Correlations of extracellular matrix metalloproteinase inducer and microvessel density to invasiveness of ameloblastoma

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Key words: ameloblastoma, matrix metalloproteinase, matrix metalloproteinase inducer, microvessel density, invasion

Background and Objective: Matrix metalloproteinases (MMPs) are involved in local invasion of ameloblastomas. This study evaluates the role of matrix metalloproteinase inducer (EMMPRIN) in angiogenesis in ameloblastomas by analyzing EMMPRIN expression and microvessel density (MVD) in ameloblastomas and odontogenic cysts.

Methods: EMMPRIN expression and MVD in 41 specimens of ameloblastoma and 40 specimens of odontogenic cyst were examined by SP immunohistochemistry.

Results: EMMPRIN was detected in all specimens of ameloblastomas and odontogenic cysts. The strong positive rate of EMMPRIN was significantly higher in ameloblastomas than in odontogenic cysts (85.4% vs. 62.5%, p < 0.05). MDV was positively correlated to EMMPRIN expression to some extent (r = 0.677, p < 0.01).

Conclusion: EMMPRIN may play an important role during the progression of ameloblastoma via controlling angiogenesis and degradation of extracellular MMPs.

Ameloblastoma is a benign odontogenic tumor commonly seen in oral and maxillofacial surgical cases. Its local invasive growth pattern, being its main biological feature, results in a higher post-operative recurrence rate. Its pathogenesis remains unclear, as well as the cause of its invasive growth is yet to be fully understood. Many studies have proven that the invasive growth of ameloblastomas is associated with Matrix metalloproteinases (MMPs), however, researches conducted on the correlations of extracellular matrix metalloproteinase inducer (EMMPRIN) and Microvessel Density (MVD) to the invasiveness of ameloblastoma are rare. The following study had focused on the EMMPRIN expression, as well as MVD statuses in ameloblastomas, and evaluated the correlation of EMMPRIN and MVD to the invasive growth of ameloblastoma.

Materials and Methods

Materials. 41 subjects of resected ameloblastoma tissue samples in 1992 to 2007 were selected from the Third Affiliated Hospital of Sun Yat-sen University, where 25 subjects were from male patients and 16 were from females; all aged between 19 and 61 years old with a median age of 39 years old. Tumor location: at mandible 31 subjects, at maxilla ten subjects. Tissue growth pattern: follicular type 23 subjects, plexiform type 18 subjects; the largest reported tumor among these subjects had invaded the ramus and partial body of the mandible unilaterally (about the size of 12 x 10 cm), while the smallest was about 2 x 2.5 cm. 40 subjects of odontogenic cysts tissue samples were selected as a control, all subjects were primarily-identified cases and had been confirmed by two pathologists.

Main reagents: immunohistochemistry SP kit (instant type), rabbit anti-human EMMPRIN polyclonal antibodies and mouse anti-human CD34 monoclonal antibody (QBEnd/10) were all purchased from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.

Methods. Immunohistochemical staining and outcome determination: SP immunohistochemistry method and DBA staining were applied. Experimental paraffin block samples were used for serial tissue sectioning, 4 mm thickness, underwent HE, as well as immunohistochemical staining respectively, applied on EMMPRIN-immunostained sections, soaked in 1 mM EDTA buffer (pH 8.0), placed in high-pressure autoclave (121°C, 2 atm) and heated for 10 min. Primary antibody was subsequently added onto the sections and left overnight at 4°C. After then, the sections were combined with rabbit anti-human EMMPRIN polyclonal antibody to undergo reaction for 45 mins, and stained by soaking them into 2 Mm H2O2 of 0.05% aminobiphenyl solution for 2–3 mins. Microwave heating method was used for CD3 staining. Outcome determination: The results of EMMPRIN immunoreactions were divided into the following 3 grades: negative (-), positive
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(+) strong positive (++); in which EMMPRIN (+) denotes for brownish granules seen in cell cytoplasm or on cell membrane, (+) for light brown and (+++) for dark brown.

Any endothelial cells or endothelial cell clusters stained brownish-yellow with CD34 antibody, could be considered as a single microvessel given that its adjacent tumor cells or connective tissues are separated. MVD was calculated in accordance to Weider’s method: The entire section was initially scanned for high-MVD areas (also known as hot spots) under low microscopic magnification. Then, brown-stained endothelial clusters were calculated under light microscope observation 200x magnification. Result was shown as mean microvessel counts from three different observed fields under 200x magnification.

Statistical method. Statistical analysis for all data was processed utilizing SPSS10.0 statistical computer software. Sample means between groups, as well as among multiple groups were compared using t-test and one-way ANOVA. Positive rate differentials between groups, as well as among multiple groups were compared using t-test or Fisher’s exact test, taken $\alpha = 0.05$, and $p < 0.05$ denoting statistical significant difference.

