Original Article

A comparative study of syndecan-1 expression in different odontogenic tumors

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ABSTRACT

Background: Expression of various cellular/molecular factors change during the course of tumor formation from odontogenic tissues of the tooth germ. Evaluation of these factors can help provide a better perception of the tumorigenesis and biologic behavior of odontogenic tumors (OTs). Syndecan-1 is a heparan sulfate proteoglycan which has not been extensively investigated in these lesions. The objective of the present study was to assess the immunohistochemical expression of CD138 in adenomatoid odontogenic tumor (AOT), ameloblastic fibroma (AF) and odontogenic myxoma (OM) and to compare it with ameloblastoma and keratocystic odontogenic tumor (KCOT).

Method: A total of 58 OTs consisting of 7 AOTs, 5 OMs, 7 AFs, 29 KCOTs and 10 ameloblastomas were immunohistochemically stained with monoclonal antibody against syndecan-1 and the percentage and intensity of the immunostained cells was assessed. Kruskal–Wallis test followed by Bonferroni analysis was used for comparisons ($P < 0.05$).

Results: Syndecan-1 was expressed in all samples except for OMs. Both percentage and intensity of syndecan-1 expression were statistically different among the studied OTs ($P < 0.001$). Pairwise comparisons showed significant difference only between OMs and each of the other tumors.

Conclusion: Syndecan-1 may be involved in the pathogenesis of AOT, AF, KCOT and ameloblastoma. However, considering the different behaviors of these tumors along with their similar expression of syndecan-1, it seems that its effect on clinical aggressiveness is limited. The significance of negative immunoeexpression of this protein in OM requires further investigation.

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1. Introduction

Syndecan-1 (CD138) belongs to a family of cell surface heparan sulfate proteoglycans.¹ This protein plays an important role in cell adhesion and cohesion.² The short cytoplasmic domain of CD138 binds to the cytoskeletal component, while the extracellular portion is capable of interacting with several growth factors and various extracellular matrix molecules.³ Syndecan-1 takes part in diverse cellular activities including migration, differentiation, cytoskeletal organization, and growth factor signaling.²

Syndecan-1 has been studied in a number of odontogenic lesions and during tooth development.^{4–8} Mesenchymal and ameloblastic cells of the tooth bud permanently express this protein as a signal transducer and matrix receptor for growth

factors.^{7,8} Syndecan-1 binds to a large number of extracellular effector molecules⁹ and its expression has been reported to be altered in solid ameloblastomas.⁵ Research on the expression of syndecan-1 in odontogenic tumors is limited and its precise role in the pathogenesis and biology of these neoplasms remains unknown.

Adenomatoid odontogenic tumor (AOT) is a benign slow growing lesion which generally affects the anterior maxillary region.¹⁰ The exact origin of this tumor is not clear, but it has been suggested to arise from the dental lamina complex or its remnants.¹⁰ Odontogenic myxoma (OM) is characterized by a benign but locally aggressive behavior, with a high rate of recurrence. This neoplasm is believed to originate from the mesenchymal component of the odontogenic apparatus.¹¹ Ameloblastic fibroma (AF) is an uncommon neoplasm of the odontogenic epithelium and mesenchymal tissues, it usually occurs in the posterior region of the mandible in children and demonstrates an indolent behavior.¹² Keratocystic odontogenic tumors (KCOTs) and ameloblastomas are both known for their infiltrative and

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aggressive behaviors. The dental lamina, its remnants or the overlying epithelium have been suggested as their possible sources.⁵

Considering that the expression of syndecan-1 in AOT, AF, and OM has not been extensively investigated, the aim of the present study was to evaluate the immunohistochemical expression of CD138 in these odontogenic tumors and to compare it with ameloblastoma and keratocystic odontogenic tumor.

2. Material and methods

The research protocol of this study was approved by the Ethics Committee of our University. A total of 58 specimens, including 7 AOTs, 5 OM, 7 AFs, 29 KCOTs, and 10 ameloblastomas, were evaluated. The H&E slides were reviewed by two oral pathologists to confirm the diagnosis.

