Expression of the Insulin-like Growth Factor-1 Receptor in Odontogenic Myxoma

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Abstract. *Odontogenic myxoma (OM) is a rare mesenchymal tumour arising in the jaws. The origin and pathogenesis of OM is poorly understood. The aim of this study was to characterize OM by immunolocalization of certain antigens in the tumour that are relevant for cellular differentiation, migration and maintenance. Materials and Methods: Five OMs were immunohistochemically investigated for expression of nestin, CD133, podoplanin, and insulin-like growth factor 1 receptor (IGF-1R). Results: OM failed to react with antibodies applied in this study, with the exception of IGF-1R in tumour cells. Discussion: OM is a poorly characterized benign, invasive tumour of the jaws. The absence of stem cell marker in OM does not exclude possible temporary expression of these antigens during certain phases of tumour development. The identification of IGF-1R in OM is shared with numerous tumours and indicates the ability of these tumour cells to respond to growth factors.*

Odontogenic myxoma (OM) is a benign tumour of the jaws with preference for teeth-bearing areas (1). The preferred theory of pathogenesis of OM postulates an odontogenic cellular origin but this hypothesis is still a subject of debate (1). Indeed, myxoma is a well-known, albeit rare, entity in other regions of the body, including both soft tissue (2) and bone (3). In particular, facial bones not involved in tooth development can also develop osseous myxoma (4). On the other hand, the close association between tumour and dentition is obvious in the majority of jaw myxomas (1). The mutational spectrum of OM differs from that for osseous myxoma (2, 5, 6). Facial extragnathic soft-tissue myxomas need special attention (7). Dental follicle and dental papilla both contain myxoid areas and thus may be confused with OM, in particular in specimens of small size (8, 9). The morphological similarities between normal dental pulp and OM are a source of speculation concerning the odontogenic origin of OM (1). Studies investigating the putative odontogenic origin of OM produced conflicting results (1, 10-13). In order to specify the OM phenotype, this study addressed the expression of certain antigens indicating stem cell properties, migration and response to growth factors.

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Materials and Methods

Samples of five formalin-fixed and paraffin-embedded OM were subjected to immunohistochemical investigation. Particular attention was paid to the expression of stem cell markers (nestin and CD133), receptors associated with the control of cellular growth and apoptosis [insulin-like growth factor 1 receptor (IGF-1R)], and cell motility (podoplanin). All investigations were performed with simultaneous incubation of positive control tissues known to express the relevant antigen in routine laboratory practice. Immunoreactivity of antibodies was assessed for intensity (weak, moderate, strong) and localization (cellular, cytoplasmatic). Characteristics of the antibodies are summarized in Table I.

Results

The samples were completely negative for the antibodies used in this study, except for diffuse cytoplasmatic positivity of tumour cells for IGF-1R in all OM cases (Figure 1). The results are summarized in Table I.

Discussion

This study revealed a diffuse expression of IGF-1R in OM. This finding is in accordance with published staining

Table I. *Antibodies, sources, and staining results of antibodies used in assessing odontogenic myxoma.*

Antibody/ target antigen	Clone	Provider	Dilution	Detection system	Staining result in odontogenic myxoma
Nestin	Rat-401	Millipore, Billerica, MA, USA	1:50	DAB (Histofine Simple Stain Max PO, Nichirei Bioscience, 414154 F)	Negative
CD133	AC133	MACS, Miltenyi Biotec, Bergisch Gladbach, Germany	1:100	DAB	Negative
IGF-1R	C-20	Santa Cruz, Dallas,Tx, USA	1:50	DAB	Positive
Podoplanin	D2-40	Dako, IR072, Glostrup, Denmark	1:100	Dako EnVision Flex detection system	Negative

reactions after application of antibody to IGF-1R in both dental pulp and ligament, and also in bone (14, 15). Consequently, this finding cannot contribute to current hypotheses on OM pathogenesis. Nevertheless, the intense IGF-1R immunoreaction of OM is plausible in view of current perspectives on the role of IGF-1R in neoplasm.

To the best of our knowledge, IGF-1R and CD133 have not been investigated in OM, and only one study has addressed nestin expression (16). Therefore, the discussion of the staining results is guided by known immunohistological identification of these antigens in different stages of tooth development and odontogenic apparatus.

