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Expression of osteonectin/secreted protein acidic and rich in cysteine and matrix metalloproteinases in ameloblastoma

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BACKGROUND: Ameloblastoma is the most common clinically-significant epithelial odontogenic tumor, and is considered a benign but locally-aggressive tumor of the craniofacial region. Osteonectin/secreted protein acidic and rich in cysteine (SPARC) is induced in response to a number of biological processes such as tumor growth and metastasis, whereas matrix metalloproteinases (MMPs) degrade the extracellular matrix and participate in various biological processes including tumor invasion and metastasis. We hypothesize that SPARC acts with MMPs for the local invasiveness of ameloblastoma. The aim of this study was to examine the association of SPARC with MMP-1, MMP-2, and MMP-9 in ameloblastoma.

METHOD: Immunohistochemical expression of SPARC, MMP-1, MMP-2, and MMP-9 as well as co-expression of SPARC and MMP-9 were examined in a cohort of 23 cases of ameloblastoma.

RESULTS: SPARC, MMP-1, -2, and -9 were detected in the cytoplasm of the ameloblastic-like columnar cells and stellate-reticulum-like cells as well as in the stromal tissues of fibroblasts and endothelial cells of our cohort of ameloblastoma patients. Furthermore, co-expression of SPARC and MMP-9 were found in 23 cases of ameloblastoma. This may be the first study to demonstrate that the expression level of SPARC was statistically correlated with MMP-9 but not with MMP-1 or -2 in ameloblastoma.

CONCLUSION: Our results suggest a putative association between SPARC and MMPs (especially MMP-9) in ameloblastoma to regulate tumor invasion.

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Keywords: ameloblastoma; matrix metalloproteinase-9; osteonectin; secreted protein acidic and rich in cysteine

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Introduction

Ameloblastoma is an epithelial odontogenic neoplasm that constitutes approximately 10% of all odontogenic jawbone tumors (1). It usually occurs in young adults; males and females are equally affected (2). Becelli et al. (3) reported that 35% of ameloblastoma patients are asymptomatic. Although ameloblastoma is usually regarded as a benign tumor, it may be locally aggressive, with a relatively high recurrence rate, causing significant morbidity and occasionally even death. Further, ameloblastoma can exhibit an invasive growth pattern in cancellous bone, with small tumor nests at a maximum of 5 mm from the gross disease (4). To the best of our knowledge, the details of the oncogenesis, cytodifferentiation, and progression of ameloblastoma are yet to be fully elucidated.

Matrix metalloproteinases (MMPs), collectively called matrixins, are proteinases that participate in extracellular cellular matrix (ECM) degradation (5). Activated MMPs are generally present in greater amounts in and around malignant tissues than in normal, benign, or premalignant tissues, with the highest expression occurring in areas of active invasion at the tumor–stroma interface (6).

MMP-1 (collagenase-1), one of the interstitial collagenases, is scarcely detected in healthy skin; however, it is expressed by basal keratinocytes in cutaneous wounds (7). Moreover, MMP-1 is commonly expressed in different types of carcinomas, including oral squamous cell carcinoma (8). Originally characterized as type IV collagenases, MMP-2 and -9 are essential proteases for basement membrane-invasive events. MMP-2, produced by mesenchymal cells, and MMP-9, secreted by inflammatory cells (macrophages and neutrophils), are now regarded as key regulators of pathological angiogenesis (9).

Osteonectin/secreted protein acidic and rich in cysteine (SPARC), first described by Termine et al. (10), is a major non-collagenous constituent of bovine and human bone. Expression of SPARC is generally high

during embryogenesis and markedly reduced or absent during normal post-natal life; however, expression occurs in response to inflammation, tissue injury, tumor growth and metastasis (11). A connection between SPARC expression and the development of various cancers has been described (12); furthermore, SPARC expression has been found to be correlated with MMP-2 and -9 activity in melanoma cells (13).

Pinheiro et al. (14) showed that MMP-1, -2, and -9 when expressed in ameloblastoma might digest the bone matrix and release mitogenic factors, which could increase tumor cell proliferation. SPARC has been shown to induce MMP-9 expression in macrophages/monocytes in the tumor microenvironment (15), and Arnold et al. (16) suggested that MMP activity in the tumor microenvironment might be related to various factors including ECM deposition, presence of other proteases and cytokines, and the production of MMP, as well as SPARC activity. Therefore, gaining an understanding of the interaction of SPARC and MMPs in ameloblastoma may enhance our knowledge of the locally-aggressive behavior of this odontogenic tumor. The aim of this study is to investigate the association of SPARC with MMP-1, -2, and -9 in a cohort of ameloblastoma patients.

Materials and methods

Sample collection

The records of a total of 23 patients (15 males; eight females) with ameloblastoma were retrieved from Kaohsiung Medical University (2002–2006) with an approved IRB (98-IRB-141), of which the mean age was 38.2 years (range, 13–62 years). All patients had primary lesions and underwent total surgical excision. The detailed clinical and histological data of these 23 patients are shown in Table 1. Twenty-two cases occurred in the mandible and only one case was found in the maxilla. In seven cases (30.4%), local recurrence was found on regular follow-up. Seven cases were of a pure plexiform type, while ten cases were of a pure follicular type; the remaining six cases were of hybrid histological subtypes. The surgical specimens were fixed in 10% buffered formalin embedded in paraffin. Four-micrometer-thick tissue sections were prepared for immunohistochemical analysis and hematoxylin and eosin staining. The histological diagnosis of ameloblastoma was rendered in accordance with the World Health Organization (WHO) guidelines for the histological typing of odontogenic tumors (17).

