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Odontogenic Myxoma—Characterisation of the Extracellular Matrix (ECM) of the Tumour Stroma

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In order to elucidate the origin of the odontogenic myxoma, the composition and the structural organisation of the extracellular matrix (ECM) of this tumour were characterised. Collagen type I, VI, procollagen type III, undulin, tenascin and fibronectin were demonstrated in biopsy material of 4 cases by polyclonal antibodies. The tumour stroma showed a pronounced reaction for collagen type I. Fibroblasts displayed an intense intracytoplasmatic reaction for procollagen type III, and collagen type I was not found in the fibroblasts of the adjacent normal oral mucosa. In contrast to the surrounding connective tissue, label for collagen type VI was weak, as was the reaction for fibronectin and tenascin. Undulin was almost undetectable. The immunohistochemical results suggest that the odontogenic myxoma is characterised by an as yet unobserved structural organisation of ECM proteins, a secretion defect of fibrillar collagens type I and III with no resemblance to physiological tooth development.

Keywords: collagen, extracellular matrix, odontogenic myxoma, odontogenic tumour

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INTRODUCTION

THE ODONTOGENIC myxoma (OM) is a rare locally aggressive, non-metastasising non-encapsulated neoplasm infiltrating bone marrow spaces. It produces copious amounts of mucoid substance with few tumour cells. This mucoid substance may be compared to that formed in the embryonic dental follicle between the cells of the primitive pulp. Histogenetically, the odontogenic myxoma has been classified as an ectomesenchymal tumour [1].

The myxoma of the jaw is clearly different from myxomatous tumours elsewhere in the skeleton or soft tissues [2]. This separation has been justified by the presence of islands of odontogenic epithelium within the neoplastic, myxomatous tissue and the frequent association with a congenitally missing tooth.

The tumour's clinical characteristics have been reviewed by Slootweg and Wittkampf [3].

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Histopathologically, the odontogenic myxoma is characterised by loose mucoid tissue that contains spindle and stellate myxoblasts. Cellular and nuclear polymorphism is rare, as is enhanced mitotic activity. Numerous delicate capillaries may be present and nests of odontogenic epithelium may be seen. Occasionally, such islands show a stellate reticulum-like appearances. Signs of inflammation are rarely observed [4].

OM is considered a benign neoplasm, although several reports have mentioned a recurrence rate after curretage of approximately 25% [5]. The malignant variant called odontogenic myxosarcoma is extremely rare, with only one case being documented in the literature [6].

Extracellular matrix (ECM) proteins have been shown to play an important role in cellular growth and differentiation by complex cell matrix interactions and inductive phenomena. Recent studies have concentrated on the distribution of ECM proteins during odontogenesis [7–9], but little is known about the ECM pattern and cell–matrix interactions in odontogenic myxomas. Previous immunohistochemical studies using vimentin as a marker demonstrated the evidence for a mesenchymal derivation for odontogenic myxoma [10–12].

Classically, the ECM comprises four classes of molecules, collagens, non-collagenous structural glycoproteins, elastin and proteoglycans/glycosaminoglycans. Collagens type I, III and V belong to the class of fibril-forming collagens, whereas collagen type VI is a minor but ubiquitous constituent of the ECM that forms microfibrils and probably serves as a flexible

anchor interconnecting collagen fibres with each other, and with cells [13, 14]. Among the ECM glycoproteins, fibronectin, tenascin and undulin share common structural features of fibronectin-like type III homology units with additional functional domains such as epidermal growth factor-like repeats (tenascin) and A domains of von Willebrand factor (undulin). The ECM directs cell migration, morphogenesis and wound healing and is strongly involved in the network of cellular communication [15].

Accordingly, ECM components are involved in the process of histo- and morphodifferentiation during tooth development, involving complex cell matrix interactions and inductive phenomena. Several studies concentrated on the pattern of the ECM in odontogenic tumours allowing the demonstration of specific alterations in its composition for a clear distinction between the tumour stroma and the adjacent normal connective tissue [14, 16]. However, little is known about the composition of the tumour stroma and the origin of tumour cells in OM.

