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Spatial distribution of osteopontin, CD44v6 and podoplanin in the lining epithelium of odontogenic keratocyst, and their biological relevance

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ABSTRACT

Background and aims: The odontogenic keratocyst (OKC) remains the most challenging jaw cyst to treat because of its locally-aggressive behaviour and high recurrence potential. Emerging evidence suggests that osteopontin, its receptors CD44v6 and integrin α_v, and podoplanin, have a role in the local invasiveness of this cyst. However the spatial distribution characteristics of these pro-invasive markers in the lining epithelium of OKC, and their association with the clinicopathologic parameters of OKC are largely unexplored. This study sought to address these issues in comparison with dentigerous cysts (DCs) and radicular cysts (RCs) and to evaluate their biological relevance.

Methods: A sample consisting of 20 OKC cases, 10 DCs and 10 RCs was subjected to immunohistochemical staining for osteopontin, CD44v6 and integrin α_v, and podoplanin, and semiquantitative analysis was performed.

Results: All factors (except integrin α_v) were detected heterogeneously in the constitutive layers of the lining epithelium in all three cyst types. Key observations were significant upregulation of CD44v6 and podoplanin in OKC compared to DCs and RCs, suggesting that these protein molecules may play crucial roles in promoting local invasiveness in OKC ($P < 0.05$). Osteopontin underexpression and distribution patterns were indistinctive among all three cysts indicating its limited role as pro-invasive factor. Clinical parameters showed no significant correlations with all protein factors investigated.

Conclusions: Present findings suggest that an osteopontin^{low} CD44v6^{high} and podoplanin^{high} immunoprofile most probably represent epithelial signatures of OKC and are markers of local invasiveness in this cyst.

1. Introduction

Among the various types of odontogenic cysts encountered in the jawbones, the odontogenic keratocyst (OKC) remains the most challenging to treat because of its locally-aggressive behaviour and high recurrence potential [1,2]. OKC was previously reclassified a neoplasm by the 2005 World Health Organization (WHO) Working Group and renamed keratocystic odontogenic tumour [3]. However the 2017 WHO Consensus Panel's decision to re-instate it as an odontogenic cyst attests to our lack of understanding of its biological nature [1]. The dentigerous cyst (DC), on the other hand, is the most common developmental odontogenic cyst while the radicular cyst is the most common inflammatory odontogenic cyst [4-6]. Both these cyst types are innocuous in nature and seldom recur after removal.

Odontogenic cysts are osseo-destructive lesions with different growth characteristics [7]. OKCs tend to grow in an antero-posterior direction within the medullary cavity of the jaw bone, without causing obvious bone expansion. DCs and RCs, on the other hand, tend to expand equally in all directions, following a unicentric ballooning pattern. In recent years, significant progress has been made in identifying pro-invasive factors implicated in the growth and progression of these cysts. Among them, osteopontin (OPN), their receptors (CD44v6 and integrin α_v) and podoplanin (PDPL) have generated much research interest [8-16]. These are multifunctional glycoproteins involved in mediating bone metabolism, immune regulation, wound healing, cell survival, cell-cell interactions, cell adhesion and migration, and tumour progression [17-19]. They are also chemo-attractant for osteoclast precursors and can modulate osteoclastic activity [20].

Current evidence suggests that OPN, its receptors CD44v6 and integrin α_v, and podoplanin, have a role in the local invasiveness of OKC [8-16]. However the immunophenotypic distribution characteristics of these proteins in the lining epithelium, and association of expression levels with the clinicopathologic parameters of OKC are largely undefined. This study sought to address these issues in comparison with DCs and RCs and to evaluate their biological relevance.

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Table 1
Associations between osteopontin, CD44v6 and podoplanin expression levels and clinicopathologic parameters in twenty patients with odontogenic keratocyst.

Variables	n = 20	Osteopontin				*P* value	CD44v6				*P* value	Podoplanin				*P* value
		−	+	++	+++		−	+	++	+++		−	+	++	+++	
Age (years)[a]						0.525					–					0.352
≤ 28	11	7	4	0	0		0	0	0	11		0	2	7	2	
> 28	9	5	3	1	0		0	0	0	9		0	2	3	4	
Gender						0.703					–					0.870
Male	8	5	3	0	0		0	0	0	8		0	2	4	2	
Female	12	7	4	1	0		0	0	0	12		0	2	6	4	
Ethnicity						0.411					–					0.530
Malays	6	3	3	0	0		0	0	0	6		0	1	4	1	
Chinese	6	5	0	1	0		0	0	0	6		0	0	3	3	
Indian	6	3	3	0	0		0	0	0	6		0	2	2	2	
Others	2	1	1	0	0		0	0	0	2		0	1	1	0	
Site						0.974					–					0.189
Maxilla	3	2	1	0	0		0	0	0	3		0	0	3	0	
Mandible	14	8	5	1	0		0	0	0	14		0	3	7	4	
Unknown	3	2	1	0	0		0	0	0	3		0	1	0	2	
Radiology						0.734					–					0.123
ULRL	5	3	2	0	0		0	0	0	5		0	3	1	1	
MLRL	7	4	2	1	0		0	0	0	7		0	1	4	2	
Unknown	8	5	3	0	0		0	0	0	8		0	5	3	0	
Root resorption						0.827					–					0.202
Absent	15	9	5	1	0		0	0	0	15		0	3	6	6	
Present	5	3	2	0	0		0	0	0	5		0	1	4	0	
Cyst size (cm)						0.456					–					0.355
< 2	5	2	2	1	0		0	0	0	5		0	0	2	3	
2–4	5	3	2	0	0		0	0	0	5		0	2	2	1	
> 4	10	7	3	0	0		0	0	0	10		0	2	6	2	