Semi-quantitative immunohistochemistry

Following tissue sectioning, staining was performed using a standard avidin-biotin peroxidase complex (ABC) method (18). Rabbit monoclonal antibodies against human MMP-1 (Cat. No. ab52631) and rabbit polyclonal antibodies against human MMP-2 (Cat. No. ab52756) and MMP-9 (Cat. No. ab38898) proteins were obtained from Abcam Corporation, Cambridge, UK, the specificities of which have been established in previous studies (14). Rabbit polyclonal antibody (Calbiochem-Novabiochem International, Cambridge, MA, USA; Cat. No.499255) was used for the identification of SPARC protein. Tissue sections were mounted on gelatin-chrome alum-coated slides; following deparaffinization in xylene (twice) and rehydration in a decreasing-concentration ethanol series (absolute, 95%, 70% and 30% ethanol, and then water), tissue sections were microwave-treated three times (5 min each time) in a citrate buffer (10 mM; pH 6.0) in order to retrieve antigenicity. Endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol for 60 min. Prior to immunohistochemical staining, a 10% solution of normal rabbit serum was applied for 60 min to tissue sections in order to inhibit non-specific staining; these sections were subsequently incubated with antibodies against MMP-1, -2, -9 (1:100 each) and SPARC (1:1000) overnight at 4°C. After subsequent rinsing with TBS (three times, 10 min each), the tissue sections were then incubated for 60 min at room temperature with biotin-conjugated goat anti-rabbit IgG (Vector, Burlingame, CA, USA; 1:100), following which all sections were again washed with Tris-buffered saline (TBS; three times, 10 min each) and then incubated with an avidin–biotin complex conjugated to horseradish peroxidase (Dako, Santa Barbara, CA, USA) for a further 60 min. After washing with TBS (three times, 10 min each), peroxidase binding was visualized as a brown reaction product via a benzidine reaction. The sections were then counterstained with Mayer's hematoxylin. Each set of

Table 1 Clinical and histological data of the 23 ameloblastoma patients in this study

Case No.	*Gender*	*Age*	*Location*	*Histology subtypes*
1[a]	Male	59	Mandible	Follicular/ Plexiform
2	Female	13	Mandible	Plexiform
3	Male	14	Mandible	Follicular/ Acanthomatous
4[a]	Male	61	Mandible	Follicular
5[a]	Male	61	Mandible	Follicular
6	Male	36	Mandible	Follicular
7	Male	62	Mandible	Follicular
8	Female	22	Mandible	Plexiform
9	Male	32	Mandible	Follicular/ Acanthomatous
10	Male	16	Mandible	Plexiform
11	Female	16	Mandible	Plexiform/ Acanthomatous
12	Female	39	Mandible	Follicular
13	Female	23	Mandible	Plexiform/ Acanthomatous
14	Male	56	Mandible	Follicular
15[a]	Male	59	Mandible	Plexiform/ Acanthomatous
16	Female	24	Mandible	Follicular
17	Female	27	Mandible	Plexiform
18	Male	23	Mandible	Plexiform
19[a]	Male	55	Mandible	Follicular
20[a]	Male	60	Maxilla	Plexiform
21	Male	16	Mandible	Follicular
22	Female	59	Mandible	Plexiform
23	Male	46	Mandible	Follicular

[a]Local recurrence.

experiments included a human oral squamous-cell carcinoma specimen known to express MMP-1, -2, -9 and SPARC, which served as a positive control and ensured the reproducibility of the staining process; a negative control in which the primary antibody step was omitted was also included in each set of experiments. Tissue sections were analysed using a Nikon Eclipse E600 microscope (Nikon, Lewisville, TX, USA).

The immunostained sections were compared with the corresponding hematoxylin and eosin-stained sections in order to establish a topographic relationship between positive-stained areas and histopathological diagnoses. The percentage (P) of positive-stained tumor cells was determined semi-quantitatively by assessing the entire tumor section, and each section was assigned to one of the following categories: 0 (0–4%), 1 (5–24%), 2 (25–49%), 3 (50–74%), or 4 (75–100%). The intensity (I) of immunostaining was determined as 0 (negative), 1 (light yellow), 2 (yellow-brown), or 3 (dark brown). Staining intensity was judged relative to unstained tumor cells of the sample. Finally, an immunoscore was calculated by multiplying the percentage of positive cells (P) by the staining intensity score (I), as proposed by Krajewska et al. (19). The immunoscores for SPARC and the MMPs were then evaluated and classified into four groups: (+) score 0–2, (++) score 3–5, (+++) score 6–8, and (++++) score 9–12, scored by the first two authors independently. When there was disagreement between the scores assigned by the evaluators, a consensus was reached by discussion. Interobserver agreement was evaluated using kappa statistics (20) and assessed according to the calculated kappa value: a kappa value of less than 0.40 was considered as showing poor agreement; one of 0.40–0.59, fair agreement; one of 0.60–0.74, good agreement; and one of 0.75–1.00, excellent agreement.

