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ANATOMICAL PATHOLOGY

Epithelial-to-mesenchymal transition in ameloblastoma: focus on morphologically evident mesenchymal phenotypic transition

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Summary

The ameloblastoma is the most common and clinically significant odontogenic epithelial neoplasm known for its locally-invasive behaviour and high recurrence risk. Epithelial-to-mesenchymal transition (EMT) is a fundamental process whereby epithelial cells lose their epithelial characteristics and gain mesenchymal properties. EMT induction via transcription repression has been investigated in ameloblastoma. However, morphologically evident mesenchymal phenotypic transition remains ill-defined. To determine this, 24 unicystic (UA), 34 solid/multicystic (SA) and 18 recurrent ameloblastoma (RA) were immunohistochemically examined for three EMT-related mesenchymal markers, alpha smooth muscle actin (α-SMA), osteonectin and neuronal cadherin (N-cadherin). All three factors were heterogeneously detected in ameloblastoma samples (α-SMA, *n*=71/76, 93.4%; osteonectin, *n*=72/76, 94.7%; N-cadherin, *n*=24/76, 31.6%). In the tumoural parenchyma, immunoreactive cells were not morphologically distinct from their non-reactive cellular counterparts. Rather, α-SMA and osteonectin predominantly labelled the cytoplasm of central polyhedral > peripheral columnar/cuboidal tumour cells. N-cadherin demonstrated weak-to-moderate circumferential membranous staining in both neoplastic cell types and cytoplasmic expression in spindle-celled epithelium of desmoplastic amelobastoma. For all tumour subsets, α-SMA and osteonectin scored significantly higher in the stroma > parenchyma whilst α-SMA was overexpressed along the tumour invasive front > centre ($p<0.05$). Stromal N-cadherin scored higher in SA > UA and RA > UA ($p<0.05$). Other clinicopathological parameters showed no significant associations. Taken together, acquisition of mesenchymal traits without morphologically evident mesenchymal alteration suggests partial EMT in ameloblastoma. Stromal upregulation of these proteins in SA and RA implicates a role in local invasiveness.

Key words: Solid/multicystic ameloblastoma; unicystic ameloblastoma; recurrent ameloblastoma; immunohistochemistry; epithelial-mesenchymal transition; mesenchymal phenotype.

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INTRODUCTION

The ameloblastoma is the most common and clinically significant odontogenic epithelial neoplasm known for its local invasiveness, aggressive behaviour and high recurrence risk. In the 2005 World Health Organization (WHO) classification, four main subtypes of ameloblastoma were identified; namely, solid/multicystic, peripheral/extraosseous, unicystic (UA) and desmoplastic.[1] With the dawn of the new 2017 WHO classification, solid/multicystic ameloblastoma was renamed ameloblastoma, and desmoplastic ameloblastoma reclassified as its histological variant.[2] As much of this work was carried out before year 2017, the 2005 WHO classification, terminologies and diagnostic criteria for ameloblastoma are adhered to here.[1]

Epithelial-to-mesenchymal transition (EMT) is a complex process that enables the dynamic and potentially reversible change from an epithelial to a mesenchymal morphology of individual cells to facilitate their migration through the extracellular matrix.[3,4] Physiological EMT is essential for proper gastrulation and organ development while pathological EMT is involved in tissue fibrosis, wound healing and cancer progression.[4–6] The central event of EMT is loss of cell adhesion molecules notably E-cadherin, and gain of mesenchymal properties.[5] E-cadherin downregulation is mediated by transcriptional repressors such as Snail 1 (Snail), Snail 2 (Slug), Zinc finger E-box-binding homeobox 1 (ZEB1) and ZEB2, and Twist, a basic helix-loop-helix transcription factor.[3,5,6] In EMT, the hallmark of mesenchymal phenotypic transition includes elevated expressions of vimentin, alpha smooth muscle actin (α-SMA), osteonectin and neuronal cadherin (N-cadherin).[5,6]

The potential role of EMT in the development and progression of ameloblastoma has gained growing interest. Most previous studies (including ours) focussed on E-cadherin transcriptional repression in this neoplasm.[7–12] Snail, Slug, SIP and Twist overexpression induce EMT by suppressing epithelial genes including E-cadherin in ameloblastoma to drive tumour development and progression.[7,9–11] In contrast, little attention has been paid to the mesenchymal morphology transition associated with this process.[12,13] The aim of this study was to determine the EMT-related mesenchymal phenotypic attributes of ameloblastoma. To this end we investigated for expression of three such protein factors, α-SMA, osteonectin and N-cadherin in the tumour epithelium and stromal cells in 76 cases of ameloblastoma, assessed for

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any associated mesenchymal morphological alterations in these immunoreactive tumour cells, and determined their relevance.