For immunohistochemical detection, 3- μ m thick paraffin embedded tissue sections were mounted on 3-poly-L-lysine-coated glass slides. The samples were dewaxed in xylene and hydrated in graded alcohols (100%, 90% and 70%, 5 min each) and then washed in distilled water. For antigen retrieval, the sections were treated with citrate buffer (pH 6.0) in a microwave for 14 min and endogenous peroxidase activity was inhibited by 3% hydrogen peroxide for 10 min. The sections were then incubated with the primary monoclonal antibody anti-human syndecan-1 (clone MI15 Dako Corporation, Carpinteria, CA, USA, dilution 1:100) at room temperature for 1 h followed by application of EnVision System (Dako Cytomation, Glostrup, Denmark) for 30 min at room temperature. Human tonsillar tissue was used as positive control, and specimens in which the relevant primary antibody was omitted served as negative controls.

The percentage of positive neoplastic cells was assessed and classified into four groups: 0: (0%); 1: 1–10%, 2: 11–50% and 3: >50%.⁵ The staining intensity was evaluated by using the following scores: low, intermediate, and high.

2.1. Statistical analysis

Kruskal–Wallis test was used to compare the expression of syndecan-1 among different lesions and Bonferroni method was applied for the adjustment of *P*-value in multiple comparisons. The results were considered significant when *P* was less than 0.05.

3. Results

All ameloblastomas were immunopositive for syndecan-1. The staining intensity of this protein was low in two samples while the other eight cases exhibited intermediate to high immunoreactivity in the cytoplasm, mainly in the stellate reticulum-like cells. However, in some areas, the peripheral columnar cells also showed cytoplasmic syndecan-1 expression (Fig. 1). Syndecan-1 staining intensity was variable in KCOTs, but all specimens were positive. In most cases, cytoplasmic/membranous immunoreaction of syndecan was seen through the full thickness of the epithelial lining. Nevertheless, focal areas of the surface layer or basal cells in some samples were negative for this protein (Fig. 2). Syndecan-1 immunoreaction was detected in all specimens of AOTs and AFs, and demonstrated moderate to strong intensity. In AOTs, immunostaining of syndecan-1 was prominently localized to the spindle-shaped cells that were adjacent to the duct-like structures as well as the peripheral cords (Fig. 3). The whorl-like structures of AOTs sometimes showed CD138 immunoreaction. The AFs revealed marked expression of syndecan-1 in both the neoplastic epithelial and mesenchymal components (Fig. 4). Staining was usually cytoplasmic/membranous in AOTs and ameloblastic fibromas, but nuclear staining was seen in spindle-shaped cells

Fig. 1. Syndecan-1 immunoexpression in a representative section of ameloblastoma. Note positive cytoplasmic staining of the stellate reticulum (scale bar, 0.1 mm).

Fig. 2. An example of syndecan-1 immunostaining in keratocystic odontogenic tumor with cytoplasmic and membranous staining of the entire epithelial thickness. Basal cells in some areas show negative reaction to this marker (scale bar, 0.1 mm).

Fig. 3. Immunohistochemical expression of syndecan-1 in a representative section of adenomatoid odontogenic tumor demonstrating positive staining in spindle-shaped cells outside the ductal and whorl-like structures (scale bar, 0.1 mm).

of AOT and the ectomesenchymal cells of AFs. CD138 staining in OM was negligible (below 1%, very faint and in some samples) and therefore considered negative.

Differences in the intensity and percentage of syndecan-1 expression among odontogenic tumors were statistically significant

Fig. 4. Representative section of ameloblastic fibroma stained with monoclonal antibody against syndecan-1. Immunopositivity is observed in both epithelial and mesenchymal components (scale bar, 0.1 mm).

(both $P < 0.001$). In pairwise comparisons of these lesions, significant differences were only found between OMs and the other tumors (Table 1).

4. Discussion

Odontogenic tumors are classified into epithelial, mesenchymal, or mixed neoplasms according to the type of odontogenic tissue(s) involved in their development.¹³ These lesions encompass a heterogeneous group that is categorized from hamartomatous to benign and rare malignant neoplasms with variable aggressiveness.¹⁴ The molecular pathogenesis of odontogenic tumors remains unclear¹⁵ and the assessment of cellular markers can lead to a better understanding of the biologic behavior of these lesions. The interaction of different parts of the tooth germ tissue and their inductive influences are duplicated at various degrees in odontogenic tumors. Therefore different cellular and molecular factors may be retained or eliminated in the process of tumorigenesis.

