INTERSPECIES SIMILARITIES BETWEEN CANINE AND HUMAN MAMMARY CANCER

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ABSTRACT

The study investigates the interspecies similarities between canine and human mammary cancer in the sense of innovative predictive and prognostic tumour markers. Surgical resection specimens with diagnosed spontaneous primary mammary cancer obtained from 100 female canine patients were included in this study. Expression of carbonic anhydrase IX (CAIX) enzyme and human epidermal growth factor receptor 2 (HER2) expression was evaluated immunohistochemically. The study was completed with investigation of Ki67 expression and proliferation with marker of myogenous differentiation. Histopathological grading was performed using the Nottingham/modified Bloom-Richardson system. As in humans, our analysis of canine mammary cancer has shown that CAIX positivity in tumour cells significantly correlates with higher levels of HER2 immunoreactivity (P = 0.001), and increased tumour grade (P < 0.001). The percentage of smooth muscle actin (SMA) positive cases was significantly higher (P = 0.002) in the group of mammary carcinomas with CAIX positivity compared to the tumours that were negative. Using antibody Ki67 proliferative activity was not significantly different between mammary tumours that were CAIX positive and CAIX negative. Canine mammary gland carcinomas may, therefore, represent valuable animal models for the study of hypoxic signaling pathways involved in mammary carcinogenesis in humans.

INTRODUCTION

Mammary cancer is a heterogeneous oncological disease characterized by variability in histopathological characteristics, in biological behaviour, and in the possibilities of therapeutic management (Bergholtz et al. 2022). Besides the analysis of human epidermal growth factor receptor 2 (HER2) expression and other commonly used predictive and prognostic markers in mammary cancer, the study of hypoxic signalling pathways has gained attention in oncological research because of possible therapeutical implications (Gloyeske et al. 2015; Ahn et al. 2020; Bonacho et al. 2020). In the normal cell, HER2 is a key component of a complex signalling network and plays a critical role in the regulation of tissue development, growth, and differentiation (Sundaresan et al. 1999). In such studies, carbonic anhydrase IX (CAIX) has emerged as one of the most promising biological markers of hypoxia in mammary carcinoma tumour cells (Campos et al. 2022). CAIX expression occurs when tumour growth exceeds vascularization due to hypoxia (Maxwell et al. 1999). However, the association between CAIX expression and the degree of differentiation, the HER2 status, as well as other predictive and prognostic biomarkers has not yet been elucidated.

Ki67marker is strongly associated with tumour cell proliferation and growth. The nuclear protein (pKi67) is an established prognostic and predictive indicator for the assessment of biopsies from patients with cancer (Li et al. 2015). The prognostic value of pKi67 has been investigated in a number of studies as a reliable marker in tumours including those of the mammary glands (de Azambuja et al. 2007; Li et al. 2015; Davey et al. 2021).

Smooth muscle actin (SMA) has long been used as a myoepithelial marker in human mammary pathology diagnosis as a sensitive marker of myogenous differentiation (Zaha 2014). It is known that invasive carcinomas lack the myoepithelial cell layer that normally surrounds benign mammary gland tumours (Yeh and Mies 2008).

Study analysis

In order to identify causal molecular events driving carcinogenesis and to determine the association between biomarkers characterizing the tumour cells of mammary cancer, the development of cross-species comparison analysis.

Animal model

Dogs as a potential animal model for the study of mammary cancer because of the interspecies similarities in the sense of aetiology and pathogenesis of this common malignancy.

HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY

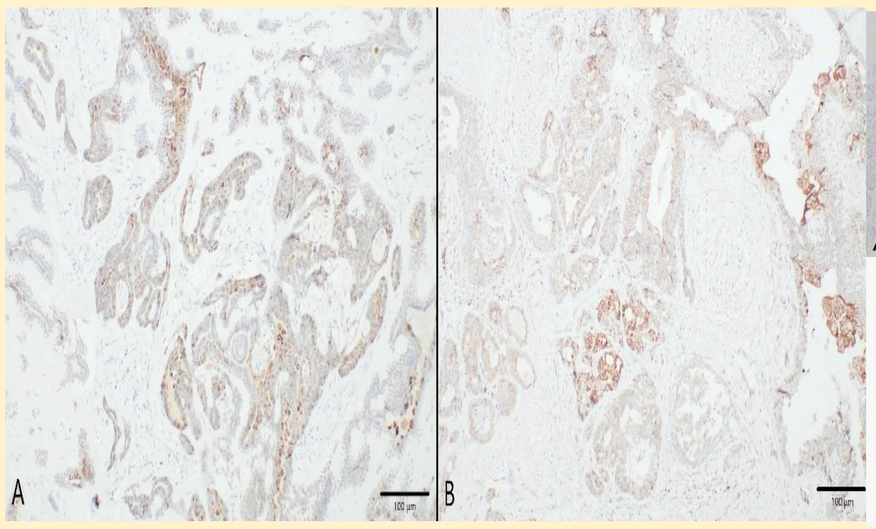

Fig. 1. A, B – Canine mammary carcinomas: carbonic anhydrase IX (CAIX) positivity defined as cytoplasmic staining present in at least 5% of tumour cells (CAIX immunohistochemistry, bar 100 µm)

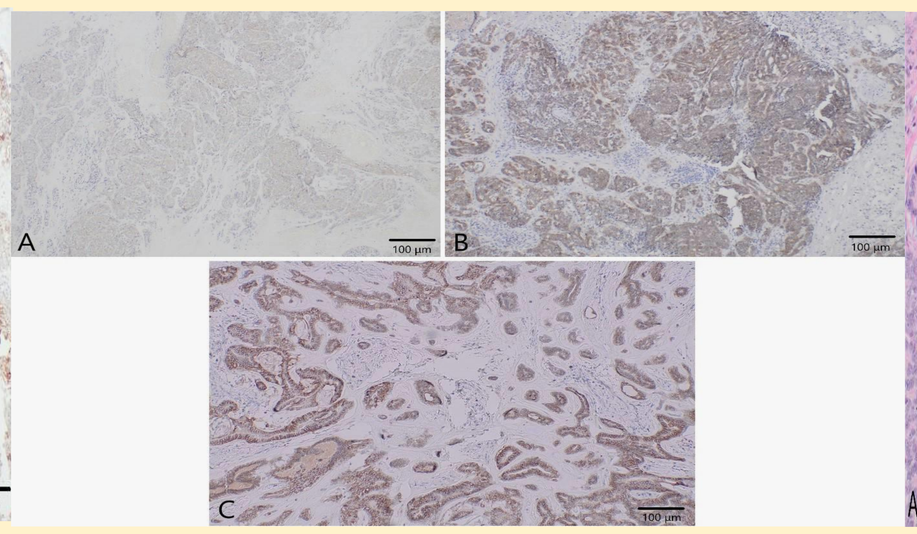

Fig. 2. Canine mammary carcinomas: three degrees of human epidermal growth factor receptor 2 (HER2) positivity: A – degree 1+; B – degree 2+; C – degree 3+; (HER2 imunohistochemistry, bar 100 µm)

