified with Masson's trichrome stain [139]. Metaplastic cells in the spontaneous and chemically induced mixed mammary tumors were positive for keratin [139]. Myoepithelial cells in these tumors were also

positive for keratin and actin but negative for vimentin and desmin indicating their epithelial differentiation [139].

Mixed Mammary Tumors in Rabbits

Malignant mixed tumors of the mammary gland are unusual in rabbits and to the best of our knowledge only one case of a mixed mammary tumor has been reported. The 8-month old animal had a large lobulated mass in the mammary gland region. Microscopically, malignant epithelial cells and malignant connective tissue components were described [140].

Mixed Mammary Tumors in Other Large Domestic Animals

We found only one reported case of a mammary gland CS in one 16-year-old female horse. The mass was discovered by the owner during regular cleaning. Histopathological and immunohistochemical examinations reported that the tumor was a CS [141]. There are no reports of mixed mammary tumors in other large domestic animals.

Mixed Mammary Tumors in Wild Eutherian Mammals

Malignant mixed tumors of the mammary gland are unusual in wild eutherian mammals. There has been only one case of mixed mammary tumor reported in a female gray squirrel (*Sciurus carolinensis*) [142]. The morphology of this tumor was similar to the CSs seen in domestic animals (i.e. dogs and cats) [142].

Mixed Mammary Tumors in Non-Domestic Cats

Most mammary tumors in zoo cats are malignant, resembling those that occur in domestic cats [143]. One case of a malignant mixed mammary tumor has been reported in a captive lion (*Panthera leo*) [144]. The tumor had tubulopapillary, solid and cartilaginous elements and a mitotic index of 50 per 10 consecutive high-power fields. One case of a mixed tumor has been also reported in a captive tiger (*Panthera tigris*) and that tumor contained myoepithelial cells and adenocarcinomatous cells with squamous metaplasia [145].

Mixed Mammary Tumors in Marsupials and Monotremes

There are no reports of mixed mammary tumors in marsupials or monotremes. They do not develop mixed mammary tumors. Although marsupials and monotremes do share some features of mammary gland development (mammary hairs, areolar patches) with eutherian mammals, but in marsupials, the teat usually does not differentiate from mammary cells, but each teat commences as an individual analge [146].

Histogenesis of Mixed Mammary Tumors in Eutherian Mammals

The histogenesis of the different components of mixed mammary tumors is not fully understood. In terms of MBC, epithelial mesenchymal transition (EMT) is considered to be a key process in tumor progression [147]. Gwin et al. demonstrated that MBCs with chondroid differentiation, showed both an epithelial and a mesenchymal element, which included a cartilaginous matrix with morphological features analogous to EMT [147]. EMT is characterized by loss of epithelial phenotype proteins such as cytokeratins and E-cadherin and gain of mesenchymal proteins including vimentin [117, 148] .

Several studies have suggested a monoclonal origin of the epithelial and stromal components in human MBCs [149–152] and other studies have suggested that these tumors could be derived from myoepithelial cells [153, 154]. Whether the cell origin is luminal or myoepithelial may not be crucial, as recent data has suggested that the mutations in *PIK3CA* were present in luminal or myoepithelial cells in MBCs. *PIK3CA* is a subunit p110 alpha of phosphatidylinositol 3-kinase and it is one of the most commonly mutated genes in breast cancer [155].

Similar to human MBCs, the source of the various elements of mixed neoplasia in dogs is still unknown [43]. It has been suggested that the chondroid tissue in canine mixed mammary tumors is derived from epithelial cells [156, 157]. In addition to the epithelial cells, myoepithelial cells are present in MBCs and it has been suggested that these cells have a role in the origin of this type of tumor [39, 125, 158–162]. One hypothesis is that these elements arise from stem cells that have a high capacity for divergence [158, 163, 164]. In contrast, other studies have suggested that cartilage and/ or bone in MBCs are produced from the mesenchymal connective tissue [43, 156, 165].