MATERIALS AND METHODS

Tumour sample

This study was approved by the Faculty of Dentistry Medical Ethics Committee [DF OP1201/0001(L)]. Archived annotated samples of 76 ameloblastoma cases [24 unicystic (UA), 34 solid/multicystic (SA), two desmoplastic (DA) and 18 recurrent ameloblastomas (RA)] were retrieved from the Oral Pathology Diagnostic and Research Laboratory, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia. New haematoxylin and eosin stained slides of these cases were reviewed by a qualified oral pathologist (CHS) and histopathological diagnosis rendered according to the 2005 WHO criteria.[1] Formalin-fixed, paraffin-embedded surgical specimens with adequate intratumoural tissue and tumour invasive front formed the case selection inclusion criterion. Patients' clinical data (age, gender and race), and tumour data (site, duration, radiographic findings and tumour size) were recorded. As follow-up data on treatment outcome was poor, this clinical parameter was excluded from analysis.

Immunohistochemistry

New 5 μm thick tissue sections were cut, dried, deparaffinised and rehydrated following standard protocols. Immunohistochemical staining was performed as previously described.[9] The primary antibodies used were mouse monoclonal anti-α-SMA (M085101, 1:1000; Dako Corporation, USA), rabbit polyclonal to anti-osteonectin (ab14174, 1:1000; Abcam Inc, USA) and mouse monoclonal anti-N-cadherin (M 361301, 1:50; Dako). α-SMA immunoreactivity of blood vessel wall within specimens was used as internal control. Breast carcinoma tissues known to express osteonectin and N-cadherin served as positive controls. For negative control, sections were treated as above but without the primary antibody. Isotype-matched mouse monoclonal IgG (ab18457; Abcam) at appropriate concentrations (1:1000 and 1:50) was used in isotype controls. All control sections were negative.

Immunohistochemical assessment

Digitised images (Olyvia DotSlide Virtual Slide System; Olympus Imaging Inc., Japan) of all sections in each case were obtained for descriptive and semiquantitative evaluation. In the latter, for each section, four fields at ×200 magnification were randomly selected according to their tissue compartment (parenchyma and stroma) and topographic location (tumour centre and invasive front). Immunoreactivity was scored according to methods described previously with minor modifications.[14–16]

α-SMA expression score was based on staining intensity and extent in tumoural parenchyma and stroma.[14] Score 0 = negative except for positive blood vessel wall; 1+ = scanty layers of α-SMA positive stromal spindled cell focal around tumour parenchyma (<10% cells); 2+ = multilayers of α-SMA positive stromal spindled cell surrounding tumour parenchyma (10–30% cells); and 3+ = intense α-SMA positive stromal spindled cell encroaching tumour parenchyma (>30% cells) (Supplementary Fig. 1A–D, Appendix A).

Osteonectin immunostaining was graded according to the intensity and percentage of neoplastic and/or stromal cells showing cytoplasmic staining.[15] Score 0 = negative; 1+ = mild staining in focal areas (<25%); 2+ = moderate staining in significant areas (25–50%); and 3+ = strong staining in predominant areas (>50%) (Supplementary Fig. 1E–H, Appendix A).

N-cadherin expression was quantified according to the extent of circumferential cytoplasmic membrane stained and the percentage of neoplastic cells exhibiting membranous staining.[16] Score 0 = negative; 1+ = faintly perceptible staining (≥10% cells); 2+ = moderate complete circumferential membrane staining (≥10% cells); 3+ = strong complete membrane staining (≥10% cells) (Supplementary Fig. 1I–L, Appendix A).

Statistical analysis

Statistical analysis was performed using SPSS software version 12.0 (SPSS, Chicago, USA). Student's *t* test or analysis of variance (ANOVA) compared differences in patients' mean ages. Chi square test assessed statistical significance of associations between clinicopathological parameters and expressions of individual or combined mesenchymal phenotype-related proteins. Wilcoxon signed ranks test evaluated protein expression rates in mean ranks by tissue compartments and topographic locations whereas

Table 1 Patients' characteristics

Variables	Ameloblastoma (*n*=76) (%)		
	SA	UA	RA
Total (%)	34 (100)	24 (100)	18 (100)
Age, years			
Mean (range)	28.5 (8–63)	21.8 (3–39)	40.7 (10–76)
Gender			
Male (*n*=45)	20 (58.8)	13 (54.2)	12 (66.7)
Female (*n*=31)	14 (41.2)	11 (45.8)	6 (33.3)
Tumour site			
Maxilla (*n*=4)	3 (8.9)	0 (0)	1 (5.5)
Mandible (*n*=66)	30 (88.2)	24 (100)	12 (66.7)
Others (*n*=6)[a]	1 (2.9)	0 (0)	5 (27.8)
Tumour duration, months			
Mean (range)	26.3 (1–180)	9.5 (1–72)	76.8 (12–240)
Tumour size, cm			
Mean (range)	5 (0.7–8)	5.8 (1.8–11.5)	3.3 (1.5–5.4)
Radiological findings (*n*=48)			
ULRL (*n*=6)	3 (10.7)	3 (18.8)	0 (0)
MLRL (*n*=23)	14 (50)	6 (37.5)	3 (75)
RLNOS (*n*=13)	7 (25)	5 (31.2)	1 (25)
Others (*n*=6)	4 (14.3)	2 (12.5)	0 (0)
Root resorption (*n*=48)			
Absent (*n*=39)	23 (82.1)	13 (81.2)	3 (75)
Present (*n*=9)	5 (17.9)	3 (18.8)	1 (25)
Histological variants			
	Plexiform: 29 (85.3) Follicular: 2 (5.9) Mixed: 1 (2.9) Desmopl: 2 (5.9)	Luminal: 9 (37.5) Intralum: 7 (29.2) Mural: 8 (33.3)	Plexiform:11 (61.1) Mural: 4 (22.2) Desmopl: 3 (16.7)

