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Immunolocalization of podoplanin in benign odontogenic tumours with and without ectomesenchyme

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ABSTRACT

Objectives: To investigate podoplanin expression in epithelial odontogenic tumours with and without ectomesenchyme and verify the association between its immunoexpression and proliferative activity in keratocystic odontogenic tumours (KCOTS) and orthokeratinized odontogenic cysts (OOCs).

Design: Eight ameloblastomas, nine adenomatoid odontogenic tumours, twenty KCOTS, five OOC, one calcifying epithelial odontogenic tumour, two ameloblastic fibromas, four ameloblastic fibro-odontomas and five calcifying cystic odontogenic tumours were immunohistochemically analysed with anti-podoplanin antibody. For KCOTS and OOC, the cell proliferation index was determined with Ki-67 immunostaining and compared by Spearman correlation coefficient.

Results: Podoplanin was expressed in the peripheral odontogenic epithelium of most tumours. Ectomesenchyme was negative, except for odontoblasts. KCOTS exhibited positive podoplanin expression while in OOC it was absent/weak. There was statistically significant correlation ($p = 0.006$) between podoplanin expression and cellular proliferation index of KCOTS and OOC.

Conclusion: Podoplanin seems to be related to the proliferative activity of KCOTS and may have a role in the process of local invasion of odontogenic tumours with and without ectomesenchyme.

1. Introduction

Podoplanin is a mucin-type glycoprotein firstly identified in podocytes.[1] This protein has been widely used as a lymphatic endothelial cell marker once it is expressed in lymphatic vessels but not in blood vessels.[2] It has been demonstrated that podoplanin causes actin cytoskeleton rearrangement through RhoA GTPase activation to phosphorylate ezrin, promoting epithelial–mesenchymal transition and facilitating cell

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migration.[3] Podoplanin is found in various healthy and diseased tissues, including oral benign and malignant tumours.[4–13]

Recent investigations have focussed in studying its expression in the epithelium of benign odontogenic tumours.[5,6,8,12–14] These investigations demonstrated that podoplanin immunostaining is basically found in the epithelial cells located in the invasion front of ameloblastomas, keratocystic odontogenic tumours (KCOTS), adenomatoid odontogenic tumours and calcifying epithelial odontogenic tumours.[6,8,12,14] On the other hand, central epithelial cells of those tumours present slight or negative podoplanin expression. In the same way, more mature and less active locations, *i.e.* squamous metaplasia areas, acanthomatous and granular cells of ameloblastomas and supra-basal layers of KCOTS lack podoplanin staining.[6,8,12,14] In odontomas, the podoplanin expression was detected in developing and mature odontoblasts and secretory ameloblasts while mature ameloblasts did not express podoplanin.[5]

An investigation to verify if podoplanin expression could be a useful parameter for reclassification of the keratocystic odontogenic tumour from cyst to tumour status was recently published.[8] The authors compared qualitatively the podoplanin expression in 46 keratocystic odontogenic tumours and 11 orthokeratinized odontogenic cysts (OOCs). They concluded that the podoplanin immunoreaction was higher in KCOTS than in OOCs, probably because KCOTS has more of a neoplastic character, with more aggressive progression and local invasiveness.[8]

Despite of the numerous studies about the presence of podoplanin expression in various oral tissues and tumours, little is known about its physiologic or pathologic function. Sawa et al.[15] suggested an association of podoplanin in cellular proliferative activity due to its expression in tooth germ, which is present in cells with high mitotic activity, *i.e.* in dental lamina, terminal portion of Hertwig sheath and pre-ameloblasts. Tsuneki et al.[13] found that podoplanin-positive cells are located within areas with PCNA-positive cells in ameloblastomas, keratocystic odontogenic tumours, adenomatoid odontogenic tumours, and calcifying cystic odontogenic tumours.[13] On the other hand, a previous study conducted by our research group has showed absence of significant correlation between podoplanin and epithelial odontogenic proliferative activity in ameloblastomas reinforcing that the exact role of this protein in the benign odontogenic tumours needs to be elucidated.[14]

In view of the above considerations, the aim of this study was to investigate the expression of podoplanin in two groups of odontogenic tumours: those exclusively composed by epithelial neoplastic components and those composed by epithelial and ectomesenchymal tumoral cells. Additionally, we verified the possible association between podoplanin immunoexpression and the proliferative activity in keratocystic odontogenic tumours and orthokeratinized odontogenic cysts.

2. Materials and methods

Fifty-four odontogenic tumours were selected from the archives of the Laboratory of Pathology, Bauru School of Dentistry – University of São Paulo, Brazil, for the current study:

Odontogenic epithelium without ectomesenchyme:

- 8 ameloblastomas (AM): 4 follicular and 4 plexiform subtypes;
- 9 adenomatoid odontogenic tumours (AOT);
- 20 keratocystic odontogenic tumors (KCOTS);
- 5 orthokeratinized odontogenic tumors (OOC); and
- 1 calcifying epithelial odontogenic tumors (CEOT).

Odontogenic epithelium with ectomesencyhme:

- 2 ameloblastic fibromas (AF);
- 4 ameloblastic fibro-odontomas (AFO); and
- 5 calcifying cystic odontogenic tumours (CCOT).

The tumours were stained with haematoxylin–eosin and reviewed according to the World Health Organization histological classification of odontogenic tumours.[16] This study was approved by the Research Ethics Committee of the Bauru School of Dentistry, University of São Paulo (process number 99/2010).

2.1. *Podoplanin and Ki-67 expression in odontogenic tumours*

A formalin-fixed 4-μm section of epithelial odontogenic tumours was taken from the pathology archive for immunohistochemistry analysis of anti-podoplanin and anti-Ki-67 antibodies expressions by odontogenic cells. Only KCOTS and OOC were submitted to the Ki-67 antibody reaction.

After antigen retrieval using 10 mM citrate buffer, pH 6.0, in a domestic pressure cooker (Nigro, model Eterna 4(1/2) L, Brazil) for 4 min, endogenous peroxidase activity was blocked by incubation in 3% H_2O_2 for 20 min. Each epithelial odontogenic tumour section was incubated overnight at 4 °C with the primary monoclonal anti-podoplanin antibody (D2-40 clone, code#3619-1; Dako North America, Inc., Carpinteria, CA, USA), dilution 1:200 or anti-Ki-67 antibody (MIB-1 clone, Dako North America, Inc., Carpinteria, CA, USA code#7240-1), dilution 1:100, in phosphate-buffered saline (PBS) with bovine serum albumin (Sigma, cod#A2153, St Louis, MO, USA) solution to block a non-specific reaction. Then, each section was incubated with Advance HRP Link System (Dako North America, Inc., Carpinteria, CA, USA code #K4067) for 30 min at 37 °C. Both antibodies (podoplanin and Ki-67) were detected using 3.3′-diaminobenzedine tetrahydrochloride (Sigma, Inc., St. Louis, MO, USA cod#D-5637). Sections were counterstained with Mayer's haematoxylin before being dehydrated and cover slipped. Staining each sample without adding anti-human primary antibody was performed as a negative control and human palatine tonsils for both antibodies were stained for positive controls. Intensity of the staining was graded as absent, weak (≤25% of epithelial odontogenic positive cells) and strong (>25% of epithelial odontogenic positive cells).

For evaluation of proliferative activity of odontogenic epithelial cells from KCOTS and OOC, the labelling index (number of positive cells/total cells × 100) of Ki-67 staining was obtained. A computerized system of capturing images (Axiocam camera, Zeiss) attached to a light microscope (Axioskop 2 Plus, Zeiss) was used for this purpose. At least