In the current research, for the first time, we evaluated CD138 expression in AOT, AF, and OM and compared it with ameloblastoma and KCOT. Our findings indicated its expression in all the studied samples excluding OM which did not demonstrate immunopositivity. No statistical difference in the expression of syndecan was observed between these tumors except for OM. These observations suggest that CD138 may play a role in the pathogenesis of most odontogenic tumors of the jaws, regardless

of their biological behavior. In accordance with previous researches,^{4,5,16} we found the same pattern of CD138 expression in ameloblastoma and KCOT, but we were not able to find similar studies on the expression of this protein in the other evaluated proteins to compare their expression patterns. Syndecan expression in AOTs was more prominent in the spindle-shaped and elongated cells immediately adjacent to luminal and rosette-like structures and nodules of cuboidal cells. Ultrastructural study of AOT has shown two different types of epithelial cells: polygonal or columnar cells, which formed nodules or duct-like structures and spindle-shaped cells.¹⁷ There are some structural differences such as the number of tonofilaments, desmosomes and lysosomes between these two cell types¹⁷ that may be responsible for increased expression of syndecan in the spindle-shaped cells of AOT. Ameloblastic fibroma showed CD138 expression in both epithelial and mesenchymal components. This was similar to the reactivity pattern of syndecan in the early bell stage of normal tooth development.⁸ Therefore this protein may be essential for the reciprocal interaction between epithelial and ectomesenchymal tissue in AF. In the present study, OM revealed negative immunoreaction to CD138. This result can be interpreted in more than one manner: there is either the possibility that CD138 may not have a significant role in the development of OM or its elimination might be one of the factors that leads to the development of this tumor. Evaluation of the immunoreactivity of CD138 in other odontogenic ectomesenchymal tumors could provide additional information on the biochemical effect(s) of syndecan-1 in the pathogenesis of these neoplasms, especially considering the fact that its expression has been reported in the mesenchymal component of tooth germs. Regarding that CD138 was found in both epithelial and mesenchymal components of AF, but not in OM, it can be postulated that may be the inductive characteristic of odontogenic epithelium in some way affects its mesenchymal expression.

Syndecan is known as a cell surface molecule, but some studies have shown the localization of this marker in the nucleus.^{2,18,19} It is interesting that nuclear expression of CD138 was detected in some cases of AOTs and AFs in the present study. The precise molecular mechanism of nuclear translocation of syndecan is not entirely elucidated. However, the occurrence of this event may depend on the interaction of syndecan with tubulin.¹⁶ The ectodomain of CD138 can be released from the cell surface and binding with heparan sulfate chains could transfer syndecan to the nucleus.¹⁹ Purushothaman et al.²⁰ demonstrated that the presence of syndecan-1 in the nucleus of myeloma cells could inhibit the HAT activity and histone acetylation. In odontogenic tumors like AOT and AF, further investigations are needed to identify the role of the nuclear localization of CD138.

Table 1
Immunohistochemical staining of CD138 in odontogenic tumors.

	N	Percentage scores N (%)				Intensity scores N (%)		
		0	1	2	3	Low	Intermediate	High
Keratocystic odontogenic tumor	29	0 (0)	0 (0)	3 (10.4)	26 (89.6)	1 (3.5)	10 (34.5)	18 (62)
Ameloblastoma	10	0 (0)	2 (20)	0 (0)	8 (80)	2 (20)	4 (40)	4 (40)
Adenomatoid odontogenic tumor	7	0 (0)	0 (0)	1 (14.3)	6 (85.7)	1 (14.3)	5 (71.4)	1 (14.3)
Ameloblastic fibroma	7	0 (0)	0 (0)	0 (0)	7 (100)	0 (0)	3 (42.9)	4 (57.1)
Odontogenic myxoma ^a	5	5 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

^a Adjusted $P < 0.001$: comparison of CD138 percentage in odontogenic myxomas and keratocystic odontogenic tumors.

Adjusted $P = 0.05$: comparison of CD138 percentage in odontogenic myxomas and ameloblastomas.

Adjusted $P = 0.01$: comparison of CD138 percentage in odontogenic myxomas and adenomatoid odontogenic tumors.

Adjusted $P = 0.01$: comparison of CD138 percentage in odontogenic myxomas and ameloblastic fibromas.

5. Conclusion

In the present study, syndecan-1 was detected in all the selected odontogenic tumors except for OM. Considering the variations in aggressiveness of these neoplasms, from indolent and slow-growing in AOT to invasive and infiltrative in KCOTs and ameloblastomas, it seems that CD138 may have a significant role in the pathogenesis of these tumors but its effect on the clinical behavior of OTs is limited although its precise biologic effects of CD138 and its importance in these neoplasms remains debatable.

Conflicts of interest

The authors have none to declare.

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