Statistical analyses

Statistical analyses were performed using JUMP 7.0 software (SAS Institute Inc, Cary, NC, USA). The statistical significance of differences in immunoscore between SPARC, MMP-1, -2 and, -9 were analyzed by Pearson's χ^2-test. The immunoscores of these four proteins with recurrence were also compared using Pearson's χ^2-test. *P*-values lower than 0.05 were considered to indicate statistical significance.

Double immunohistochemistry

Double immunohistochemistry with SPARC and MMP-9 antibodies was performed using the EnVision Doublestain System kit (Dako) according to the manufacturer's instructions in order to examine the co-expression of these two proteins in ameloblatoma specimens. In brief, slides were incubated overnight at 4°C with anti-SPARC antibody (1:1000) after antigen retrieval and then incubated with labeled polymer-HRP-anti-mouse and anti-rabbit antibodies for 30 min at room temperature. Peroxidase activity was detected with the enzyme substrate 3,3′-diaminobenzidine tetrachloride. After quenching the enzyme reaction, slides were incubated in double stain block at room temperature for 5 min to block endogenous phosphatase. The slides were then incubated overnight at 4°C with anti-MMP-9 antibody (1:100). After washing, slides were incubated with labeled polymer-alkaline phosphatase anti-mouse and anti-rabbit antibody for 30 min at room temperature. Fast Red chromogen substrate solution was used to visualize anti-MMP-9 antibody. The sections were then counterstained with Mayer's hematoxylin.

Results

Interobserver agreement between the two observers was excellent for the assessment of immunoscores, with a kappa value of 0.92. The immunohistochemical expression scores for each case of ameloblastoma are summarized in Table 2. All ameloblastoma samples showed expression of MMP-1, -2 and -9 and SPARC with different intensities; these proteins (MMP-1, -2, -9 and SPARC) were mostly located in the cytoplasm of the ameloblastic-like columnar cells and stellate-reticulum-like cells, as well as in the stromal tissues of fibroblasts and endothelial cells. Furthermore, co-expression of SPARC and MMP-9 proteins was noted in the cytoplasm of the ameloblastic-like columnar cells and stellate-reticulum-like cells, also in the stromal tissues of fibroblasts and endothelial cells.

SPARC expression was found to be correlated significantly with MMP-9 expression (Table 3) but not with MMP-1 and -2. Recurrence was not statistically significant in terms of the immunohistochemical expression of

Table 2 Immunoscores of SPARC, MMP-1, 2 and 9 for the 23 ameloblastoma patients in this study

	Immunoscores			
Case no.	*SPARC*	*MMP-1*	*MMP-2*	*MMP-9*
1[a]	++	++	++	+++
2	+++	++	++++	++++
3	+	++	+	++
4[a]	++++	++	++	++++
5[a]	+++	++	++++	++++
6	+++	++	+	+++
7[a]	+++	++	++	++++
8	+++	+++	+++	++++
9	+++	++	++	++++
10	++	+++	++	+++
11	++++	+++	++++	++++
12	++++	+	+	++++
13	++++	+	++	++++
14	+++	+	++	++++
15[a]	++	++	++	++
16	+++	++	+	++++
17	+++	+	+++	++++
18	++++	+++	+	++++
19[a]	++++	+	+	++++
20[a]	+++	+	++	++++
21	++++	++	+++	++++
22	++++	++	++	++++
23	++++	++	++	++++

SPARC, secreted protein acidic and rich in cysteine; MMPs, Matrix metalloproteinases; +, score 0–2; ++, score 3–5; +++, score 6–8; ++++, score 9–12.
[a]Local recurrence.

Table 3 *P*-value for Pearson's χ^2-test to identify associations between the immunoscores of SPARC, MMP-1, -2 and -9 in this study

	SPARC	*MMP-1*	*MMP-2*	*MMP-9*
SPARC		0.8122	0.5984	0.0003*
MMP-1	0.8122		0.8374	0.5062
MMP-2	0.5984	0.8374		0.8644
MMP-9	0.0003*	0.5062	0.8644	

SPARC, secreted protein acidic and rich in cysteine; MMPs, matrix metalloproteinases.
*Statistical significance ($P < 0.05$).