Nestin. Nestin is an intermediate filament strongly associated with early phases of central nervous system and muscle development (17, 18). Furthermore, nestin can be expressed in other developing and permanent tissues (19). Nestin is expressed in a temporo-spatial pattern during odontogenesis (19). However, nestin was not found to be expressed in rodent dental papilla in studies on dentinogenesis using a three-week old incisor (20). Simultaneously stained preodontoblasts and odontoblasts expressed nestin, but apical bud stained very weakly (20). Re-expression of nestin in teeth was demonstrated in pathological conditions, such as carious and injured teeth (19). Futhermore, a recent study revealed a substantial proportion of OMs express nestin. Immunoreaction for nestin in OM was restricted to four out of nine tumours (16).

Fujita *et al*. explained the immunoreactivity of odontogenic tumours for nestin with reference to the neural crest origin of ectomesenchymal tissues comprising these tumours (16). However, the origin of OM is not yet clear and the poor immunological response of OM to several antibodies has so far failed to reveal the cells giving rise to OM.

In this study, both nestin and CD133 expression were undetectable. Expression of these stem cell markers cannot be excluded in early phases of tumour development or phases of accelerated growth, but simultaneous failure of reactivity to both antibodies by these tumours are unlikely due to technical faults. Indeed, only a minority of OM proved to exhibit immunoreactivity in the study of Fujita *et al*. (16).

Podoplanin. Podoplanin is a mucin-type transmembrane glycoprotein originally identified in kidney podocytes. Podoplanin is homologous to T1α-2, an antigen expressed on the apical surface of alveolar type I cells (21, 22). Podoplanin is expressed in lymphatic but not in blood vessel endothelial cells (23). Expression of podoplanin has been revealed in different organs, including odontogenic tissues (24, 25). Podoplanin function is unknown but experimental data support the hypothesis that this protein acts in cell motility. OMs are locally invasive tumours (1). It is likely that tumour cells in OM use other pathways to allow cell migration.

IGF-1R. IGF-1R is a transmembrane (tyrosine kinase) receptor activated by the hormones insulin-like growth factor 1 and 2. IGF-1 is involved in mitosis and development of mammalian cells (26). IGF-1R expression in OM is a new finding.

IGF-1R and teeth. IGF-1R is a relevant factor in tooth development and maintenance (27, 28). However, there are only scarce data in the literature that do not draw an adequate picture of the impact ofIGF-1R on odontogenesis and possible deviations from physiological pathways. During enamel formation, diffuse cellular IGF-1R staining was noted during active secretory phases of amelogenesis but this changed to a vesicular or granular staining pattern during ameloblast transition towards enamal maturation (15). These findings were interpreted as temporo-spatially variant expression those treated with IGF-1 and distribution of IGF-1R during odontogenesis (29, 30). However, normal dental pulp maintains IGF-1R expression (31). Besides IGF-1R expression in teeth, experimental evidence revealed locally increased IGF-1R expression of the jaws following tooth extraction (32).

IGF-1-treated tooth germs developed larger volumes than those treated with other growth-promoting substances (30). Interestingly, IGF-1-treated stem cells from apical papilla (SCAP) promoted osteogenic differentiation *in vitro*, whereas untreated SCAP mainly developed dentin-pulp complex-like structures (33). In contrast to osteoclasts, odontoclasts of