Results

EMMPRIN expressions in ameloblastoma and odontogenic cysts. Immunohistochemical staining showed EMMPRIN (+++) and (+) in all ameloblastoma and odontogenic cyst tissues, hence there are no negative results shown. In ameloblastoma tissues, EMMPRIN (+++) and (+) were found concentrated mainly on columnar cells or cuboid cells’ membrane and/or in cytoplasm surrounding the tumor cell nests, whilst polygon cells present medially were generally, non-immunoresponsive (Fig. 1); In odontogenic cyst tissues, EMMPRIN (+++) and (+) were found mainly in basal cells, whilst in odontogenic cyst tissues with EMMPRIN (+++), the middle cell layers were also found immunoresponsive.

Comparison of EMMPRIN (+++) expression between ameloblastomas and odontogenic cysts. 35 in 41 (85.37%) subjects with ameloblastoma showed EMMPRIN (+++), 25 in 40 subjects with odontogenic cyst showed EMMPRIN (+++) ($p = 0.024$). However, statistical EMMPRIN (+++) expression difference between the two types of ameloblastomas was not significant (Table 1).

CD34 expressions and MVD counts in odontogenic cysts and two types of ameloblastoma tissues. Multiple endothelial cells as well as endothelial clusters stained yellow with CD34 antibody could be found in the interstitials of odontogenic cyst and the two types of ameloblastoma tissues (Fig. 2); endothelial cells or clusters stained yellow with CD34 antibody could be considered as a single microvessel given that its adjacent tumor cells or connective tissues are separated. The MVD counts were $24.0 \pm 5.7$ in odontogenic cyst tissues, $21.2 \pm 5.8$ in follicular type of ameloblastoma tissues, and $25.8 \pm 9.0$ in flexiform type of ameloblastoma tissues, with no significant difference ($p = 0.085$); it was significantly lower in EMMPRIN (+) ameloblastoma tissues than in EMMPRIN (+++) ameloblastoma tissues ($18.6 \pm 2.3$ vs. $23.6 \pm 6.5$, $p = 0.026$). EMMPRIN expression was positively correlated to MVD count ($r = 0.67$, $p < 0.01$).

Discussion

Extracellular Matrix (ECM) is a dynamic reticulated structure present in between cell to cell, which is formed up by collagen, glycoprotein, proteoglycan and other macromolecular substances. This structure does not only participate in various cellular physiological processes, but also, it is involved in some pathological processes such as tumor invasion, metastasis and etc. Hence, the degradation of MMPs in ECM has become an important study aspect in oncology researches. There have been numerous reports found regarding researches associated with MMPs in ameloblastoma, however, the relationship between ameloblastoma and

![Figure 1. Expression of EMMPRIN in ameloblastoma (SP x400). (A) Strong expression of EMMPRIN is detected on membrane of tumor cells in ameloblastoma. (B) Weak expression of EMMPRIN is detected on membrane of tumor cells in ameloblastoma.](image)

<p>| Table 1 EMMPRIN expression in ameloblastomas and odontogenic cysts |
|-------------------------|---------------------|---------------------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>EMMPRIN expression (cases (%) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odontogenic cyst</td>
<td>40</td>
<td>15 (37.5)</td>
</tr>
<tr>
<td>Ameloblastoma</td>
<td>41</td>
<td>6 (14.6)</td>
</tr>
<tr>
<td>Follicular type</td>
<td>23</td>
<td>3 (13.0)</td>
</tr>
<tr>
<td>Flexiform type</td>
<td>18</td>
<td>3 (16.7)</td>
</tr>
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EMMPRIN as an MMP stimulating factor remains unclear, and studies associated with this subject is also rarely seen. EMMPRIN, also known as CD 147, tumor collagenase stimulatory factor, M6 antigen and etc., is a transmembrane glycoprotein with a molecular weight of about 58 KD, and is also one of the immunoglobulin super-family members. It plays a main role in participation of cell-cell recognition, its abundance on tumor surfaces is one of its main features, and it induces massive MMPs production from tumor cells as well as from their peripheral fibroblasts. EMMPRIN has been found to have stronger expressions in various tumors such as lung cancers, bladder carcinomas and breast cancers if compared with normal control tissues. On the other hand, recent oncology studies have also revealed that combination of EMMPRIN and its effect cells (fibroblasts, endothelial cells, etc.,) could induce MMPs production, and result in significant MMPs increase in content and activity. These indicate that EMMPRIN promotes tumor invasion and metastasis through regulating MMPs under-attached to the expression of MMPs. Hence, they have put forward a new mechanism of neovascularization, that is, the tumor cell-matrix interaction mediated by EMMPRIN, produced by tumor cells, directly promotes MMPs and VEGF production, and promotes neovascularization of tumors.

Currently, there are no related study reports known regarding the correlation of odontogenic cyst EMMPRIN to MVD. Our study has concluded that the expressions of EMMPRIN had been shown positive in ameloblastoma tissues as well as in odontogenic cyst tissues, and EMMPRIN was mainly expressed in epithelial cells nearby their basal membrane, and also strong expressions of EMMPRIN in ameloblastoma tissues were found more prominent than in odontogenic cyst tissues. In addition, the positive EMMPRIN expression in ameloblastomas has a certain degree of correlation to the density of microvessel distribution.

Acknowledgements
Grant National Natural Science Foundation of China (No. 30672409).

References
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