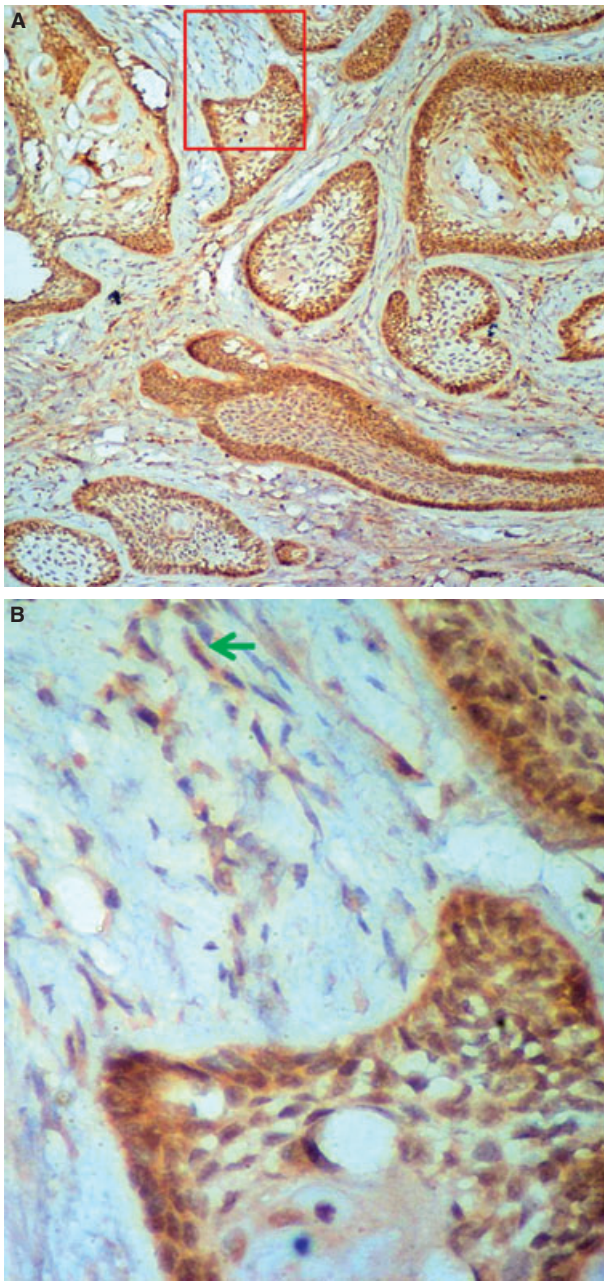

Figure 1 Representative sample (case 5) of the immunohistochemical expression of matrix metalloproteinase-9 (MMP-9) (A, ×100; B, ×400) in ameloblastoma. Insect rectangle indicates area shown in (B); MMP-9 proteins are also present in the stromal region (B, arrow) (avidin–biotin peroxidase complex staining).

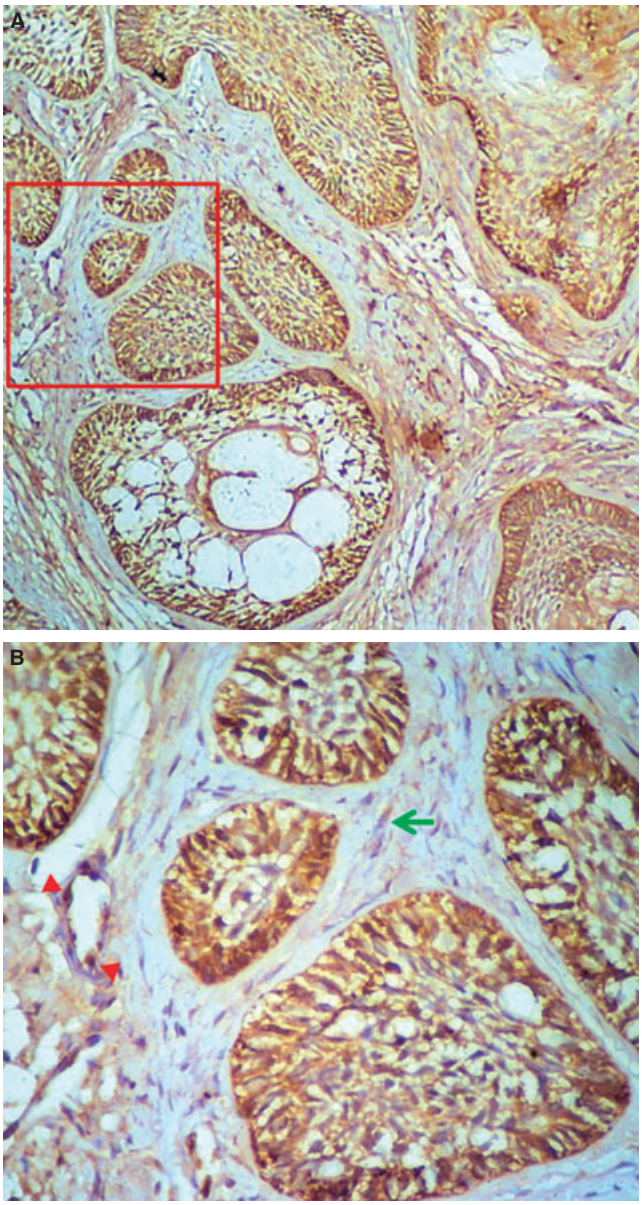

Figure 2 Representative sample (case 5) of the immunohistochemical expression of secreted protein acidic and rich in cysteine (SPARC) (A, ×100; B, ×400) in ameloblastoma. Insect rectangle indicates area shown in (B); SPARC proteins are also present in the stromal region (B, arrow). Positive staining of the endothelial cell was around capillary (B, arrowhead) (avidin–biotin peroxidase complex staining).