The aim of the present study was to characterise the composition and the structural organisation of the ECM in OM to elucidate the pathogenesis and origin of this tumour.

MATERIALS AND METHODS

Source and preparation of tissues

Specimens of OM were taken from 4 patients (two males, two females) whose median age was 25 years (range 19–34). In 3 patients the OM was localised in the mandible, in 1 case in the molar region of the maxilla. In 1 case, the tumour was associated with an impacted tooth.

After surgery, excised specimens were fixed in 10% formalin, embedded in paraffin and sections of 5 μm were prepared. Paraffin sections were stained with haematoxylin-eosin (H&E) and Alcian blue.

Antigens and antibodies

Collagen type I and the aminoterminal propeptide of collagen type III were isolated from neutral salt-extracted monkey and bovine skin, respectively. Undulin was purified from monkey skin and collagen VI obtained from human placenta [17]. Production of monospecific antisera has been described in detail [18]. Hyperimmune sera raised in rabbits were affinity purified and the resulting antibodies were tested for monospecifity by radioimmunoassays and western blotting [18].

Monospecific antisera to human tenascin and fibronectin were obtained from Telios Pharmaceuticals (La Jolla, California, U.S.A.). Monospecifity of these antisera was verified by immunoprecipitation and western blotting.

Immunolabelling

Paraffin sections were dried for 24 h at 50°C, deparaffinised in xylol (four times for 5 min) and alcohol. Sections were then incubated with 0.1% trypsin (Sigma, Germany) in phosphate-buffered saline (PBS; pH 7.2) for 10 min. All antibodies were used at a concentration of 10–20 μg/ml in PBS. Sections were incubated with primary antibodies for 40 min and after washing in PBS peroxidase-conjugated goat-anti-rabbit antibodies (Dianova, Hamburg, Germany; dilution 1:200) were applied for another 40 min. Immunostaining was visualised using 5.0 mg AEC (3-amino-9-ethylcarbazole; Dianova) in 8 ml PBS. Sections were counterstained with haematoxylin [14].

RESULTS

Conventional histopathology

The tumour stroma consisted of collagenous connective tissue and loose myxoid tumour stroma with typical angular and stellate cells bearing thread-like cytoplasmic processes. Bundles of reticular collagen fibres spread throughout the mucoid ground substance of the tumour, consisting predominantly of acid mucopolysaccharides. Odontogenic epithelium was not observed. The unequivocal diagnosis of odontogenic myxoma was established in all 4 cases.

ECM components

Collagen type I showed a weakly organised, fibrous network. At the transition to the adjacent tissue of the uninvolved oral mucosa a pronounced and more intense staining and the formation of well-organised collagen fibre bundles was noted (Fig. 1). The fibroblasts of the tumour

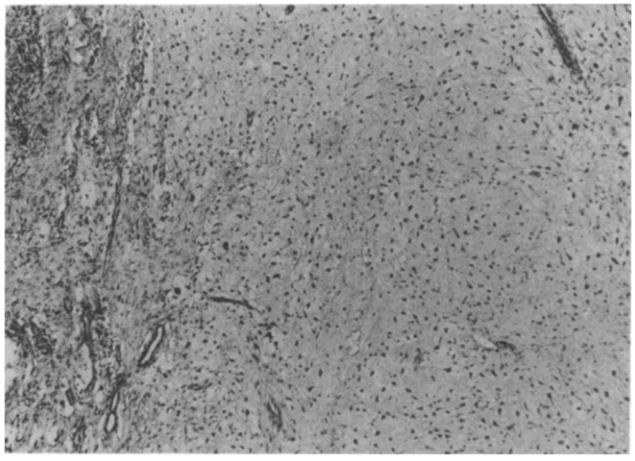

Fig. 1. Collagen type I (×50): diffuse, filamentous organisation. Pronounced and intense staining and the formation of collagen fibre bundles at the border of the adjacent tissue of the oral mucosa (left part of the micrograph).