SA, solid/multicystic ameloblastoma; MLRL, multilocular radiolucency; RA, recurrent ameloblastoma; RLNOS, radiolucency not otherwise specified; Intralum, intraluminal; Desmopl, desmoplastic; UA, unicystic ameloblastoma; ULRL, unilocular radiolucency.
[a] A case each of recurrent ameloblastoma was from the chin, infratemporal, pre-auricular, submental and submandibular region.

Kruskal–Wallis test compared protein expression differences in mean ranks between three independent samples. The significance level was set at $p<0.05$.

RESULTS

Patient characteristics

The demographic profile and tumour data of SA, UA and RA cases are shown in Table 1. The mean age of all patients was 28.9±15.32 years and age range 3–76 years. Their overall male-to-female ratio was 1.5:1. Most (86.8%) were mandibular tumours. A painless, slow-growing swelling was the chief presenting complaint (n=55/76, 72.3%). Sixty cases (78.9%) were pre-operatively diagnosed as ameloblastomas. Known treatment modalities were enucleation or curettage (n=24/60, 40%), segmental or marginal resection (n=18/60, 30%), surgical excision (n=15/60, 25%), hemi-mandibulectomy (n=2, 3.3%) and partial maxillectomy (n=1/60, 1.7%).

Immunohistochemical findings

Results are shown in Tables 2 and 3 and illustrated in Fig. 1–4. Subcellular localisation for all three proteins, α-SMA, osteonectin and N-cadherin, was cytoplasmic and/or membranous.

α-SMA expression

α-SMA was heterogeneously detected in most ameloblastoma samples (n=71/76, 93.4%). Neoplastic epithelial cells expressing α-SMA were not morphologically distinct from their non-reactive cellular counterparts. Instead, α-SMA predominantly stained central polyhedral cells (Fig. 1A,B 4A) and less frequently peripheral columnar/cuboidal cells (Fig. 1C). In the desmoplastic ameloblastoma, the hypercellular spindled epithelium was often intensely stained (Fig. 1D) while the UA lining epithelium was weak-to-absent (Fig. 1E,F). Stromal α-SMA positive spindled cells formed single/multilayers that completely/incompletely encircled tumour islands (Supplementary Fig. 1B,C, Appendix A). Vascular endothelium was α-SMA positive (Fig. 1A,4A).

Osteonectin expression

Osteonectin was the most widely expressed protein in tumour sample (n=74/76, 94.7%). Regardless of its immunoreactivity

Table 2 Associations between α-smooth muscle actin, osteonectin and N-cadherin expression levels with clinicopathological parameters in 76 patients with ameloblastoma

Variables	n=76	Immunoreactivity scores and p values													
		α-Smooth muscle actin				p value	Osteonectin				p value	N-cadherin				p value
		0	1+	2+	3+		0	1+	2+	3+		0	1+	2+	3+	
Age, years						0.960					0.297					0.329
≤27[a]	41	3	17	19	2		1	20	17	3		31	9	1	0	
>27	35	2	15	17	1		3	11	16	5		21	12	2	0	
Gender						0.804					0.390					0.370
Male	45	3	21	20	1		3	15	21	6		28	15	2	0	
Female	31	2	12	15	2		1	16	12	21		24	6	1	0	
T. site						0.162					0.151					0.505
Maxilla	4	1	1	1	1		0	3	0	1		4	0	0	0	
Mandible	65	3	29	31	2		3	28	28	6		42	20	3	0	
Others	7	1	2	4	0		1	0	5	1		6	1	0	0	
T. duration, months	n=52					0.998					0.672					0.077
< 6	17	1	8	8	0		0	9	8	0		16	1	0	0	
6–12	17	1	7	8	1		1	8	6	2		10	7	0	0	
> 12	18	1	7	9	1		2	6	7	3		9	7	2	0	
Radiological findings	n=48					0.734					0.453					0.972
ULRL	6	1	2	3	0		1	2	3	0		4	2	0	0	
MLRL	23	0	10	11	2		1	11	6	5		15	6	2	0	
RL NOS	13	1	5	6	1		1	6	6	0		9	3	1	0	
Others	6	0	4	2	0		0	3	3	0		4	2	0	0	
Root resorption	n=48					0.081					0.526					0.064
Absent	39	2	14	21	2		3	18	15	3		28	10	1	0	
Present	9	0	7	1	1		0	4	3	2		4	3	2	0	
T. size, cm	n=43					0.673					0.697					0.076
< 2	4	1	1	2	0		0	1	3	0		1	3	0	0	
2–4	12	1	3	7	1		1	4	5	2		7	3	2	0	
< 4	27	1	15	10	1		1	15	8	3		21	6	0	0	
T. subtypes						0.818					0.056					0.231
UA	24	1	12	11	0		0	8	15	1		20	4	0	0	
SA	34	3	14	15	2		2	19	9	4		19	13	2	0	
RA	18	1	6	10	1		2	4	9	3		13	4	1	0	
Topographic location						**0.002**					0.317					0.102
T. centre	76	24	46	4	2		12	43	19	2		62	12	2	0	
T. front	76	19	45	10	2		13	42	19	2		58	16	2	0	

