

DISTRIBUTION EXPRESSION PROFILES OF PODOPLANIN AND β -CATENIN IN LESIONS OF AMELOBLASTOMA AND ODONTOGENIC KERATOCYST

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ABSTRACT

Background and objectives: Ameloblastomas and odontogenic keratocysts are benign odontogenic lesions of an epithelial origin that displayed behavior of local destruction and high recurrence. Podoplanin is designated as an endothelial lymphatic marker and it is linked to invasion and cell motility; its role together with β -catenin still not entirely elucidated in both odontogenic lesions.

Materials and methods: Both Podoplanin and β -catenin were immunohistochemically inspected in archival paraffin blocks of ameloblastoma and odontogenic keratocyst.

Results: Podoplanin and β -catenin are strongly expressed in all layers of follicular ameloblastoma; however, in odontogenic keratocysts; podoplanin is only expressed in basal cell layer.

Conclusions: Podoplanin is expressed in ameloblast like cells in ameloblastomas and basal cells of odontogenic keratocysts. It might be associated with cell migration and local invasiveness of both lesions. Besides; both podoplanin and β -catenin varied expression in ameloblastoma subtypes and odontogenic keratocyst might be useful in odontogenic lesions diagnosis.

KEY WORDS: Podoplanin, β -catenin, ameloblastoma, odontogenic keratocyst, odontogenic

INTRODUCTION

Ameloblastomas (ABs) and odontogenic keratocysts (OKCs) are benign odontogenic lesions that appear in both maxilla and mandible with distinguishing clinical consequences ⁽¹⁾. AB is considered as main clinically imperative tumor with increased rate of recurrence after treatment

by curettage as described in various reported studies ⁽²⁾. Though, up to now its varied molecular and biological behavior remains still not entirely clarified. OKC is designated as cyst that commonly accompanying to oral region; its distinctive growth behavior is related to enzymatic activity and proliferation potential of epithelial lining ⁽³⁾.

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Cell cytoskeleton and extracellular matrix are found to be a complicated dynamic network of macromolecules playing principal role in the preservation of a precise microenvironment functions including cellular adhesion, differentiation, proliferation⁽⁴⁾ and angiogenesis⁽⁵⁾; as well as diverse stromal architecture that is described in ABs and OKCs⁽⁶⁾.

Podoplanin, transmembrane glycoprotein of 38-kDa, was described primarily in podocytes of kidneys then commonly applied as lymphatic marker for endothelial cells⁽⁷⁾. The characteristic immunohistochemical of podoplanin patterns of expression have been correlated to stem cell role that might be associated with development of malignancy⁽⁸⁾.

β -catenin is designated as a protein with several functions that chiefly associated with maintaining of adhesion of cells by creating complexes with another adhesion molecule as E-cadherin⁽⁹⁾, both in charge to retain epithelial cell homeostasis. β -catenin downregulation in cell membrane correlates with tumor invasiveness and metastasis⁽¹⁰⁾. Furthermore, β -catenin mutations are linked to different cancer types including colon, gastrointestinal, ovarian cancer, pancreatic and prostate cancers⁽¹¹⁾.

The reported data of both podoplanin and β -catenin motivated us to reveal their collective role in cell migration and local invasiveness in ABs and OKCs.

MATERIAL AND METHODS

Samples

The current study was carried out on 60 paraffin blocks previously diagnosed as follicular ameloblastoma (AB) and odontogenic keratocyst (OKC) (30 each). Blocks were retrieved from Department of Oral Pathology archival files, Faculty of Dentistry, University of Tanta. These paraffin blocks were cut at 5 μ m on serial sections from were used for both conventional H&E as well as

immunohistochemistry. Design and procedures of the present study followed the research guidelines adopted by Committee of Research Ethics, Faculty of Dentistry, University of Tanta. The records (paraffin-embedded tissue blocks) were supplied from Department of Oral Pathology, Faculty of Dentistry, University of Tanta after taking head of Department of Oral Pathology written approval.

Antibodies

Rabbit polyclonal antibodies against Podoplanin (D2-40) (Abcam, USA) and mouse monoclonal antibodies against β -catenin were obtained from (Dako, UK).