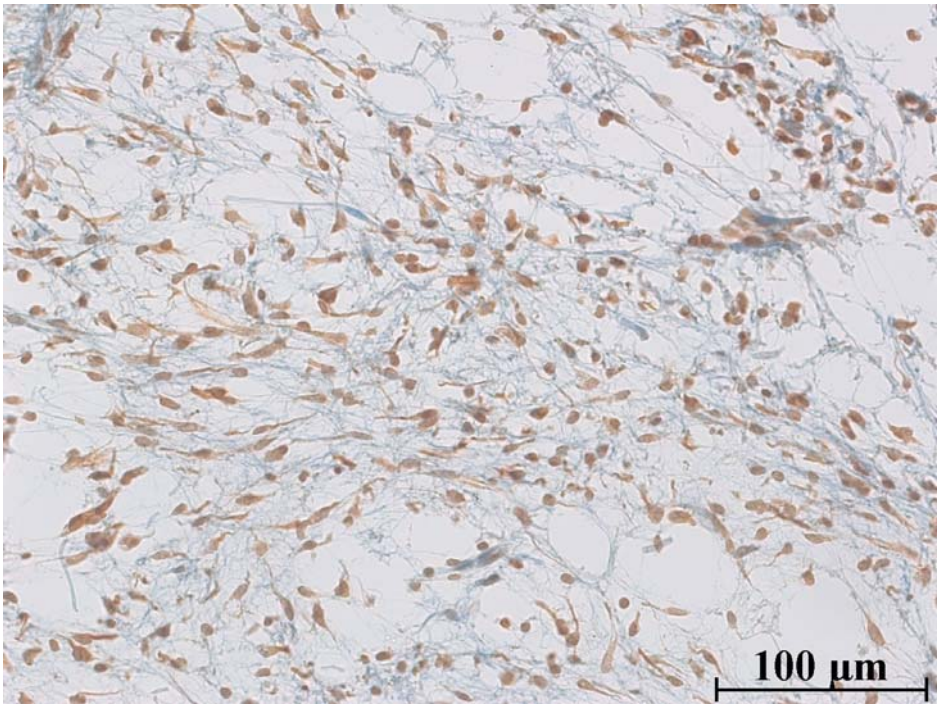

Figure 1. *Immunolocalization of IGF-1R during odontogenesis.*

deciduous teeth do not express IGF-1R. This finding was interpreted as part of the resorption process of deciduous teeth (34).

Caviedes-Bucheli *et al*. found IGF-1R to be statistically significantly more highly expressed in human pulp from wisdom teeth with incomplete root development (35). These authors concluded that IGF-1R has both mineralizing and pulp-repairing functions in teeth. On the other hand, according to these authors, IGF-1R was significantly more highly expressed in pulp cells of teeth with complete root development (35).

In contrast to IGF-1R expression in cementocytes of deciduous teeth, this antigen was not expressed in mature premolars and molars (36). Moderate to weak IGF-1R expression was restricted to the body of odontoblasts and periodontal ligament cells (36).

In the periodontium, IGF-1R expression increased with experimentally induced tooth movement (37). Resorption of bone and tooth was associated with IGF-1R-expressing odontoclasts and periodontal ligament cells during phases of experimental orthodontic tooth movement (38). With respect to odontogenic tumour development in general, it should be noted that periodontal ligament cells with the capacity to develop into cementoblasts and osteoblasts are involved in experimental mineralized nodule formation, an IGF-1R expression-associated process (39).

IGF-1R and bone. IGF1 plays a fundamental part during skeletal development (14). With respect to the alveolar process of the jaw, IGF-1R was expressed in endothelial cells, the extracellular matrix and osteoblasts but not by osteocytes in an experimental model of osseous wound healing following tooth extraction (32).

IGF-1R and tumours. IGF-1R protects tumour cells from apoptosis (40) and is involved in cellular proliferation, differentiation, and motility (41). IGF-1R is a key element in several phases of cancer (42). In the head and neck region, IGF-1R expression predicts clinical outcome in advanced-stage oral squamous cell carcinoma (43). There are only sparce data on IGF-1R expression in odontogenic tumours. The intensity and distribution of membraneous IGF-1R expression in cellular elements differed slightly in ameloblastic tumours compared to tooth germs but were predominantly noted in odontogenic epithelial cells close to the basement membrane (44). Weak IGF-1R staining was noted for some fibroblasts and endothelial cells in dental papillae and dental follicles that were simultaneously studied. On the other hand, IGF-1R expression was strong in ameloblastomas and metastasizing ameloblastoma (44). However, these findings currently are not relevant for therapeutic approaches. Targeting IGF-1R for the treatment of tumours is challenging due to the great structural similarity between IGF-1R and insulin receptor (45).

Conclusion

OM is a poorly defined neoplasm of the jaws. The immunohistochemical characterization of OM is still in its beginning. IGF-1R expression denotes the property of this tumour entity to respond to paracrine growth factors.

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