Chi square test was used except for topographic location where the p value was determined by Wilcoxon signed rank test. Bold values indicate statistical significance ($p<0.05$).

Expression levels: 0, negative; 1+, mild; 2+, moderate; 3+, strong.

MLRL, multilocular radiolucency; RA, recurrent ameloblastoma; RL NOS, radiolucency not otherwise specified; SA, solid/multicystic ameloblastoma; T, tumour; UA, unicystic ameloblastoma; ULRL, unilocular radiolucency.

[a] 27 years is median age for patients with ameloblastoma.

Table 3 Tumour subtypes and expression levels according to tissue compartment and topographic location

Markers	Tumours	T. parenchyma			T. stroma			T. centre			T. periphery		
		Mean rank	χ statistics (df)	*p* value	Mean rank	χ statistics (df)	*p* value	Mean rank	χ statistics (df)	*p* value	Mean rank	χ statistics (df)	*p* value
α-SMA	SA	40.18			36.25			40.53			40.31		
	UA	35.58	0.784 (2)	0.676	37.83	1.473 (2)	0.479	32.92	3.084 (2)	0.214	35.15	1.062 (2)	0.588
	RA	39.22			43.64			42.11			39.56		
OSN	SA	34.65			39.26			42.82			43.15		
	UA	39.96	2.638 (2)	0.267	41.29	1.648 (2)	0.439	36.46	3.255 (2)	0.196	35.69	3.488 (2)	0.175
	RA	43.83			33.33			33.06			33.47		
NCAD	SA	40.01			43.41			41.50			42.82		
	UA	36.46	0.691 (2)	0.708	31.00	9.351 (2)	**0.009**	34.58	3.066 (2)	0.216	32.58	5.545 (2)	0.063
	RA	38.36			39.22			38.06			38.22		

p value was determined by Kruskal Wallis test. Bold values indicate statistical significance ($p<0.05$).
df, degrees of freedom; T, tumour.

status, the tumour epithelium demonstrated comparable cellular morphology. Osteonectin expansively labelled the central polyhedral cellular compartment, and may appear to disrupt the peripheral columnar/cuboidal cell layer and to abut onto the adjacent immunoreactive stroma (Fig. 2A,C, Fig. 4B). Loosely-arranged granular cells were variably osteonectin positive (Fig. 2B), desmoplastic subtype neoplastic epithelium stained weak-to-absent (Fig. 2D) and UA lining epithelium was moderate-to-absent (Fig. 2E,F). Stromal osteonectin diffusely labelled spindled cells and fibrous tissues (Fig. 2C,E,F).

N-cadherin expression

About a third (n=24/76, 31.6%) of study sample expressed N-cadherin. In N-cadherin-positive tumours, staining was membranous in both central polyhedral and peripheral columnar/cuboidal neoplastic epithelial cells (Fig. 3A). However, morphological alteration was not evident. N-cadherin labelling was moderate in cytoplasm of spindled cell areas in desmoplastic variant (Fig. 3D), weak-to-absent in SA and UA lining epithelium (Fig. 3B,C,E,F). Stromal mast cells

