The role of CD44 adhesion factor in canine mammary carcinomas

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Abstract

CD44 is an adhesion molecule implicated in the progression of human breast cancer. The purpose of this study was to describe CD44 antigen expression in canine mammary carcinomas and to evaluate its prognostic significance in relation to other clinico-pathological variables.

A reduction in CD44 expression was significantly related to variables such as tumour size and adherence to underlying tissues but was not related to tumour location or to ulceration of the overlying skin. Complex (grade I) and anaplastic (grade III) carcinomas exhibited more intense expression of this antigen than did some tubulopapillary and most solid carcinomas (grade II). Although reduced CD44 expression was associated with infiltrative growth and vascular invasion in solid carcinomas, intense expression was also observed in anaplastic tumours. Although overall these findings suggest a role for this adhesion factor in canine mammary tumour development and progression, the complexity and apparently paradoxical nature of some of the findings currently limit the use of this immunohistochemical marker as a prognostic indicator.

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Introduction

CD44 antigen represents a polymorphic family of immunologically related cell surface proteoglycans and glycoproteins which participate in cell to cell and cell to matrix adhesion, in lymphocyte activation and homing, and in cell migration (Goodison et al., 1999). Of the 20 exons that contribute to the CD44 molecule, the first and terminal five are constant, coding for an intra-cellular domain that interacts with the signalling molecules and the cytoskeleton, and for an extra-cellular domain that recognises hyaluronan, respectively (Naor et al., 1997). The 10 exons between these regions are variables as they are subject to alternative splicing.

Differential utilisation of this variable region generates multiple isoforms of CD44, termed CD44v, of different molecular size (Naor et al., 1997), the smallest of which is termed standard CD44 (CD44s). Standard CD44 antigen is expressed in a wide range of normal tissues whereas the expression of variant isoforms of CD44 is highly restricted in normal tissue (Martegani et al., 1999).

CD44 antigen expression patterns have been implicated in facilitating breast cancer progression through altering tumour cell adhesion characteristics and therefore facilitating vascular and tissue invasion (Hanahan and Weinberg, 2000; Bourguignon, 2001; Mine et al., 2003; Draffin et al., 2004). Although the relationship between the CD44 expression and other clinico-pathological characteristics of human breast cancer has been the subject of debate (Bankfalvi et al., 1998; Bourguignon, 2001; Morris et al., 2001; López et al., 2005), most studies suggest that CD44 expression correlates with tumour metastasis and is therefore a useful prognostic indicator (Bankfalvi et al., 1998; Mine et al., 2003; Draffin et al., 2004; López et al., 2005).

Despite the study of CD44 in human breast cancer, little is known of the expression of this adhesion factor in normal or neoplastic canine mammary tissue. The aim of the current study was to define CD44 expression in the normal canine mammary gland by immunohistochemistry, and to investigate if this expression was altered in mammary carcinomas. Furthermore, the potential value of CD44 as a prognostic indicator was assessed by correlating its expression with other clinico-pathological features of these tumours.

Material and methods

Case selection and clinical features

A total of 63 mammary carcinomas were obtained from 49 female dogs aged from 4 to 15 years, of pure and mixed breeds. Normal-appearing mammary glands from four female dogs (two pregnant and two lactating animals) that had died from road traffic accidents or poisoning were included as controls. The following clinical features of each tumour were recorded: its location within the mammary gland chain; the presence of overlying skin ulceration; and adherence to underlying tissues. The tumours were also classified by size according to the World Health Organization (WHO) clinical staging system (Owen, 1980).

Histopathological and immunohistochemical examinations

All samples were formalin-fixed and wax-embedded. Sections of 3 μm thickness were stained by the haematoxylin and eosin method and were examined and classified as mammary carcinomas in terms of their histological type in accordance with the WHO classification system (complex, tubulopapillary, solid and anaplastic) (Misdorp et al., 1999). A further classification of the tumours based on histological grade (increasing evidence of anaplasia) into grades I, II and III was also carried out (Misdorp, 2002).