In terms of CSs in cats, the histogenesis of this tumor type has been outlined in two hypotheses: (1) the multiclonal theory proposes that both epithelial and mesenchymal elements came from two or more stem cells; (2) the monoclonal theory proposes that the epithelial and the mesenchymal elements came from totipotent stem cells that can participate in several routes of terminal differentiation [32].

Conclusion

To summarize, spontaneously arising mixed tumors of the mammary gland occur in humans and non-human eutherian mammals (see Table 3). Mixed mammary tumors occur with low frequency in humans but are more common in dogs. They are rarely reported in cats, rodents, rabbits, horses and have not been reported in marsupials or monotremes. In humans, mammary gland mixed tumors can be benign (i.e. pleomorphic adenoma of breast) or malignant (i.e. metaplastic breast

Table 3 Summary comparison of mixed mammary tumors between different species

	Mixed mammary tumors					
Species	Type of mixed mammary tumor	Frequency	Age of onset	Subtypes	Histological features	
					Epithelial element	Mesenchymal element
Humans	Benign "Pleomorphic adenoma"	< 100 cases have been described	23 – to 78-year-old	Pleomorphic adenoma	Cuboidal to columnar cells with low mitotic activity	Chondroid and/ or osseous tissues
	Malignant "Metaplastic breast cancers"	≈ 0.25 to 1% of all breast cancers	55 –to 60-year--old	A purely subgroup	Spindle-cell carcinoma Squamous-cell carcinoma Low-grade adenosquamous carcinoma Myoepithelial carcinoma	
				A mixed subgroup	Carcinoma with mesenchymal differentiation [chondroid, osteoid and other types of messenchymal differentiation (carcinosarcoma)]	
Dogs	Benign	≈ 40%–50% of all mammary benign tumors	6 – to 10-year--old	Benign mixed tumor	Benign ductal and/or acinar epithelial cells and myoepithelial cells	Cartilage and/or bone formation combined with myxoid fibrous tissue
	Malignant	≈ 20% to 32% of all mammary malignant tumors		Carcinoma in benign mixed tumor	Tumor derived from a malignant transformation of the epithelial element of a benign mixed tumor	Cartilage and/or bone formation combined with myxoid fibrous tissue
				Carcinosarcoma	Malignant epithelial element (could be ductal adenocarcinoma)	Malignant mesenchymal element (cartilage and/or bone formation)
Cats	Malignant	No statistics have been documented	The age of both cats was 13	Carcinosarcoma	Malignant epithelial element	Malignant mesenchymal element (cartilage and/or bone formation)
Rodents	Malignant	No statistics have been documented	N/A	Mixed adenocarcinoma	Ductal adenocarcinoma	Malignant mesenchymal element (fibrous tissue)
Rabbits	Malignant	No statistics have been documented	8-month-old	Carcinosarcoma	Malignant epithelial cells	Malignant myxoid matrix and cartilage pieces
Pony mare	Malignant	No statistics have been documented	16-year-old	Carcinosarcoma	Malignant epithelial element	Malignant mesenchymal element
Captive lions	Malignant	No statistics have been documented	N/A	Malignant mixed mammary tumor	Tubulopapillary epithelial elements	Cartilaginous tissue
Captive tiger	Malignant	No statistics have been documented	14-year-old	Malignant mixed mammary tumor	Myoepithelial cells and adenocarcinomatous cells with squamous metaplasia	None
Marsupials and Monotremes	There are no reports of mixed mammary tumors					

N/A, not available

carcinoma). Benign mixed mammary tumors are usually associated with a good prognosis, but the malignant form is associated with a poorer prognosis. This comparative review has highlighted the similarity of CMMTs to MBCs and PAB in humans. Mixed mammary tumors are more common in dogs than humans and this larger sample pool together with their histological similarity could make them a very useful natural animal model. CMMTs could help to increase the knowledge of how this tumor type can progress from benign to malignant and possibly identify new therapeutic targets.

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