MMP-1, -2, -9 and SPARC. Representative samples of MMP-9 and SPARC stainings are shown in Figs 1 and 2, whereas representative sample of co-expression of SPARC and MMP-9 proteins is shown in Fig. 3.

Discussion

The ECM acts as a protective barrier for blocking tumor invasion and metastasis, and degradation of the ECM is a critical step in the process of cancer cell invasion and metastasis. A number of proteins including ECM-degrading enzymes, growth factors, cytokines and

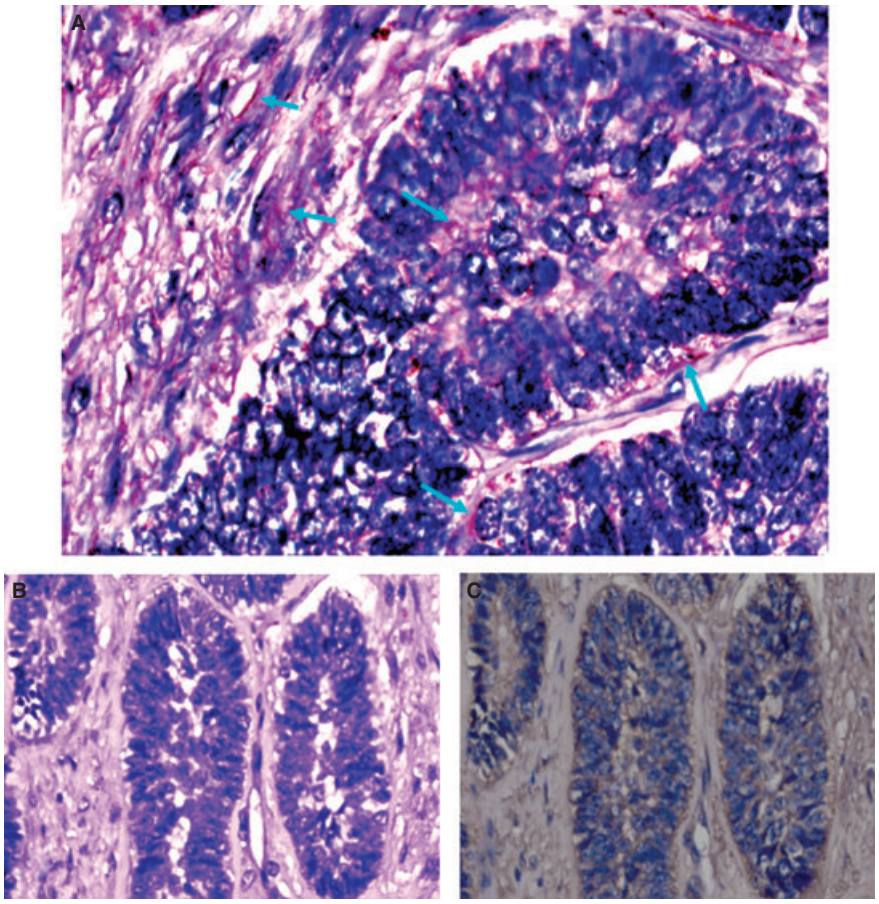

Figure 3 Representative sample (case 5) of the ameloblastoma showing co-expression of secreted protein acidic and rich in cysteine (SPARC) and matrix metalloproteinase-9 (MMP-9) in the tumor island as well as stromal region (light blue arrows) (A, ×400). Control stainings of MMP-9 (B, ×200) & SPARC (C, ×200).

proteins related to ECM are involved in the process of cancer cells crossing tissue boundaries.

Matrix metalloproteinases are ECM-degrading enzymes and play important roles in tumor invasion and metastasis (21). Where MMPs and tissue inhibitors of metalloproteinases (TIMPs) are imbalanced is where pathological conditions occur, such as rheumatoid arthritis (22), periodontal disease (23) and tumor invasion (24). Recently, correlation of MMPs with survival rate in cases of oral cancer has also been reported (25, 26).

Matrix metalloproteinase-1 is one of the three mammalian collagenases (MMP-1, -8, and -13) and belongs to the group of interstitial collagenases, which can degrade fibrillar collagens I, II, III, VII and X, gelatin, and proteoglycan core protein (27). The MMP subfamily of gelatinae, which includes gelatinae-A (MMP-2) and gelatinae-B (MMP-9), can cleave collagen IV and the structural components of basement membranes as a preface to invasion. Abnormal expression of MMP-1, -2 and -9 has been found to be correlated with aggressiveness in many tumors (28, 29). In the current study, co-expression of SPARC and MMP-9 were also noted in the cytoplasm of the ameloblastic-like columnar cells and stellate-reticulum-like cells as well as in the stromal tissues of fibroblasts and endothelial cells of our cohort of ameloblastoma patients. In addition, not only MMP-9 but also MMP-1 and -2 were found to be expressed in the stromal and tumor cells of ameloblastoma tissues. This finding is consistent with the results of Pinheiro et al. (14) but contrasts with those of Kumamoto et al. (30). The production of MMPs by tumor cells might constitute a 'pre-invasive niche' (31), which are related to the invasiveness of the ameloblastoma. This hypothesis is also supported by silver-stained nucleolar organizer region staining results (14), which suggest that ameloblastoma cells synthesize soluble growth factors and cytokines that construct a microenvironment for neoplastic projection infiltrating into the jawbone.