Immunohistochemistry Protocol

Immunohistochemical staining were completed by means of ChemMate Envision system (Dako) in serial sections, as elucidated in previous study (5). In brief, deparaffinized sections were rehydrated by means of graded alcohols, after that activities of endogenous peroxidase were obliterated using 0.003% hydrogen peroxide within methanol at room temperature for half an hour, and then rinsed with 0.01 M phosphate-buffered saline (PBS, pH 7.4). Sections for D2-40 and β -catenin were autoclaved in citrate buffer (pH 6.0) at 121°C for 10 min. At that time, incubation of sections with 5% milk protein in 0.01 M PBS containing 0.05% Triton X-100 (Morinaga Milk Industry Co. Ltd, Tokyo, Japan) for one hour at 37°C were performed to block non-specific protein binding sites. Then, they were incubated with proper primary antibody dilution, 1:100 for D2-40 and 1:200 for β -catenin at 4°C overnight. Lastly, after incubations, the sections were rinsed with PBS and incubated with the Envision reagents at room temperature for one hour and then treated with 0.02% 3,3'-diaminobenzidine in 0.05 M Tris-HCl buffer (pH 7.6) containing 0.005% hydrogen peroxide to check the results. At end, hematoxylin counterstain of sections was completed. Antibodies (primary) were changed with pre-immune IgGs (mouse or rabbit) as control.

Immunohistochemistry result evaluation

Screening of tissue sections at lower magnification, three random fields from each case were designated using a 40 \times objective lens high power. Capturing of every category illustrative area using Nikon Eclipse microscope equipped with digital camera DXM1200C (Nikon, Japan) were completed. Number of cells with single nucleus that were positive for D2-40 and β -catenin were manually counted in a 0.25 \times 0.25 mm unit area on serial sections. Immunostaining results were scored as defined by Bencze J et al⁽¹²⁾. Intensity of color staining was rated as follows: Strong-3, moderate-2, weak-1 then none-0.

Statistical assessment

Obtained data in the present study were gathered and assessed statistically using the “SPSS 20” with probability value (p -value with 0.05) to calculate level of significance (Statistical Package for the Social Sciences) (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Podoplanin and Beta catenin expression in follicular ameloblastoma

In ameloblastoma (AB) (follicular) that reveal peripheral palisaded and reverse polarity cells (ameloblast like) and central cells (stellate

reticulum) with fibrous stroma inbetween (Fig. 1A); D2 40 displayed strong membranous expression in all cells (Fig. 1B); while β -catenin reveals weak cell membrane expression in peripheral ameloblast like cells and weak expression pattern in central cells (Fig. 1C) (Table 1).

Podoplanin and Beta catenin expression in plexiform ameloblastoma

In ameloblastoma (AB) (plexiform) that reveals less prominent peripheral cells and central stellate reticulum cells with inflammatory and vascular stroma inbetween (Fig. 2A). D2 40 displayed strong membranous expression in all cells (Fig. 2B); while β -catenin reveals moderate cell membrane expression in all cells (Fig. 2C) (Table 1).

Podoplanin and Beta catenin expression in Odontogenic keratocyst

In odontogenic keratocyst (OKC) that consists of cystic cavity with epithelial lining comprised of palisaded basal cells and suprabasal cells with underlying fibrous storma (Fig. 3A). D2 40 exhibited strong membranous expression only in peripheral palisaded basal cells but show no expression in suprabasal cells including peripheral parakeratinized cells (Fig. 3B); while β -catenin is strongly expressed in all cell layers in a membranous pattern (Fig. 3C) (Table 1).



Fig (1) A photomicrograph of H&E-stained tissue sections exhibit ameloblastoma (AB) (follicular) that reveals peripheral palisaded and reverse polarity ameloblast like cells and central stellate reticulum cells and fibrous stroma inbetween (A); D2 40 displayed strong membranous expression in all cells (B); while β -catenin reveals weak cell membrane expression in peripheral ameloblast like cells and weak expression pattern in central cells (C). Hematoxylin and eosin (HE) (A) and immunoperoxidase stains for D2 40 (B), β -catenin (C); (A) \times 40; (B, C) \times 100.