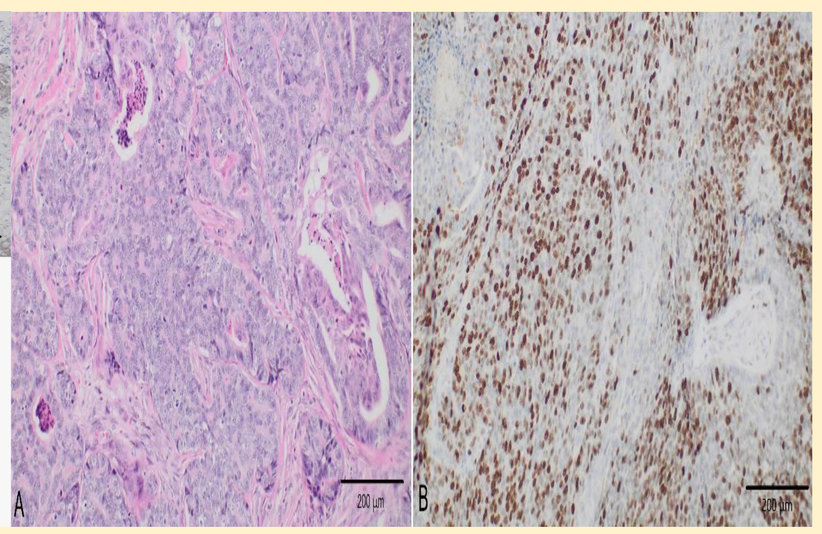

Fig. 3. A - grade 3 canine mammary carcinoma (haematoxylin-eosin), B - corresponding Ki67 positivity in tumour cells (Ki67 immunohistochemistry, bar 200 µm

MATERIAL METHODS

Histopathology

- Tissue samples with diagnosed spontaneous primary mammary cancer obtained from 100 female canine patients.
- Excised samples were fixed in 10% neutral buffered formalin and paraffin embedded
- 5-µm thick sections were stained with haematoxylin-eosin
- Tumours were allocated to specific histopathological subtypes in accordance with the World Health Organization (WHO) classifications of mammary tumours (WHO Classification of Tumours Editorial Board 2019).
- Histopathological grading was performed using the Nottingham/modified Bloom-Richardson system (Meyer et al. 2005)
- Mammary cancer samples were allocated to one of three categories of histological grade: **well-differentiated (grade 1), less-differentiated (grade 2), or poorly differentiated (grade 3)**. For analytical purposes, a two-tiered system was used which divided the sample into 'poorly-differentiated' (grade 3) and 'better-differentiated' (grade 1 and grade 2) categories.

Immunohistochemical analysis

- Statistical analysis: result obtained from IHC analysis of CAIX were correlated with results of HER2, SMA proliferative activity determined by Ki67 and with degree of tumour differentiation. Statistical analysis was performed using two- samples test of the equality of proportions with the continuity correction. A p value below 0.05 was considered significant.

RESULTS

- Invasive ductal carcinoma of no specific type represents 87/100 (87%) cases,
- Less commonly were subtypes included the **tubular carcinoma subtype** 8/100 (8%) and **invasive lobular carcinomas** 5/100 (5%)
- Considering degree of the differentiation: 51 mammary carcinomas (51%) 'better differentiated' (grade 1or grade 2) and 49 cases (49%) were grade 3 tumors.
- Carbonic anhydrase IX positivity was demonstrated in 62 mammary carcinomas (62%),
- HER2 expression with positivity at 2+ /3+ degrees was detected in 43 mammary carcinomas (43%)
- Borderline or negative result 1+ or 0 degree were observed in 57 cases (57%)
- SMA positivity was demonstrated in 40 mammary carcinomas (40%)
- A high proliferative score Ki67 ≥ 14% was find in 53 cases and lower than 14% was detected in 47 mammary carcinomas (47%).

- Nottingham/ modified Bloom- Richardson system showed for expression of **CAIX IX** significantly correlated with poor differentiation of the evaluated tumors; In 48 cases **(77.4%)** were positive at the grade of 3; in 5 cases with CAIX negative were categorized as poorly differentiated, grade 3 mammary carcinomas which was significant at p < 0.001.