Proteins related to the ECM, such as SPARC, have been identified as being involved in the regulation of embryogenesis (32) and cancer cell progression (12). Furthermore, it has been reported that, during normal tooth development, SPARC was not expressed in ameloblasts as well as in inner and outer enamel epithelium, cells of the stratum reticulare and stratum intermedium but SPARC was expressed in odontoblasts and cementoblasts (33). Therefore, we regard that normal tooth buds need not necessary to be included in the current study and SPARC expression in

ameloblastoma is an overt pathologic phenomenon. The current study, to the best of our knowledge, may be the first to identify the expression of SPARC in ameloblastoma tissues.

High SPARC expression levels indicate poorer prognoses in some tumors, for example, those of the colon, lung, esophagus and pancreas (12); in contrast, SPARC expression in neuroblastoma inhibits angiogenesis and impairs tumor growth (34). Thus, the differing influence of SPARC in different tumors reflects that the function of SPARC may be tissue-specific. Moreover, we investigated the relationship between the presence of SPARC and MMP-1,-2 and -9 in this odontogenic tumor, and our results showed that SPARC expression is significantly associated with MMP-9 expression in ameloblastoma but not with MMP-1 and -2. These findings may be interpreted as being suggestive of participation of SPARC in ECM turnover in the ameloblastoma microenvironment; moreover, SPARC may interact with MMPs (probably chiefly MMP-9) to induce a cascade of neoplastic cells to invade the pericellular microenviroment. This notion has been supported by other studies that have reported the association of SPARC expression and MMPs induction. For instance, Tremble et al. (35) reported that SPARC induced MMP-1, -3 and -9 in rabbit fibroblasts; Shankavaram et al. (36) indicated that, in human monocytes, SPARC affected the expression of MMP-1 and -9; Gilles et al. (37) found that MMP-2 was induced by recombinant-SPARC in an invasive cell line but not in a non-invasive cell line; and Kunigal et al. (38) observed that SPARC increased MMP-9 expression level in glioblastoma cell lines. In addition, based on the results of a previous study (39), MMPs are a potential cofactor of SPARC due to the fact that, first, SPARC was cleaved by various MMPs, and second, SPARC expression was associated with expression of MMP-1 and -2 in tumor tissue specimens. Thus, in line with these aforementioned studies, our findings imply that, possibly in association with MMPs, participation of SPARC in the proteolysis of the ECM may be one of the factors contributing to the locally-aggressive behavior of ameloblastoma.

Angiogenesis is a critical factor for the invasion of solid tumors. Immunohistochemical detection of VEGF (40) and MMP-9 (14) has been reported in ameloblastoma, and the roles of VEGF in angiogenesis include augmentation of the initiation of angiogenesis and increasing vascular permeability (41). Kato et al. (42) reported that VEGF increased the SPARC level in human vascular endothelial cells. MMP-9 regulates the vascular architecture for stabilization of the vessel (43); furthermore, MMP-9 recruits the endothelial stem cells in the bone marrow (44). Some bioactive SPARC fragments could stimulate angiogenesis (45). In the present study, SPARC was expressed not only by tumor cells but also by fibroblasts and endothelial cells within the stroma. Therefore, we hypothesized that MMP-9 and SPARC contribute to the growth of blood vessels required in the local invasiveness of ameloblastoma. The putative pathways by which SPARC, MMP-9 and VEGF are associated in ameloblastomas were depicted in Fig. 4. On the other hand, SPARC has been detected

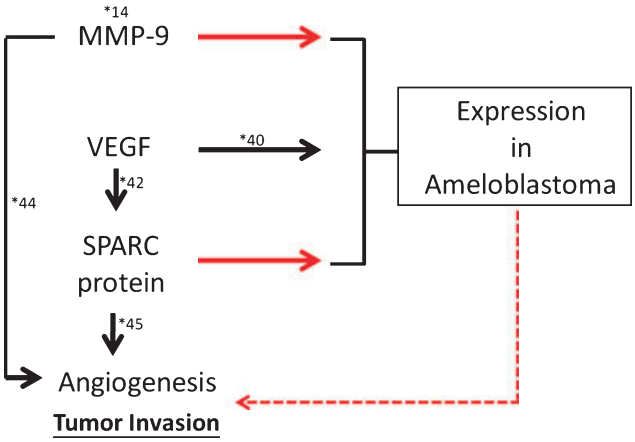

Figure 4 Putative association between secreted protein acidic and rich in cysteine (SPARC) and matrix metalloproteinase-9 (MMP-9) in ameloblastoma with respect to angiogenesis for tumor invasion. MMP-9 contributes to the local invasiveness of ameloblastoma (14) and recruits the endothelial stem cells in bone marrow (44). Vascular endothelial growth factor (VEGF) is an important mediator of angiogenesis in ameloblastoma (40). Induction of SPARC by VEGF in human vascular endothelial cells (42, 45) plays a role in tumor angiogenesis. The co-expressed MMP-9 and SPARC in ameloblastoma described in the present study (red arrow) may contribute to the angiogenesis required for the local invasiveness of ameloblastoma (red dotted arrow). *Reference numbers.

in the Golgi apparatus in cultured cells (46). Hence, we hypothesized that the cytoplasmic SPARC staining noted in the current study possibly occurred within the secretory vesicles of cells.