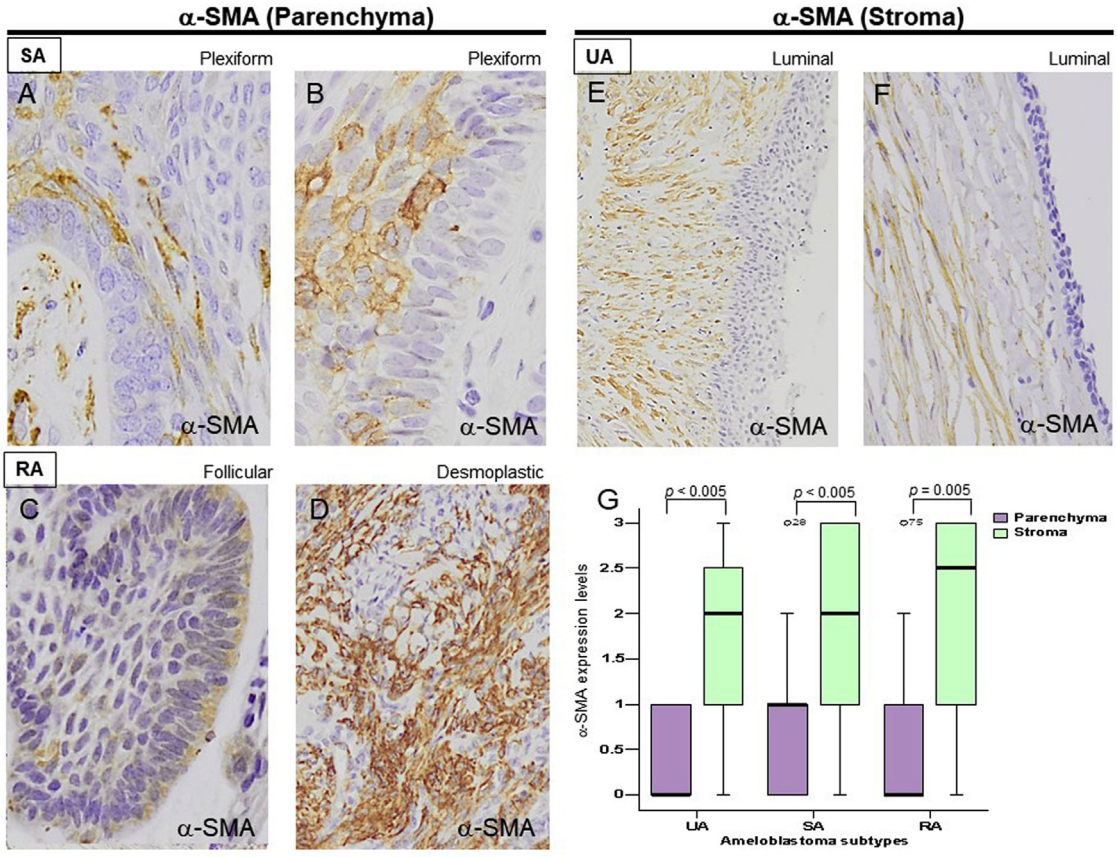

Fig. 1 Distribution patterns of α-smooth muscle actin (α-SMA) by cell type and subcellular protein localisation in the parenchyma and stroma of ameloblastoma subtypes. (A,B) Plexiform solid/multicystic ameloblastoma (SA). (C) Follicular recurrent ameloblastoma (RA). (D) Desmoplastic RA. (E,F) Unicystic ameloblastomas (UA). (G) Box plots show data distributed in relation to median values for α-SMA expression levels in ameloblastoma subtypes stratified by parenchyma and stroma. Level of significance is set at $p<0.05$.