ULRL, unilocular radiolucency; MLRL, multilocular radiolucency; *P* value was determined by Chi square test ($P < 0.05$). Integrin α_v results are not shown because they are consistently negative.

[a] Median age 28 years in odontogenic keratocyst sample was used as cut-off point for binary measurements of patients' ages.

2. Materials and methods

2.1. Samples

The source of the study sample was from the archives of the Oral Pathology Diagnostic and Research Laboratory, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia. Twenty cases of OKC, 10 cases each of DC and RC were reviewed by a qualified oral pathologist (SCH) and selected according to established criteria [1,3,6]. Patient characteristics namely age at presentation, gender, ethnicity and anatomic location of cyst were recorded [ethics approval: DF OP1201/0001(L)].

From the archival formalin-fixed, paraffin-embedded tissue blocks of these cases, new consecutive 5 μm thick sections were prepared for staining with hematoxylin-eosin, and for immunohistochemistry.

2.2. Immunohistochemistry

Immunohistochemistry was performed using the Envision™ method. Briefly, deparaffinized sections of 5 μm thickness were pretreated for antigen retrieval by microwaving (99 °C) in 10 nM of citrate buffer (pH 6, 20 min). These sections were then immersed in 0.3% methanol containing 3% hydrogen peroxide for 20 min, to block endogenous peroxidase, and rinsed in 0.05 M Tris-buffered saline (TBS) (5 min, two times) before immersing in blocking solution (Dako Corporation, Carpinteria, CA, USA) for 20 min at room temperature. Sections were incubated with the following optimally diluted primary antibodies for 30 min at room temperature: goat polyclonal anti-OPN (ab36125, 1:200; Abcam Inc., Cambridge, MA, USA), mouse monoclonal to anti-CD44v6 (ab78960, 1:500; Abcam), anti-integrin α_v (ab16821, 1:500; Abcam) and anti-PDPL (ab10288, 1:500; Abcam). Immunoreactions were performed using the Envision Kit (Dako). The antigenic sites were visualized using diaminobenzidine (DAB) substrate chromogen (Dako) and counterstained with Mayer's hematoxylin. Known positive sections of mucoceles with macrophages for OPN, tonsil for CD44v6, peripheral giant cell granuloma for integrin α_v and lymphangioma for PDPL, were used as positive controls. In addition, positive labeling for OPN in bone trabeculae and PDPL in lymphatic endothelial cells of study sample sections served as internal positive controls. For negative control, sections were treated as above but without the primary antibody. All the control sections were negative.

2.3. Immunohistochemical analysis

The distribution patterns and immunoreactivity for all markers were evaluated by descriptive and semi-quantitative methods. Digitized images (Olyvia DotSlide Virtual Slide System, Olympus Imaging Inc., Tokyo, Japan) of all slides in each case were assessed by two investigators. The sections were systematically sampled and the lining epithelium of OKCs, DCs and RCs was divided into three constitutive layers, basal, spinous and keratin/surface zones. Five hotspots/fields in each epithelial layer were randomly selected at × 200 magnification. The level of expression for OPN, CD44v6, integrin α_v and PDPL was quantified according to the percentage of immunoreactive epithelial cells present: (−) negative when none of the epithelial cells were positively stained in the cytoplasm, membrane or nucleus; (+), < 25% cells positive; (++), 25–50% cells positive; and (+++), > 50% cells positive. Areas of inflamed epithelium were avoided.

2.4. Statistical analysis

All statistical analyses were performed using SPSS software version 12.0 (SPSS Inc., Chicago, IL). Chi square test or Fisher exact (when the assumptions were not met) was used to analyse expression rates of each protein in OKC, DC and RC. The Kruskal-Wallis was used to evaluate protein expression differences in mean ranks between various tissue types. Spearman correlation analysis was used to identify any possible correlation between the expression scores of these proteins. *P* value