J. Comp. Path. 2017, Vol. ■, 1–14

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NEOPLASTIC DISEASE

Canine Mixed Mammary Tumour as a Model for Human Breast Cancer with Osseous Metaplasia

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Summary

Canine mixed mammary tumours (CMMTs) and human metaplastic breast carcinomas (HMBCs) share several histopathological features and risk factors. In both species, these tumours display epithelial and stromal components. HMBCs are rare malignant tumours, but CMMTs are one of the most common mammary tumours in dogs and are more often benign than malignant. In this study, benign ($n = 88$) and malignant ($n = 13$) CMMTs were characterized using specific antibodies against oestrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, cytokeratin 5/6, cytokeratin AE1/AE3, vimentin, Ki67, E-cadherin and p63. Cartilage and bone matrices associated with benign and malignant CMMTs were characterized using specific antibodies against BMP4, Runx2, Sox9 and osteopontin. The current study suggested that CMMTs are of epithelial origin, but display a myoepithelial-like differentiation. The findings suggest key roles for Sox9, Runx2 and BMP4 in chondrogenesis and bone formation in CMMTs. The high expression of osteopontin in CMMTs appears to be unrelated to tumour malignancy.

Keywords: dog; human breast cancer; immunohistochemistry; mixed mammary tumour

Introduction

Breast cancer is the most common form of neoplasia in women globally, with more than 1.3 million cases diagnosed every year (Pezzi *et al.*, 2007). The most common type of breast cancer, accounting for 85% of cases, is invasive ductal carcinoma (IDC) (Pezzi *et al.*, 2007). Metaplastic breast cancer (MBC) is unusual. Of the 365,464 diagnosed cases of breast cancer reported to the US National Cancer Database between 2001 and 2003, 892 were MBCs (Pezzi *et al.*, 2007). Therefore, the incidence of MBCs was 0.24% (Pezzi *et al.*, 2007; Chuthapisith *et al.*, 2013). MBC encompasses a group of tumours in which neoplastic epithelium differentiates into mesenchymal components (i.e. osseous, chondroid or spindle cells) (Chuthapisith *et al.*, 2013). MBCs do not express oestrogen receptor (OR), progesterone receptor (PR) or human epidermal growth factor receptor 2 (HER2) (triple negative), but may express cytokeratin 5/6 (CK5/6) (Zhang *et al.*, 2015). This tumour type is aggressive, as patients may develop extensive systemic metastasis (Huvos *et al.*, 1973). The pathogenesis of MBC is not well understood (Dantas Cassali *et al.*, 2012). The absence of OR, PR and HER2 expression renders hormone therapy and targeted (anti-HER2) therapy ineffective. So far, there is no successful therapeutic regimen that is effective for MBCs (Rakha *et al.*, 2015).

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0021-9975/$ - see front matter

http://dx.doi.org/10.1016/j.jcpa.2017.03.005

Please cite this article in press as: Saad ES, et al., Canine Mixed Mammary Tumour as a Model for Human Breast Cancer with Osseous Metaplasia, Journal of Comparative Pathology (2017), http://dx.doi.org/10.1016/j.jcpa.2017.03.005

The molecular classification of breast cancer categorises tumours into five subtypes and this profiling has been claimed to lead to better clinical outcomes (Hu *et al.*, 2006). The subtypes are determined on the basis of differing expression of 'intrinsic' genes. The subtypes are: luminal A-like, luminal B-like, basal-like, HER2 overexpressing and normal breast tissue-like (Perou *et al.*, 2000). The classification can assist in deciding which patients will benefit from different therapies. For instance, patients with luminal A-like subtype tumours only need hormonal treatment (Eroles *et al.*, 2012). Chemotherapy is the optimal therapy for luminal B-like subtype, HER2-positive subtype and triple-negative tumours, and HER2-positive tumours are treated with anti-HER 2 therapy (Eroles *et al.*, 2012). Variations in gene expression patterns among these subtypes indicate fundamental changes in their cellular biology and that these variations give rise to different outcomes (Sorlie *et al.*, 2003). People with basal-like cancers have the poorest survival, as they are triple-negative cancers lacking OR, PR and HER2, so the only treatment available is chemotherapy (Sorlie *et al.*, 2001).