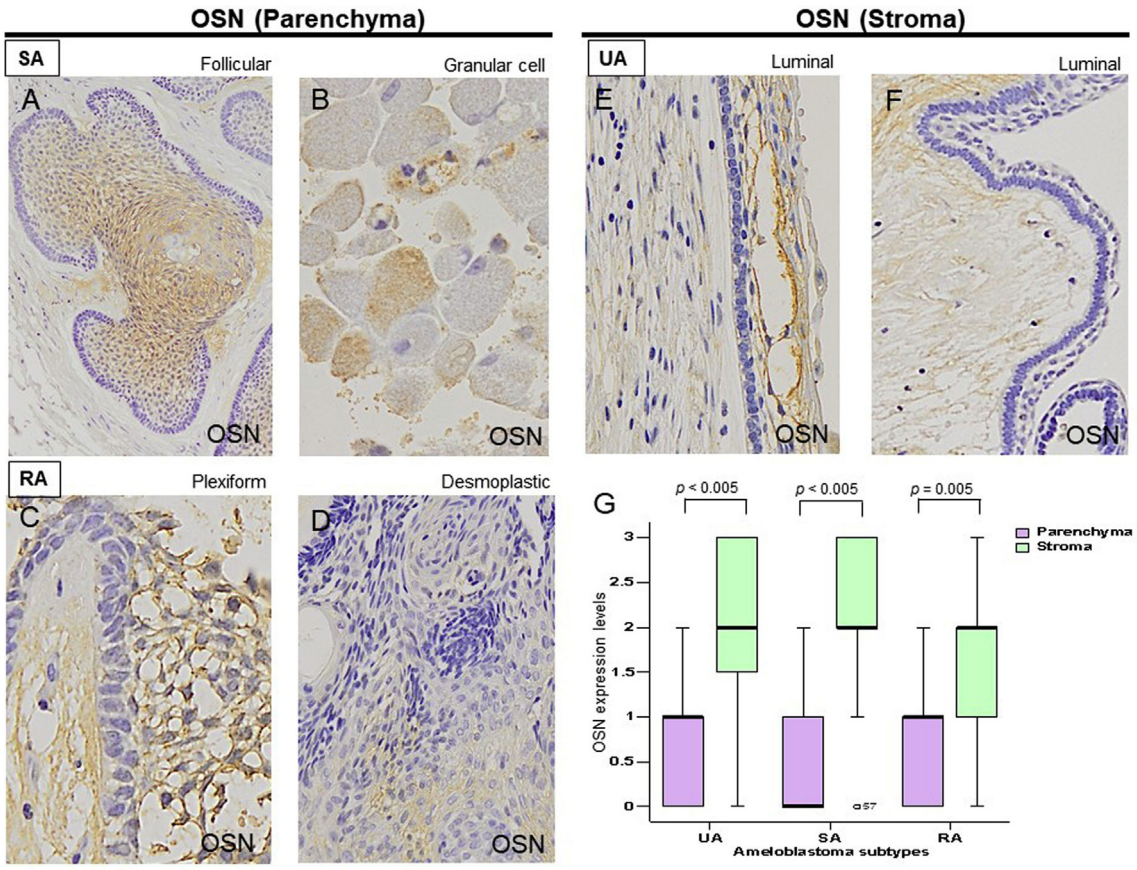

Fig. 2 Distribution patterns of osteonectin (OSN) by cell type and subcellular protein localisation in the parenchyma and stroma of ameloblastoma subtypes. (A) Follicular solid/multicystic ameloblastoma (SA). (B) Granular cell SA. (C) Plexiform recurrent ameloblastoma (RA). (D) Desmoplastic RA. (E,F) Unicystic ameloblastomas (UA). (G) Box plots show data distributed in relation to median values for OSN expression levels in ameloblastoma subtypes stratified by parenchyma and stroma. Level of significance is set at $p<0.05$.

(Fig. 3C), and bone cells (Fig. 3E, inset) were N-cadherin positive.

Statistical findings

For all tumour subtypes, a high α-SMA expression was detected along the tumour invasive front compared to the tumour centre (Table 2) (p=0.002), while significantly higher α-SMA (p=0.000) and osteonectin (p=0.000) expression levels were observed in the stroma > parenchyma (Fig. 1G,2G). N-cadherin expression rate was significantly higher in the tumoural stroma of SA > UA (p=0.002) and RA > UA (p=0.016), and along the invasive front of SA > UA (p=0.018) (Tables 2 and 3). The relative proportion of parenchyma and stroma, parenchyma only and stroma only, exhibiting positive or negative expression of these three markers was not significantly different among all three tumour groups (p>0.05). Except for a significant association between tumour size and combined α-SMA$^+$/osteonectin$^+$ expression (p=0.012), no significant correlations were found between all three markers' expression singly or in combination, with other clinicopathological parameters (p>0.05).

DISCUSSION

There is mounting evidence that loss of E-cadherin itself without a mesenchymal phenotype may not be associated with invasive behaviour, whereas a mesenchymal phenotype regardless of E-cadherin expression is associated with invasive behaviour in some cancers.[17] This underpins the focus of our current investigation. The question raised was whether EMT activity in ameloblastoma is accompanied by a shift towards a morphological mesenchymal phenotype. To address this, we examined by immunohistochemistry 76 ameloblastoma cases of various subtypes for expression of three EMT-related mesenchymal markers, α-SMA, osteonectin and N-cadherin. The rationale was to delineate their distribution characteristics and to elucidate their relevance.

Conceptually, complete EMT refers to morphologically evident conversion from a full epithelial state to a full mesenchymal state.[17–19] During this lineage transformation, epithelial cells lose their epithelial properties and acquire mesenchymal traits with loss of apical-basal polarity, loss of cell-cell contact and disassembly of its actin cytoskeleton.[3,6] Incomplete or partial EMT, on the other hand, refers to evident existence of intermediate hybrid epithelial and mesenchymal phenotypes.[20,21] In the present study, a key observation was aberrant expression for α-SMA, osteonectin and N-cadherin by the various neoplastic epithelial cells of ameloblastoma subtypes. However, these cells mainly retained their epithelioid morphology, comparable in feature with their non-reactive neoplastic counterparts.[9] For instance, in the classic ameloblastoma, the central tumour cells remained predominantly polyhedral while the peripheral tumour cells were columnar/cuboidal regardless of their α-SMA, osteonectin or N-cadherin expressivity. Recent advances propose that EMT represents a continuum whereby