Additional dewaxed sections were examined immunohistochemically by an avidin-biotin-peroxidase complex (ABC) method with diaminobenzidinetetrachloride as chromogen substrate (Vector Laboratories). Dewaxed and rehydrated sections were incubated with the primary antibody IM7, a rat monoclonal pan-specific anti-CD44 antibody that recognises the extra-cellular constant domain (Santa Cruz Biotechnology), at a 1:400 dilution. Histologically normal canine mammary tissue served as a positive control tissue and primary antibody was omitted from the procedure to establish negative controls.

Tissue sections were examined independently by two veterinary pathologists. A number of neoplastic cells labelling positively for CD44 antigen were counted in 10 high power fields (magnification factor of 400) for each tissue section and were scored semi-quantitatively as <10%, between 10% and 70%, or >70% cells positive (Saddik and Lai, 1999).

Statistical methods

To assess potential associations between CD44 antigen expression and the other clinico-pathological variables examined, the data were grouped in contingency tables and were analysed using the Chi-square test or Fisher exact method for comparison of proportions. Analyses were performed using SPSS 14.0 for Windows software and differences were considered significant when $P < 0.05$.

Results

CD44 antigen expression in normal mammary gland

The CD44 expression pattern within mammary alveoli was dependent on the activity status of the gland. In developing alveoli of the pregnant dogs, most myoepithelial cells and the basal and some lateral surfaces of epithelial cells labelled positively (Fig. 1A). During lactation, myoepithelial and scattered epithelial cells at the base of alveoli expressed CD44 (Fig. 1B). In inactive glands, only few myoepithelial cells expressed CD44. At all stages of glandular development, antigen staining was largely membranous in both myoepithelial and epithelial cells.

CD44 expression in the interlobular and intralobular ducts was not dependent on the activity status of the gland. Intense antigen staining was observed on the lateral surfaces of the ducts and at the interface between the ductal epithelium and myoepithelium. CD44 expression was usually absent from the apical epithelial surface and from the basal aspect of the myoepithelium in contact with the underlying stroma.

CD44 expression in canine mammary carcinomas

The correlation of CD44 expression relative to selected clinical and histopathological criteria is summarised in Tables 1 and 2, respectively. A reduction in CD44 expression was significantly associated with increasing tumour size and with adherence to underlying tissues. Thus, tumours with diameter >5 cm exhibited significantly lower CD44 expression than did tumours of <3 cm diameter ($P < 0.05$). Likewise, tumours with adherence to underlying tissues exhibited significantly lower CD44 expression than did tumours without adherence ($P < 0.05$) (Table 1). No statistically significant association was found between CD44 expression and either ulceration of the overlying skin or with tumour location.

Grading of the 63 cases based on histological type identified 25 complex, 17 tubulopapillary, 17 solid and four anaplastic carcinomas. Comparison of immunolabelling patterns between these tumour subtypes identified significantly lower antigen expression in solid relative to complex ($P < 0.0001$), tubulopapillary ($P < 0.05$) and anaplastic
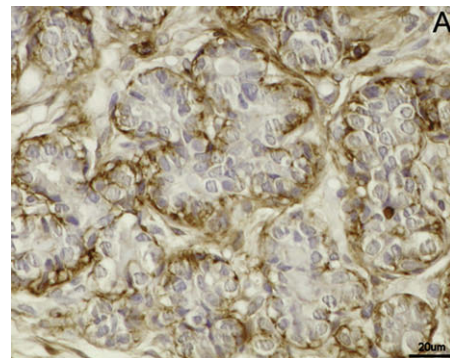

Fig. 1A. Photomicrograph illustrating normal canine mammary gland from a pregnant female. Developing alveoli exhibit membrane-associated CD44 antigen staining of most of the epithelial and myoepithelial cells. ABC-Peroxidase method. Bar = 20 μm.