Table. 1 Monoclonal antibodies used in immunohistochemical analysis of 100 canine mammary carcinomas

Specifity	Clone	Dilution	Source
CAIX mouse MoAb	CAIX/SAV BA	1:200	Agilent DAKO (Santa Clara, USA
HER mouse MoAb	c-erbB-2 Oncoprotein	1:200	Agilent DAKO (Santa Clara, USA
SMA mouse MoAb	1A4	Ready to use	Agilent DAKO (Santa Clara, USA
Ki67 mouse MoAb	MIB-1	Ready to use	Agilent DAKO (Santa Clara, USA

CAIX- carbonic anhydrase IX; HER-human epidermal growth factor receptor 2; SMA-smooth muscle actin; Ki67 mouse MoAb

Table 2. Distribution of evaluated markers in the analysed samples of 100 canine mammay carcinomas

Clinicopathological variables		Number (%)
CAIX	Positive Negative 2+ or 3+	62 (62.0) 38 (38.0) 43 (43.0)
HER2	1+ or 0	57 (57.0)
SMA	Positive Negative	40 (40.0) 60 (60.0)
Ki67	≥ 14% < 14%	53 (53.0) 47(47.0)
Tumor differentiation	Grade 3	53 (53.0)
Tumor differentiation	Grade 1/ Grade 2	47 (47.0)

DISCUSSION

Comparative cross- species studies are of immense importance for the purpose of identification of casual molecular events driving carcinogenesis and to determine the association between biomarkers characterizing tumor cells of mammary cancer.

In our study we determined the expression rate of novel predictive/ prognostic markers in tumour tissue of canine mammary gland carcinomas and compared it with the results of the corresponding human mammary cancer.

Inadequate blood supply in the tumor tissue is a result of uncoordinated and inappropriate formation of blood vessels in the rapidly proliferating and growing tissue. Hypoxia is a major clinical importance because it is associated with resistance to the most chemoterapeutical approaches (Ilardy, et al., 2014). Malignancies with histomorphological evidence of hypoxia have been found to be more often poorly differentiated , high grade tumours (van Kuijk et al. 2016).

Evaluation of the hypoxic state in malignat tumors has clinical implications there is a ongoing search for IHC markers determining this tumour feature (Lock et al., 2013) Generali et al. (2006) demonstrated that CAIX positivity correlates with HER2 overexpression in the tumour tissue of human breast cancer. Also, amplification of HER2 occurs in nearly 25% of all breast cancer types and enhance its aggressiveness (Wolff et al. 2013).

A positive correlation between a higher degree of CAIX epression and higher levels of HER2 immunoreactivity in tumour tissue of human mammary cancer was also evidenced by results presented by authors (Bartošová et al., 2002).

In our study, we have shown that significant association between CAIX positivity and higher level of HER2 overexpression seems to be present also in canine mammary gland carcinoma. These results suggest that dogs share similar pathogenetic mechanism implicated in the process of mammary carcinogenesis as those in humans. The data which are related to amplification and/or molecular factors leading to HER2 overexpression. SMA positive tumour stroma was higher in the tumour group characterized as CAIX expression than in the CAIX negative group. In women, it seems that SMA- rich stroma has been linked to resistance to trastuzumab (Kim et al., 2018; Vathiotis et al. 2021). Brennan et al. (2006) concluded that CAIX expression in breast cancer tumor cells is associated with the large tumor size, higher grade and overall poor prognosis in premenopausal women.

CAIX positivity was also linked to resistance to chemotherapy (Brennan et al, 2006). By other authors as Furlejova et al. 2014, van Kuijk et al. 2016 also showed to the association between CAIX positivity and lower overall survival , poor differentiation, and the presence of necrosis in tumour tissue. Our result suggest that a similar relationship is present also in spontaneous canine mammary gland carcinoma.

Other facts was prooved according to the Nottingham/ modified Bloom-Richardson system were been determined proportion of cases with grade 3 mammary cancer with result that was higher in groups of tumours characterized by CAIX positivity compared to the CAIX- negative tumor group. In conclusion our study has shown that the expression CAIX and other novel predictive and prognostic markers in canine mammary gland carcinomas is comparable to the corresponding human breast cancer. As is the case in humans, CAIX positivity in tumour cells is significantly correlated with higher degree of HER2 immunoreactivity and higher tumour grade determined by the Nottingham/ modified Bloom- Richardson system. Mammary carcinomas CAIX positive were mostly SMA positive. Canine mammary gland carcinoma therefore may represent a valuable animal model for the study of hypoxic signaling pathways.

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