Matrix Metalloproteinase-9 (MMP-9) Expression in Different Subtypes of Ameloblastoma

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Abstract

Background Ameloblastoma is a common benign odontogenic tumor of the jaw with a local invasive and highly destructive behavior and can develop in any age, with peak prevalence in 3rd–4th decade. Ameloblastoma can be divided into six histological types: follicular, plexiform, acanthomatous, desmoplastic, granular, and basal cell. Matrix metalloproteinase-9 (MMP-9) (92-kD *gelatinase/type IV* collagenases = gelatinase B) is involved in bone resorption by degradation of extracellular matrix and osteoclasts recruitment. Recent studies have found that MMP-9 is expressed by ameloblastoma and has a role in ameloblastoma local invasiveness.

Objective To analyze MMP-9 expression between different histological types of ameloblastoma.

Material and Method Forty samples of ameloblastoma were collected through consecutive sampling and the MMP-9 expression was detected using immunohistochemistry.

Result All samples showed positive MMP-9 expression with moderate to strong intensity. 82.4 % plexiform type and 83.3 % mixed type have strong immunoexpression, significantly different with follicular type with only 36.4 % ($P < 0.05$).

Conclusion Ameloblastoma plexiform and mixed type have higher MMP-9 expression than ameloblastoma

follicular type. Different MMP-9 expression may contribute in different ameloblastoma biological behavior.

Keywords Ameloblastoma · Histological subtypes · MMP-9 · Local invasion

Introduction

Ameloblastoma is a common odontogenic tumor of the jaw that comprises 11 % of all odontogenic tumors. Ameloblastoma can be found in any ages with peak prevalence in the 3rd–4th decade of life [1]. The World Health Organization (WHO) classified ameloblastoma as benign odontogenic tumor formed by odontogenic epithelium with fibrous mature stroma but without odontogenic ectomesenchyme [2]. Ameloblastoma is classified as solid or multicystic type, unicystic type, peripheral type, and desmoplastic type. According to the histological pattern, ameloblastoma can be divided into follicular type, plexiform type, acanthomatous type, granular type, basal cell type, and desmoplastic type. A review of the international literature by Reichart et al. [3] found the solid or multicystic variant to be most common, comprising 92 % of the 3,677 cases of ameloblastoma in their review, while the unicystic and peripheral variants accounted for 6 % and 2 % of the cases. From histological pattern, the most common types are follicular (33.9 %), plexiform (30.2 %), and mixed histological type (15.5 %). Ameloblastoma is a slow-growing benign tumor but locally invasive and highly destructive with a high risk of recurrence. Ameloblastoma cells can invade into the cancellous bone beyond the tumor margin [4–7]. It is recommended to perform a radical resection 1–2 cm from tumor margin to prevent recurrences.

Pinheiro et al. [8] in their study on ameloblastoma local invasiveness found that almost half of ameloblastoma cells

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are synthesizing protein. Such protein activity may participate in a mechanism that contributes to the growth pattern of ameloblastoma. This activity could involve proteolytic enzymes related to the invasive phenotype, such as matrix metalloproteinases (MMPs). MMPs are family of zinc-dependent proteinases mediating degradation of extracellular matrix (ECM) and basement membrane. MMPs are divided into five subgroups according to their substrate specificity and structural characteristic; gelatinases, matrilysins, collagenases, stromelysins, and membrane-bound MMPs (MT-MMPs) [8]. Among the MMPs, matrix metalloproteinase-9 (MMP-9) (gelatinase B, a 92 kDa type IV collagenases) is involved in the process of tumor invasion by mediating degradation of basement membrane and remodeling of ECM, and likely the most important proteinase involved in bone resorption, as osteoclasts express this enzyme at a tremendously high level [9, 10]. MMP-9 is known to have a role in pathological bone resorption [11]. MMP-9 not only contribute in bone matrix degradation but also as a regulator in initial bone resorption process [8]. Some studies correlate MMP-9 expression to ameloblastoma local invasiveness and found that MMP-9 is related to local invasiveness and tumor growth of ameloblastoma [8, 12, 13]. MMP-9 present in ameloblastoma could digest bone matrix and release mitogens, which could, in turn, increase tumor proliferation [8]. Qian and Huang [14] found that the expression level of MMP-9 in ameloblastoma was of a high discrepancy. They suspected that there is a correlation between the discrepancy of expression level of MMP-9 and the histological types and biological behavior of ameloblastoma. The aim of this study is to analyze MMP-9 expression in ameloblastoma histological subtypes in order to understand more about ameloblastoma local invasion behavior.