Nielsen *et al.* (2004) used immunohistochemistry (IHC) to classify human breast cancers. Tumours that were positive for HER2 were placed in the HER2-overexpressing group, tumours that were HER2 negative and OR positive were placed in the luminal category, HER2- and OR-negative tumours that were also positive for a minimum of one of cytokeratin 5/6 and/or HER1 were placed in the basal-like category. Any tumour that was negative for HER2, OR, CK/5/6 and/or HER1 markers was placed in the negative group (Nielsen *et al.*, 2004; Stevens, 2014).

In dogs, OR-positive tumours have also been classified as being of luminal subtype, with HER2-positive tumours being luminal B-like and HER2-negative tumours classified as luminal A-like (Gama *et al.*, 2008). Sassi *et al.* (2010) used PR expression to categorize tumours as being of luminal subtype (Sassi *et al.*, 2010). OR negativity with HER2 positivity (and PR negativity in Sassi *et al.*, 2010) specifies a HER2-overexpressing subtype (Sassi *et al.*, 2010). Basal-like tumours were similar to the HER2-overexpressing tumours except that they were negative for HER2 and positive for the basal markers (CK5/6 and p63), and the negative subtype was negative for all markers (Stevens, 2014).

Mixed tumours are uncommon in women, but they are common in dogs (Misdorp *et al.*, 1999). In contrast to human metaplastic breast cancers (HMBCs), canine mixed mammary tumours (CMMTs) are one of the most prevalent mammary tumours (50–66%) in female dogs (Misdorp, 2008). CMMTs have a complicated histological pattern as they contain both epithelial and stromal components (Goldschmidt *et al.*, 2011). Benign CMMTs are identified by the co-existence of benign glandular components, myoepithelial proliferation and admixed myxoid, cartilaginous and/or osseous tissue (Misdorp, 2008; Goldschmidt *et al.*, 2011). The myoepithelial 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 present, it can consist of osteoid or calcified bone (Goldschmidt *et al.*, 2011). Malignant CMMTs contain both benign stromal (e.g. cartilaginous and/or osseous tissues) and malignant epithelial elements, and usually have very aggressive behaviour (Misdorp, 2008; Goldschmidt *et al.*, 2011).

Similar to the situation in HMBCs, the source of various elements of mixed neoplasia in CMMTs is not well understood (Pena *et al.*, 2013). Normally, the human mammary gland is composed of two layers of cells, an internal luminal layer made up of glandular epithelial cells and an outer basal layer of myoepithelial cells, which can be cuboidal or spindle shaped, depending on their location in the mammary gland duct system and on the hormonal status of the breast (Gusterson *et al.*, 2005).

What is significantly less obvious is which cells within these tumours have a tumour-initiating cell (i.e. cancer stem cell) function and are able to maintain cell growth (Dick, 2003). It has been proposed that stem cells may be the targets of transformation during tumour histogenesis, resulting in the heterogeneity of breast cancer (Dontu *et al.*, 2004).

It has been suggested that the chondroid tissue in mixed mammary tumours is derived from epithelial cells (Dantas Cassali *et al.*, 2012), but a role for myoepithelial cells in the origin of this type of tumour has also been suggested (de Los Monteros *et al.*, 2002). In this regard, a myoepithelial ontogeny theory has been proposed, which suggests an epithelial, mesenchymal or a stem cell morphogenesis for CMMTs (Hellmen *et al.*, 2000). For HMBCs, a monoclonal origin for epithelial and stromal components has been postulated (Zhuang *et al.*, 1997) and a myoepithelial cell origin has also been documented (Reis-Filho and Schmitt, 2003).

No previous study has looked for the presence of bone morphogenetic protein 4 (BMP4), Runt-related transcription factor 2 (Runx2) and SRY-Box 9 (Sox9) in CMMTs. Only a few studies have investigated the roles of these markers in HMBCs (Barnes *et al.*, 2003; Kusafuka *et al.*, 2008) and since Sox9 and BMP4 are essential in cartilage formation (Chimal-Monroy *et al.*, 2003; Kusafuka *et al.*, 2008)

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