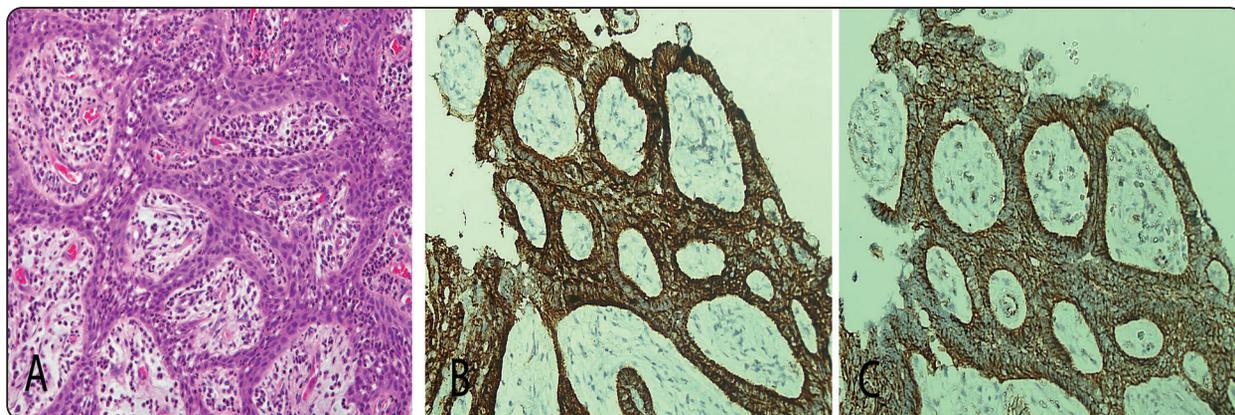


Fig. (2) A photomicrograph of H&E-stained tissue sections exhibit ameloblastoma (AB) (plexiform) that reveals less prominent peripheral cells and central stellate reticulum cells with inflammatory and vascular stroma inbetween (A). D2 40 displayed strong membranous expression in all cells (B); while β -catenin reveals moderate cell membrane expression in all cells (C). Hematoxylin and eosin (HE) (A) and immunoperoxidase stains for D2 40 (B), β -catenin (C); (A-C) $\times 200$.

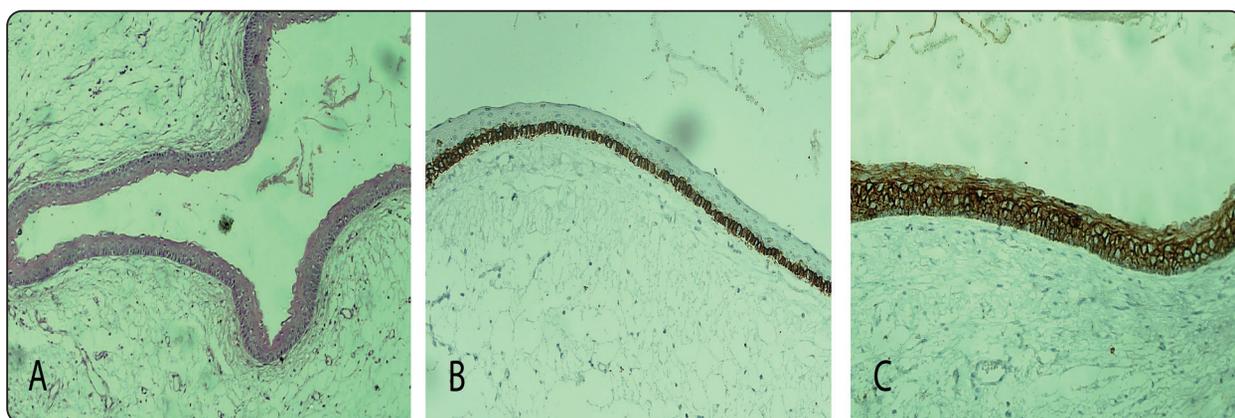


Fig (3). A photomicrograph of H&E-stained tissue sections exhibit odontogenic keratocyst (OKC) that consists of cystic cavity with epithelial lining comprised of palisaded basal cells and suprabasal cells with underlying fibrous stroma (A). D2 40 exhibited strong membranous expression only in peripheral palisaded basal cells but show no expression in suprabasal cells including peripheral parakeratinized cells (B); while β -catenin is strongly expressed in all cell layers in a membranous pattern (C). Hematoxylin and eosin (HE) (A) and immunoperoxidase stains for D2 40 (B), β -catenin (C); (A) $\times 40$; (B, C) $\times 100$.