Gelatin zymography can be utilized to identify the latent or active form of MMP-9, and Western blotting used to show whether or not SPARC has cleaved. Ikebe et al. (47) confirmed that the immunohistochemical reactivity of MMP-9 in frozen tissue is the same as that of the active form using gelatin zymography; therefore, in this study, immunohistochemical demonstration of paraffin-embedded tissues was used to investigate MMPs and SPARC expression in order to observe the localization of these proteases and SPARC in ameloblastoma. Nevertheless, the activity of MMP-9 and that of the cleaved and full-length SPARC cannot be discriminated by immunohistochemistry.

In conclusion, our results suggest a putative association between SPARC and MMPs (especially MMP-9) in ameloblastoma. Future work is needed in order to elucidate the exact mechanism of the interaction between SPARC and MMPs that modulates ECM and tissue homeostasis.

References

1. Zemann W, Feichtinger M, Kowatsch E, et al. Extensive ameloblastoma of the jaws: surgical management and immediate reconstruction using microvascular flaps. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007; **103**: 190–6.
2. Lau SL, Samman N. Recurrence related to treatment modalities of unicystic ameloblastoma: a systematic review. *Int J Oral Maxillofac Surg* 2006; **35**: 681–90.

3. Becelli R, Carboni A, Cerulli G, et al. Mandibular ameloblastoma: analysis of surgical treatment carried out in 60 patients between 1977 and 1998. *J Craniofac Surg* 2002; **13**: 395–400.
4. Gortzak RA, Latief BS, Lekkas C, et al. Growth characteristics of large mandibular ameloblastomas: report of 5 cases with implications for the approach to surgery. *Int J Oral Maxillofac Surg* 2006; **35**: 691–5.
5. Nagase H, Woessner JF Jr. Matrix metalloproteinases. *J Biol Chem* 1999; **274**: 21491–4.
6. Noel A, Jost M, Maquoi E. Matrix metalloproteinases at cancer tumor-host interface. *Semin Cell Dev Biol* 2008; **19**: 52–60.
7. Pilcher BK, Sudbeck BD, Dumin JA, et al. Collagenase-1 and collagen in epidermal repair. *Arch Dermatol Res* 1998; **290**(Suppl): S37–46.
8. Pilcher BK, Dumin JA, Sudbeck BD, et al. The activity of collagenase-1 is required for keratinocyte migration on a type I collagen matrix. *J Cell Biol* 1997; **137**: 1445–57.
9. Masson V, de la Ballina LR, Munaut C, et al. Contribution of host MMP-2 and MMP-9 to promote tumor vascularization and invasion of malignant keratinocytes. *FASEB J* 2005; **19**: 234–6.
10. Termine JD, Kleinman HK, Whitson SW, et al. Osteonectin, a bone-specific protein linking mineral to collagen. *Cell* 1981; **26**: 99–105.
11. Bornstein P, Sage EH. Matricellular proteins: extracellular modulators of cell function. *Curr Opin Cell Biol* 2002; **14**: 608–16.
12. Clark CJ, Sage EH. A prototypic matricellular protein in the tumor microenvironment-where there's SPARC, there's fire. *J Cell Biochem* 2008; **104**: 721–32.
13. Ledda MF, Adris S, Bravo AI, et al. Suppression of SPARC expression by antisense RNA abrogates the tumorigenicity of human melanoma cells. *Nat Med* 1997; **3**: 171–6.
14. Pinheiro JJ, Freitas VM, Moretti AI, et al. Local invasiveness of ameloblastoma. Role played by matrix metalloproteinases and proliferative activity. *Histopathology* 2004; **45**: 65–72.
15. Chen JJ, Lin YC, Yao PL, et al. Tumor-associated macrophages: the double-edged sword in cancer progression. *J Clin Oncol* 2005; **23**: 953–64.
16. Arnold S, Mira E, Muneer S, et al. Forced expression of MMP-9 rescues the loss of angiogenesis and abrogates metastasis of pancreatic tumors triggered by the absence of host SPARC. *Exp Biol Med* 2008; **233**: 860–73.
17. Kramer IR, Pindborg JJ, Shear M. The WHO histological typing of odontogenic tumours. A commentary on the Second Edition. *Cancer* 1992; **70**: 2988–94.
18. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 1981; **29**: 577–80.
19. Krajewska M, Fenoglio-Preiser CM, Krajewski S, et al. Immunohistochemical analysis of Bcl-2 family proteins in adenocarcinomas of the stomach. *Am J Pathol* 1996; **149**: 1449–57.
20. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; **33**: 159–74.
21. Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 2001; **17**: 463–516.
22. Okada Y, Gonoji Y, Nakanishi I, et al. Immunohistochemical demonstration of collagenase and tissue inhibitor of metalloproteinases (TIMP) in synovial lining cells of rheumatoid synovium. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1990; **59**: 305–12.