Materials and Methods

Patient and Tissue Samples

Samples were taken from 40 patients who were diagnosed with ameloblastoma (14 females and 26 males, all cases were on the mandible) in Oral and Maxillofacial Surgery Department in Cipto Mangunkusumo Hospital, Jakarta, Indonesia, between January 2008 and May 2012. Specimen are taken from the resected mandible, fixed in 10 % formalin and embedded in paraffin. The paraffin blocks were sliced into 3- μ m-thick sections and mounted on poly-L-lysine-coated slides for immunohistochemical examinations.

Immunohistochemistry (IHC)

For immunohistochemistry analysis, the tissue sections were dewaxed in xylene and rehydrated in graded ethanol.

Endogenous peroxidase activity was subsequently blocked by immersion of slides in methanol with 0.5 % hydrogen peroxide for 30 min. Sections were then microwave treated twice for 5 min each at 700 W in 0.01 M Tris EDTA (pH 9.0) and cooled down at room temperature. Non-specific conjugation was blocked with sniper block (Biocare Medical, USA) for 15 min. The slides were then incubated with primary antibody in room temperature at the proper dilution for 1 h. The primary antibody used: rat monoclonal antibody against human MMP-9 (No. 58803 Abcam, UK), diluted 1:150 in goat serum. After primary antibody incubation, the sections were incubated with universal link (Biocare Medical, USA) for 15 min, labeled with trekavidine (Biocare Medical, USA) for 15 min, then incubated with DAB as chromogen for 5 min. Then sections were counterstained with hematoxylin-eosine and examined under microscope (Olympus BX41, Japan). Formalin-fixed, paraffin-embedded samples of human breast carcinoma tissue were used as positive controls.

Staining Evaluation

The staining intensity was classified as: negative (0), weak (1), and strong (2). The expression of MMP-9 was assessed by semi quantitative analysis. The percentage of immunopositive cells was scored as 0 = 0 %, 1 = 1–50 %, 2 = >50 %. The immunoscore for MMP-9 was calculated by multiplying the percentage score and intensity score, then classified as follows: 0, negative (–); 1, 2, and 3, weak positive (+), 4 and 6, strong positive (++)

Statistical Analysis

Expressions of MMP-9 in ameloblastoma were analyzed with Chi square test according to their cells percentage, intensity, and immunoscore using SPSS version 17.0 (Chicago, IL, USA). A *P* value less than 0.05 was considered to be significant.

Results

The clinical information of all patients and their histological subtypes is summarized in Table 1. The results of immunostaining for MMP-9 are presented in Table 2. In our study, the immunostaining results showed that immunoreactivity for MMP-9 was detected in all of 40 cases in the epithelial cells of ameloblastoma (Fig. 1). MMP-9 was also present in the surrounding stromal cells but the expression in the stromal cells was not as strong as in the epithelial cells. Plexiform and mixed type ameloblastoma have a majority of >50 % immunopositive cells, 82.4 and 75 % with moderate to strong intensity. Whereas the

Table 1 Clinical information of ameloblastoma histological subtypes

	Patients	Percentage (%)
Histological subtypes		
Plexiform	11	27.5
Follicular	17	42.5
Mixed type	12	30
	40	100

Age range of the patients was 11–58 years with mean age of 35.4 year

follicular type with >50 % immunopositive cells were only 54.5 %. The staining intensity in follicular type was weak to strong staining. Immunoreaction for plexiform type and mixed type were higher than follicular type. There was a statistically significant differences in MMP-9 immunoreaction between plexiform type, follicular type, and mixed type ameloblastoma ($P = 0.017$).

Discussion

Ameloblastoma is a benign odontogenic tumor with locally invasive and highly destructive behavior that is commonly found in the third and fourth decades of life. Ameloblastoma is divided into six histological subtypes. The difference in behavior between the subtypes is still unclear. Some researchers state that there is no correlation between histological subtypes, clinical symptoms or biological behavior. But other researchers revealed some correlation between the histological subtypes, clinical and radiographical appearances [3]. Mendenhall et al. [15] stated that ameloblastoma of different histological types exhibited diverse invasion property and biological behavior. Ameloblastoma and its local invasiveness have been attracting the attention of many researchers. Qian and Huang thought that the invasive behavior of ameloblastoma is closely correlated with the bone resorption surrounding the tumor and they are two aspects of the same physiological process [13].