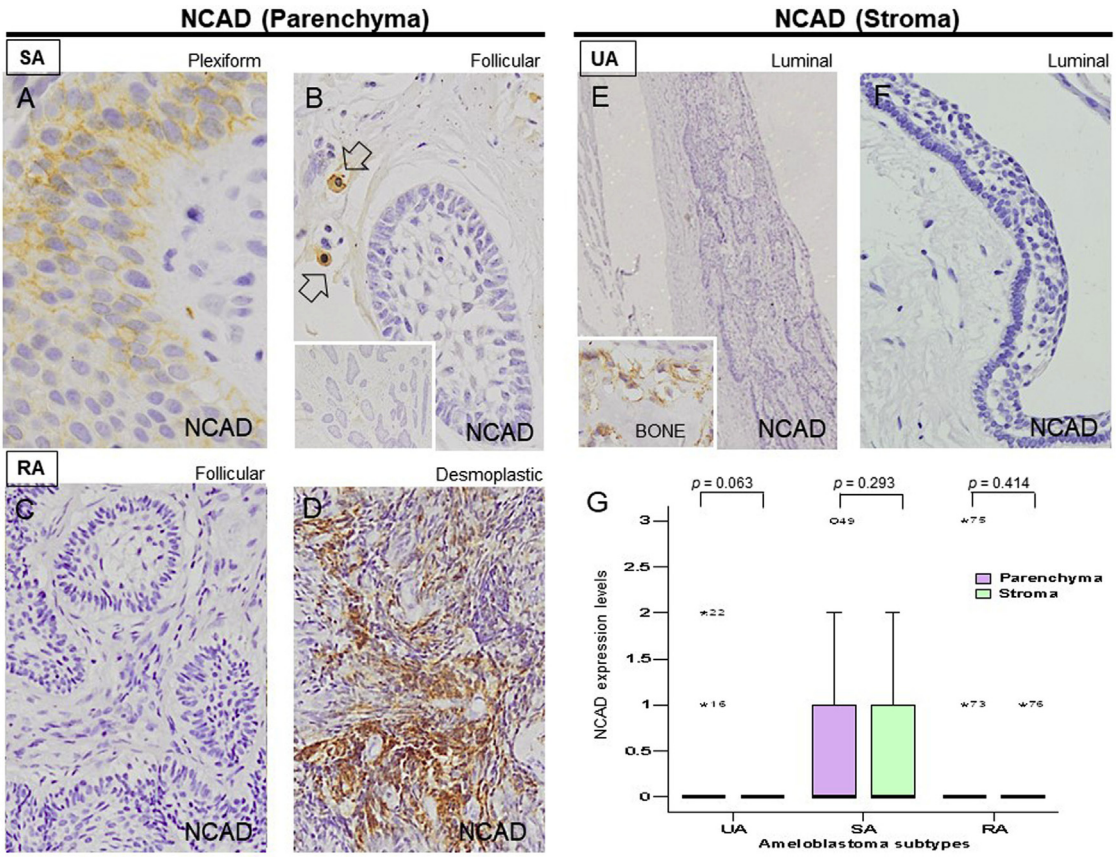

Fig. 3 Distribution patterns of N-cadherin (NCAD) by cell type and subcellular protein localisation in the parenchyma and stroma (arrows) of ameloblastoma subtypes. (A) Plexiform solid/multicystic ameloblastoma (SA). (B, B inset) Follicular SA. (C) Follicular recurrent ameloblastoma (RA). (D) Desmoplastic RA. (E, E inset, F) Unicystic ameloblastomas (UA) and osteoblasts are NCAD positive. (G) Box plots show data distributed in relation to median values for NCAD expression levels in ameloblastoma subtypes stratified by parenchyma and stroma. Level of significance is set at $p<0.05$.

cells exhibit epithelial, intermediate and mesenchymal phenotypes.[22] During this transitional process, cells move through a spectrum of intermediary phases.[6,23] Given these insights, it seems likely that these α-SMA-, osteonectin- or N-cadherin-expressing tumour cells might be transitioning cells that belong to the so-called intermediate hybrid phenotypes

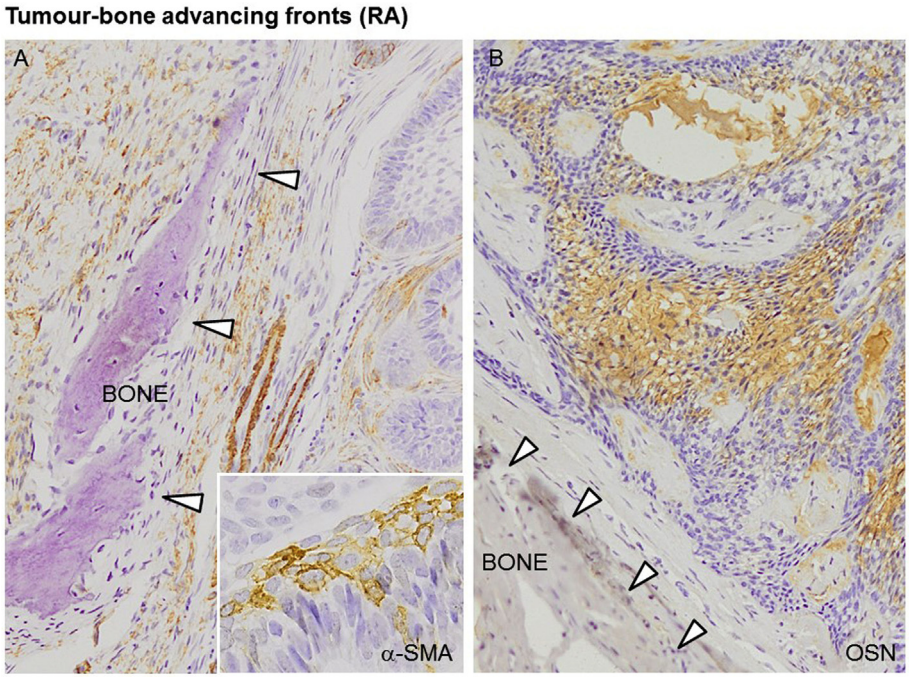

Fig. 4 Tumour-bone advancing front (arrows) of recurrent ameloblastoma (RA). (A, A inset) α-Smooth muscle actin (α-SMA) expression. (B) Osteonectin (OSN) expression.