23. Kubota T, Nomura T, Takahashi T, et al. Expression of mRNA for matrix metalloproteinases and tissue inhibitors of metalloproteinases in periodontitis-affected human gingival tissue. *Arch Oral Biol* 1996; **41**: 253–62.
24. Liotta LA, Stetler-Stevenson WG. Metalloproteinases and cancer invasion. *Semin Cancer Biol* 1990; **1**: 99–106.
25. Chiang YY, Tsai MH, Lin TY, et al. Expression profile of metastasis-related genes in invasive oral cancers. *Histol Histopathol* 2008; **23**: 1213–22.
26. Yang SF, Yang WE, Kuo WH, et al. Antimetastatic potentials of flavones on oral cancer cell via an inhibition of matrix-degrading proteases. *Arch Oral Biol* 2008; **53**: 287–94.
27. Kerrigan JJ, Mansell JP, Sandy JR. Matrix turnover. *J Orthod* 2000; **27**: 227–33.
28. Manenti L, Paganoni P, Floriani I, et al. Expression levels of vascular endothelial growth factor, matrix metalloproteinases 2 and 9 and tissue inhibitor of metalloproteinases 1 and 2 in the plasma of patients with ovarian carcinoma. *Eur J Cancer* 2003; **39**: 1948–56.
29. Minn AJ, Gupta GP, Siegel PM, et al. Genes that mediate breast cancer metastasis to lung. *Nature* 2005; **436**: 518–24.
30. Kumamoto H, Yamauchi K, Yoshida M, et al. Immunohistochemical detection of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in ameloblastomas. *J Oral Pathol Med* 2003; **32**: 114–20.
31. Kaplan RN, Riba RD, Zacharoulis S, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature* 2005; **438**: 820–7.
32. Holland PW, Harper SJ, McVey JH, et al. In vivo expression of mRNA for the Ca^{++}-binding protein SPARC (osteonectin) revealed by in situ hybridization. *J Cell Biol* 1987; **105**: 473–82.
33. Reichert T, Störkel S, Becker K, et al. The role of osteonectin in human tooth development: an immunohistological study. *Calcif Tissue Int* 1992; **50**: 468–72.
34. Chlenski A, Liu S, Crawford SE, et al. SPARC is a key Schwannian-derived inhibitor controlling neuroblastoma tumor angiogenesis. *Cancer Res* 2002; **62**: 7357–63.
35. Tremble PM, Lane TF, Sage EH, et al. SPARC, a secreted protein associated with morphogenesis and tissue remodeling, induces expression of metalloproteinases in fibroblasts through a novel extracellular matrix-dependent pathway. *J Cell Biol* 1993; **121**: 1433–44.
36. Shankavaram UT, DeWitt DL, Funk SE, et al. Regulation of human monocyte matrix metalloproteinases by SPARC. *J Cell Physiol* 1997; **173**: 327–34.
37. Gilles C, Bassuk JA, Pulyaeva H, et al. SPARC/osteonectin induces matrix metalloproteinase 2 activation in human breast cancer cell lines. *Cancer Res* 1998; **58**: 5529–36.
38. Kunigal S, Gondi CS, Gujrati M, et al. SPARC-induced migration of glioblastoma cell lines via uPA-uPAR signaling and activation of small GTPase RhoA. *Int J Oncol* 2006; **29**: 1349–57.
39. Sasaki T, Göhring W, Mann K, et al. Limited cleavage of extracellular matrix protein BM-40 by matrix metalloproteinases increases its affinity for collagens. *J Biol Chem* 1997; **272**: 9237–43.
40. Kumamoto H, Ohki K, Ooya K. Association between vascular endothelial growth factor (VEGF) expression and tumor angiogenesis in ameloblastomas. *J Oral Pathol Med* 2002; **31**: 28–34.

41. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. *Endocr Rev* 1997; **18**: 4–25.
42. Kato Y, Lewalle JM, Baba Y, et al. Induction of SPARC by VEGF in human vascular endothelial cells. *Biochem Biophys Res Commun* 2001; **287**: 422–6.
43. Chantrain CF, Shimada H, Jodele S, et al. Stromal matrix metalloproteinase-9 regulates the vascular architecture in neuroblastoma by promoting pericyte recruitment. *Cancer Res* 2004; **64**: 1675–86.
44. Heissig B, Hattori K, Dias S, et al. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell* 2002; **109**: 625–37.
45. Framson PE, Sage EH. SPARC and tumor growth: where the seed meets the soil? *J Cell Biochem* 2004; **92**: 679–90.
46. Busch E, Hohenester E, Timpl R, et al. Calcium affinity, cooperativity, and domain interactions of extracellular EF-hands present in BM-40. *J Biol Chem* 2000; **275**: 25508–15.
47. Ikebe T, Shinohara M, Takeuchi H, et al. Gelatinolytic activity of matrix metalloproteinase in tumor tissues correlates with the invasiveness of oral cancer. *Clin Exp Metastasis* 1999; **17**: 315–23.

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