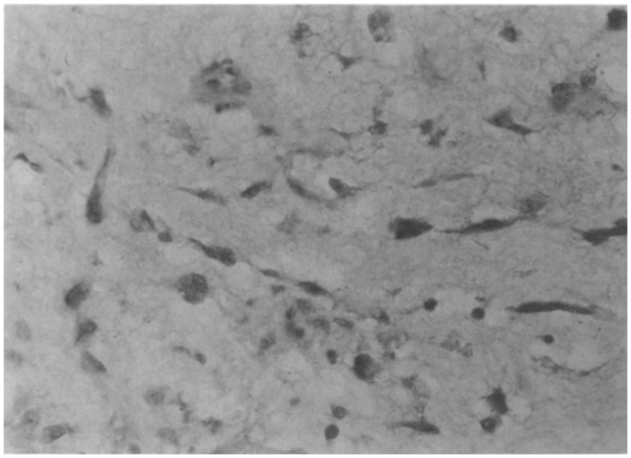

Fig. 2. Collagen type I (×315). Fibroblasts of the tumour stroma display an intense intracytoplasmatic reaction not found in the fibroblasts of the adjacent subepithelial areas of the normal oral mucosa.

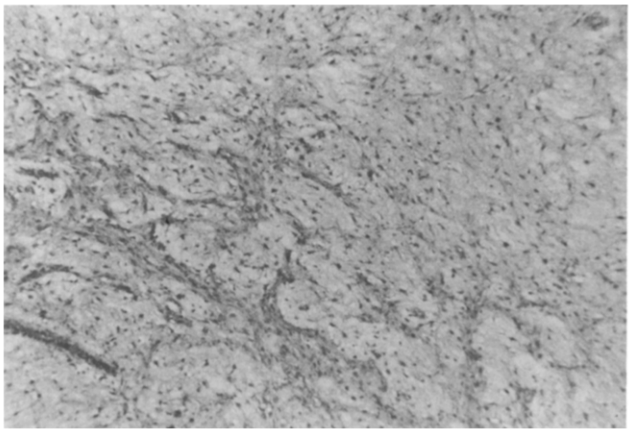

Fig. 3. Procollagen III (× 50). Procollagen III shows an intra- and extracellular pattern similar to collagen type I.

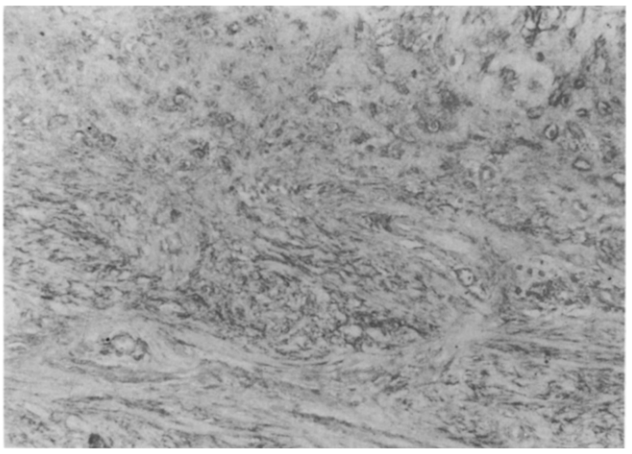

Fig. 5. Tenascin (× 100). Weak interstitial reaction within the tumour stroma which is not present in the pericellular zones.