TABLE (1) D2-40 and β -catenin expression in ameloblastoma and Odontogenic keratocyst

Histologic variants of Odontogenic lesions	Description of associated odontogenic epithelial cells	D2-40 and β -catenin		β -catenin expression
		Cellular Localization	Density	Density
Ameloblastoma follicular pattern	Prominent ameloblast-like cells	Membranous	Strong	Weak
	Prominent stellate reticulum-like cells (loosely attached)	Membranous	Strong	Weak
Ameloblastoma plexiform pattern	Less prominent ameloblast-like cells	Membranous	Strong	Moderate
	Prominent stellate reticulum-like cells (loosely attached)	Membranous	Strong	Moderate
Odontogenic keratocyst	Palisaded basal cells	Membranous	Strong	Strong
	Suprabasilar cells (firmly attached)	Membranous	Negative	Strong
	Superficial compacted parakeratin layer	Membranous	Negative	Strong

DISCUSSION

This study designated the dual appearance of podoplanin and β -catenin in ameloblastomas (ABs) and odontogenic keratocysts (OKCs). The noteworthy outcome in this study and might be of clinical relevance was that podoplanin not expressed in suprabasal cells including peripheral keratinized layer of the epithelial lining of OKC but solely in basal cells as compared to its expression pattern in AB. The additional remarkable finding was the distinctive membranous expression pattern of podoplanin both in ABs and OKCs which might be linked to cell cytoskeletal remodeling and motility during the process of growth and invasion. Remarkably, layer expression, cellular distribution and intensity of both podoplanin and β -catenin expression might be beneficial in differentiation of ABs and OKCs from other odontogenic lesions.

In ABs and OKCs, the peripheral cells that are in intimate contact with adjacent stromal cells strongly express podoplanin, while central cells that are not in physical contact with stromal cells exhibited less staining intensity as well as suprabasal cells in OKCs. These results validated the previous reports that augment the evidence of podoplanin and proliferative activity of odontogenic cells relation^(7,13). Furthermore, the enhanced podoplanin membranous expression in ABs peripheral cells, might speculate its association with remodeling of tumor cell cytoskeleton and subsequently may be valuable in odontogenic tumors classification⁽¹⁴⁻¹⁶⁾.

Interestingly, podoplanin by itself is unable to actin filaments; but require direct interaction with ezrin (ezrin-radixin-moesin (ERM) protein family that is involved in connecting proteins of cell membrane to the actin cytoskeleton), and that podoplanin overexpression in keratinocytes accompanying with marked increase in ezrin phosphorylation which then mediates the cellular motility in tumor invasion procedure⁽¹⁷⁻¹⁹⁾.

β -catenin revealed very less expression patterns in ABs but showed strong cytoplasmic

and membranous patterns in OKCs. Expression discrepancy within odontogenic lesions suggests alternate routes of β -catenin activation⁽²⁰⁻²²⁾. Nuclear as well as cytoplasmic accumulation of β -catenin is owing to either deactivation of adenomatous polyposis coli protein (APC) and/or mutations in β -catenin molecule and activation of Wnt-signaling pathway that can be used as a convenient marker to distinguish different cell conditions⁽²³⁾. Cytoplasm β -catenin appearance will precede its nuclear translocation, and consequently, will enhance cell proliferation^(24,25).

Based on reports of former studies, as well as the assumed evidence here, it may be specified that podoplanin membranous expression may be an indication for local invasion of epithelial cells in ABs. These differential patterns of podoplanin and β -catenin might explain the highly aggressive nature of ABs and the invasive behavior and high recurrence of OKC supporting its neoplastic potential.

This study came to conclude that differential podoplanin and β -catenin expression in ABs and OKCs might considerate diagnostic tool that could be useful in the diagnosis and pathogenesis of odontogenic lesions.

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