J. Comp. Path. 2014, Vol. 151, 339–346

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

www.elsevier.com/locate/jcpa

NEOPLASTIC DISEASE

Clinicopathological Significance of Immunoexpression of Claudin-1 and Claudin-7 in Feline Mammary Carcinomas

A. Rute Flores, A. Rêma, F. Carvalho, G. Lopes, A. Faustino and P. Dias Pereira

Instituto de Ciências Biomédicas de Abel Salazar, University of Porto, Rua Jorge Viterbo Ferreira N°228, 4050-313 Porto, Portugal

Summary

Claudins (CLDNs) are tight junction proteins that have a role in regulating cell adhesion and polarity, paracellular permeability, proliferation and differentiation. Several immunohistochemical studies have shown reduced expression of CLDN-1 and CLDN-7 in human and canine mammary carcinomas, suggesting that these proteins may participate in mammary carcinogenesis, invasion and metastasis. The present study characterizes expression of CLDN-1 and CLDN-7 in feline mammary carcinomas ($n = 52$) and their metastases ($n = 29$). There was an inverse association between CLDN-7 expression and histological grade of tumour. Reduced expression of CLDN-7 was significantly associated with decreased tubule formation, high proliferative activity and metastasis. No significant associations were found between CLDN-1 expression and any of these features. Evaluation of expression of CLDN-7, but not CLDN-1, may therefore provide prognostic information, assisting in the diagnosis of a subgroup of aggressive feline mammary carcinomas that share some features with the recently described 'claudin-low' subgroup of human breast cancer.

Keywords: carcinoma; cat; claudin; mammary

Introduction

Claudins (CLDNs) are tetraspan transmembrane proteins of tight junctions (TJs) that have an important role in regulating cell adhesion, proliferation and differentiation, in maintaining cell polarity in epithelial and endothelial cells and in controlling paracellular permeability (Morin, 2005; Lal-Nag and Morin, 2009). In mammals, the CLDN multigene family consists of 27 members, ranging in size from 17 to 27 kDa, which in their molecular structure have four transmembrane helices, with a cytoplasmic NH_2-terminal sequence, two extracellular domains and a cytoplasmic COOH-terminal sequence (Van Itallie and Anderson, 2006; Mineta *et al.*, 2011).

The high degree of cellular organization in normal differentiated tissues is often lost in cancer. In the course of carcinogenesis, neoplastic cells undergo a series of morphological and functional changes, involving higher proliferative activity, decreased differentiation and loss of cell polarity and adhesion, which result in lack of normal tissue architecture (Morin, 2005; Martin and Jiang, 2009; Ouban and Ahmed, 2010). Breakdown of cell–cell interactions and deregulation of the expression of junctional proteins are believed to represent critical events in neoplastic transformation, invasion and metastasis (Dhawan *et al.*, 2005). Changes in the expression pattern of CLDNs are associated with the development of different human tumours, including breast cancer (Swisshelm *et al.*, 2005; Martin and Jiang, 2009; Ouban and Ahmed, 2010).

Correspondence to: P. Dias Pereira (e-mail: pdiaspereira@yahoo.com.br).

0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2014.07.006

The exact mechanisms by which CLDNs participate in tumourigenesis are poorly understood, but the recent characterization of a new 'claudin-low' molecular subgroup of human breast cancer, associated with a poor prognosis, underlines the importance of these molecules (Hennessy *et al.*, 2009). In the last decade, several immunohistochemical studies have shown reduced expression of CLDN-1 and -7 in human breast carcinomas and associated this expression pattern with poor prognostic indicators, such as high histological grade, lymph node metastasis and shorter disease-free survival (Kominsky *et al.*, 2003; Sauer *et al.*, 2005; Szasz *et al.*, 2011; Bernardi *et al.*, 2012).

Recently, Jakab *et al.* (2008b) characterized the immunohistochemical expression pattern of CLDN-1 and -7 in canine mammary tumours. The results of that study were similar to those observed for human breast cancer, suggesting that loss of expression of CLDN-1 and -7 may lead to cellular disorientation, detachment and invasion.

Mammary tumours are the third most commonly diagnosed cancer in female cats. Approximately 85–93% of the lesions are malignant and highly infiltrative, characterized by an aggressive biological behaviour with early metastasis (Misdorp, 2002; Lana *et al.*, 2007). However, the involvement of CLDNs in feline mammary carcinogenesis is poorly understood. Recently, CLDN-2 expression was evaluated in a series of feline mammary carcinomas and a significant association between reduced CLDN-2 expression and high histological grade and metastasis was shown (Flores *et al.*, 2014). The aim of the present study was to evaluate the immunohistochemical expression of CLDN-1 and -7 in feline mammary carcinomas and their metastases and to determine whether there was an association between expression of these molecules and particular clinicopathological features of the tumours.

Materials and Methods

Specimens

Fifty-two spontaneously arising feline mammary carcinomas and corresponding regional lymph node metastases ($n = 29$), obtained by surgical excision ($n = 49$) or at the time of necropsy examination ($n = 3$), were included in this study. Samples of normal mammary tissue were obtained at the time of necropsy examination from five female cats that died of causes not related to mammary disease.

Tumour tissue was processed, embedded in paraffin wax and three consecutive histological sections (2 μm) were cut from each block. One was stained with haematoxylin and eosin (HE) for histological examination and the others were used for immunohistochemistry (IHC). All cases were classified (Table 1) by two independent observers (PDP and AF) according to the diagnostic criteria proposed by the World Health Organization (WHO) classification of mammary tumours of domestic animals (Misdorp *et al.*, 1999).

Histological grading was performed according to the Nottingham method proposed by Elston and Ellis (1996), based on the assessment of three morphological features: tubule formation, nuclear pleomorphism and number of mitoses. Each of these features was scored on a 1 to 3 scale (tubule formation: 1, >75% of tumour area; 2, 10–75% of tumour area; 3, <10% of tumour area; nuclear pleomorphism: 1, small, regular and uniform nuclei; 2, increased nuclear size and variation; 3, marked nuclear variation; mean number of mitoses per ×400 field: 1, 0–8 mitoses; 2, 9–17 mitoses; 3, >18 mitoses).

The scores for each parameter were added to obtain an overall tumour grade: grade 1 (3–5 points), well-differentiated tumour; grade 2 (6–7 points), moderately differentiated tumour; grade 3 (8–9 points), poorly differentiated tumour. Of the 52 carcinomas included in this study, 17 (33%) were grade I, 16 (31%) were grade II and 19 (36%) were grade III.

Table 1
Histological classification of the feline mammary tissues included in the study

Histological classification	*Number of samples*
Normal mammary gland	5
Malignant tumours	52
Tubulopapillary carcinoma	32
Grade I	17
Grade II	10
Grade III	5
Solid carcinoma	12
Grade II	2
Grade III	10
Squamous cell carcinoma	5
Grade II	2
Grade III	3
Anaplastic carcinoma	2
Grade II	1
Grade III	1
Lipid-rich carcinoma	1
Grade II	1
Metastatic lesions (neoplastic emboli/ lymph node metastasis)	29