endowed with mixed epithelial and mesenchymal characteristics.[17,23] These traits are purposeful for specialised capabilities such as collective cell migration. On the other hand, the significance of immunoreactive hypercellular spindled and rounded/polygonal neoplastic epithelial cells observed in our desmoplastic and granular subtypes respectively is unclear. One consideration would be that the individual tumour cells in these histological variants might also represent different stages of morphological transition in EMT.[6,20,22] Emerging evidence suggests that rounded and spindle-like morphology (fibroblastoid) are surrogate parameters of loss of polarisation and EMT.[4,23,24] Taken together, our findings seem to indicate that α-SMA, osteonectin and N-cadherin might induce partial EMT in ameloblastoma, and that the various neoplastic cellular components expressing these mesenchymal markers are probably intermediate hybrid phenotypes. This is a plausible explanation because unlike cancer EMT, the property of plasticity, specifically its mesenchymal status, is not sufficient to confer metastasis potential in benign tumours with locally invasive behaviours.[5,25]

Thus far only two EMT-related mesenchymal markers, vimentin and N-cadherin, have been investigated in ameloblastoma.[12,13] These studies reported that: (1) aberrant expression for vimentin in ameloblastoma epithelium correlates with recurrence and malignant transformation;[12] and (2) membranous N-cadherin expression in outer columnar epithelial cells implies neurodifferentiation.[13] In this study, a heterogeneous distribution pattern displayed by α-SMA, osteonectin and N-cadherin within the tumoral parenchyma was observed. Except for an elevated α-SMA expression level along the invasive tumour front, we could not detect any other correlations between expression rates and clinicopathological parameters for all three proteins investigated. With regards to the scattered mesenchymal immunoprofile, it is unclear whether the expression of mesenchymal markers by tumour cells results from proliferation of a single cell that has undergone EMT into a cluster of such cells or mesenchymal transformation of multiple tumour cell clusters.[26] The most widely expressed was osteonectin followed by α-SMA, and the least was N-cadherin. Recent evidence indicates that tumour-derived osteonectin functions as a de-adhesive molecule that represses E-cadherin and induces EMT by upregulation of its downstream target SLUG.[27,28] Furthermore, osteonectin induces expression of α-SMA and N-cadherin.[29] Aberrant N-cadherin expression in tumour cells denotes a shift from E-cadherin to N-cadherin (cadherin switch) and its subcellular protein localisation reflects the adhesive property of the tumour cell population involved. In spindled tumour cells, cytoplasmic N-cadherin expression is essential for actin cytoskeleton-N-cadherin link via its intracellular domain to promote efficient collective cell migration.[30] In contrast, membranous N-cadherin in epithelioid cells strengthens cell-cell adhesion capacity via its extracellular domain.[30,31]

α-SMA, osteonectin and N-cadherin are essentially stroma-related proteins. These factors frequently labelled spindle-shaped mesenchymal cells (myofibroblasts), mast cells, osteoblasts and osteocytes in the stroma accompanying immunoreactive tumour cells, and these findings concurred with previous reports for ameloblastoma.[8,32–35] Of relevance is the significantly higher stromal α-SMA and osteonectin scores in all tumour subtypes, and higher stromal N-cadherin in SA > RA > UA, thus fortifying the view that heterogeneous tumour progression is mirrored by an 'activated' stroma adjacent to the growing tumour.[36] Current literature identified these α-SMA- and osteonectin-expressing myofibroblasts as cancer-associated fibroblasts (CAFs) that promote invasion by secreting proteases for extracellular matrix degradation, and by inducing EMT.[14,26,27,37,38] Stromal N-cadherin activity contributes to cell motility and other invasive properties via Rho-family GTPase signalling.[5,6,16]

A limitation of this study is that only three mesenchymal markers were evaluated. However, the proteins selected for this study were the most important and representative proteins for detecting EMT mesenchymal phenotypes in ameloblastoma: α-SMA (a cytoskeketal marker), osteonectin (an E-cadherin repressor and transcription factor SLUG promoter) and N-cadherin (cadherin switch).

In conclusion, the study reported here provided immunohistochemical evidence of aberrant expression for three EMT-related mesenchymal markers by tumoural parenchyma in ameloblastoma. Acquisition of these mesenchymal traits without morphologically evident mesenchymal alteration suggests partial EMT in this neoplasm. Stromal upregulation of these proteins in aggressive subtypes implicates a role in local invasiveness. Future works should be extended to determine these characteristics in other odontogenic epithelial tumours (besides ameloblastoma) so as to better understand the role of EMT in these neoplasms.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pathol.2019.04.004.

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