MMP-9 is known for mediating degradation of basement membrane and remodeling of ECM. Studies have provided compelling evidence that MMP-9 is involved in tumor growth and bone metastases [8, 12–14, 17–21]. According to Vicente et al. [17] MMP-2 and MMP-9 are involved in angiogenesis and tumor growth, suggesting an association of the gelatinases with aggressive behavior and unpredictable clinical course in some human neoplasms. Stankovic et al. [9] found that MMP-2 and MMP-9 activity in different clinical stages of breast cancer have a significant positive association with tumor size. MMP-9 expression has also been found to correlate with the aggressiveness of head and neck carcinomas [18, 19]. MMP-9 is also considered to have an important role in bone resorption and is closely related with several osteo-destructive pathologies. [8, 20, 21]. MMP-9 act not only as solubilizers of bone matrix but also as regulators of the initiation of bone resorption [8]. Study has shown that MMP-9 is produced by osteoclast in the human bone tissues and suggest that it can degrade bone collagens in concert with MMP-1 and cysteine proteinases in the sub-osteoclastic microenvironment [16]. Several studies have been conducted to analyze the MMP-9 expression in ameloblastoma [8, 12–14, 20–22]. MMP-9 expression in ameloblastoma is higher than in other odontogenic cysts and keratocyst odontogenic tumor (KOT). This finding showed that ameloblastoma have a more aggressive behavior compared to other odontogenic cysts [23]. Kumamoto et al. [24] evaluated the correlation of MMP-9 in ameloblastoma tumor growth. They conclude that MMP-9 plays a role in regulation of tumor progression. According to Pinheiro et al. [8] MMP-9 also have a role in increasing tumor cells proliferation through mitogens release. Recent studies correlate MMP-9 with osteoclastogenesis caused by ameloblastoma [14].

In our study, all specimens showed positive immunoreactions to MMP-9. This is in accordance with the result of Henriques et al. [22] that found 95 % of ameloblastoma having positive MMP-9 immunoreactions. Most of our specimens show moderate to strong expression intensity. Yoon et al. [25] also found that ameloblastoma has

Table 2 MMP-9 immunoprofiles of ameloblastoma histological subtypes

	Immunopositive cells			Intensity			MMP-9 immunoreaction		
	0	1–50 %	>50 %	Weak	Moderate	Strong	(–)	(+)	(++)
Plexiform	0 (0)	3 (17.6)	14 (82.4)	2 (11.8)	8 (47.1)	7 (41.2)	0 (0)	3 (17.6)	14 (82.4)
Follicular	0 (0)	5 (45.5)	6 (54.5)	4 (36.4)	3 (27.3)	4 (36.4)	0 (0)	7 (63.6)	4 (36.4)
Mixed type	0 (0)	3 (25)	9 (75)	0 (0)	6 (12)	6 (12)	0 (0)	2 (16.7)	10 (83.3)
	$P = 0.267$			$P = 0.173$			$P = 0.017^*$		

() are in percentage. * Significant difference in expression between groups

Fig. 1 MMP-9 expression in ameloblastoma detected by immunohistochemistry. **A, B**, Strong and diffused MMP-9 expression with 100 % immunopositive cells (original magnification $\times 10$ and $\times 40$). **C, D**, Moderate and diffuse immunohistochemical expression of

MMP-9 (original magnification $\times 10$ and $\times 40$). **E, F**, Weak expression of MMP-9 with 30 % immunopositive cells (original magnification $\times 10$ and $\times 40$)

moderate to strong intensity of MMP-9 expression. The detection results of constant expression in all of specimens lead us to the presumption that MMP-9 played an essential role in development and progression of ameloblastoma and may be associated with bone resorption cause by ameloblastoma. From three histological subtypes, we found that ameloblastoma plexiform and mixed type have higher immunopositive cells percentage than the follicular type

and also higher immunoscore. We have not found previous studies that reviewed MMP-9 expression in ameloblastoma histological subtypes. The more exuberant expression of MMP-9 in plexiform type and mixed type suggest a possible participation of this protein in cell proliferation and differentiation and may explain greater bone resorption, higher invasion potential, and poorer prognosis than follicular type.

Conclusion

MMP-9 is expressed by ameloblastoma cells with variation among subtypes. Ameloblastoma plexiform and mixed type have a higher MMP-9 expression than other subtypes, thus they are more invasive than follicular type. But this result should be investigated further with other methods and markers and with more samples including all six ameloblastoma histological types.

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