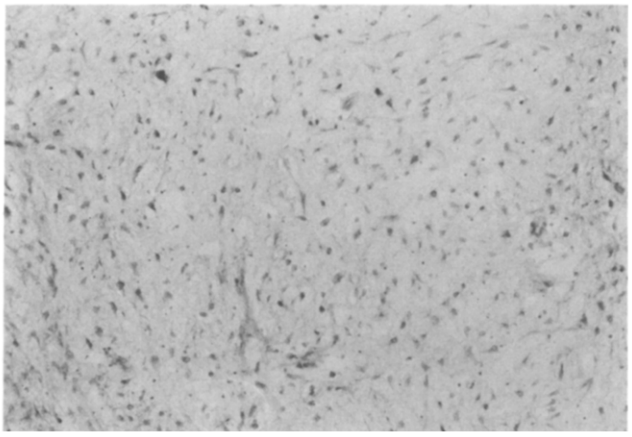

Fig. 4. Collagen type VI (× 100). The filamentous labelling in the tumour stroma allows a distinction between zones of the normal connective tissue from the tumour stroma (lower part of the micrograph).

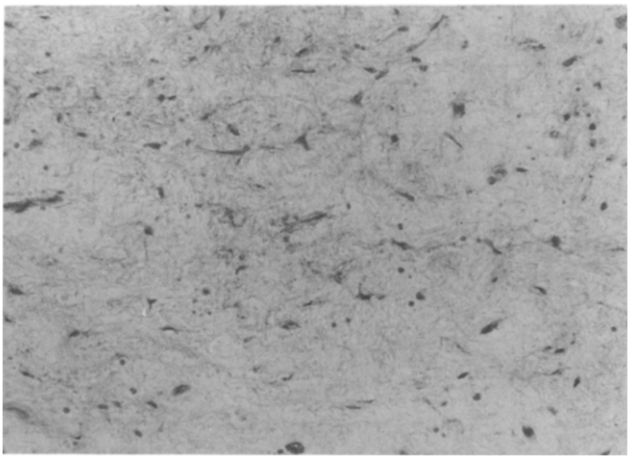

Fig. 6. Fibronectin (× 160). Weak staining compared to normal oral mucosa.

stroma presented an intense intracytoplasmatic reaction, which was not found in the fibroblasts of the adjacent subepithelial areas of the normal oral mucosa (Fig. 2). This specific reaction allowed a differentiation between tumour cells and non-tumorous fibroblasts.

Labelling for procollagen type III that shows a distribution superimposable on that of collagen III exhibited a pattern corresponding to that of collagen type I. The intracytoplasmatic staining was also evident but weaker (Fig. 3). Collagen type VI showed an amorphous pattern. Its characteristic distribution in the tumour stroma allowed a distinction between zones of individual fibre bundles positive for collagen type I and the disorganised tumour stroma. In these areas, which were similar to collagen positive for collagen I fibres, neither labelling for collagen type VI nor positivity in fibroblasts was evident (Fig. 4).

Tenascin showed a weak interstitial reaction within the tumour stroma, which was contrary to the collagens not pronounced in the pericellular zones (Fig. 5). Fibronectin was much less detectable compared to normal oral mucosa. Label for fibronectin was weak (Fig. 6). Undulin was almost undetectable within the tumour stroma.

DISCUSSION

The histogenesis of OM of the jaw is still a matter of debate. A previous immunohistochemical study using an antibody to vimentin provided evidence for the non-controversial mesenchymal nature of the tumour cells [10], with inhomogeneity within this cell population [11].

Our immunohistochemical findings demonstrate a characteristic organisation of ECM proteins in OM which allows a clear distinction between the tumour stroma and the adjacent oral mucosa. The major cross-striated collagen fibrils consisting predominantly of collagens type I and III were rarely found in the tumour, with most areas lacking these two major

collagenous proteins of soft connective tissues. However, the tumour cells showed a strong intracellular staining for collagens type I and III, a phenomenon which has not been reported before, and is suggestive of a defect of collagen deposition that is effective at the level of protein export and deposition.

The specificity of this intracellular label is underlined by the observation that mesenchymal cells in the adjacent normal connective tissue did not show intracellular staining. In the few fibre bundles of the tumour stroma, which were labelled by antibodies to collagens type I and III, the adjacent fibroblasts did not show intracellular staining, indicating that these cells did not show the preformed defect in collagen secretion and may represent normal subepithelial connective tissue. Therefore, our results indicate that antibodies to collagens type I and III might allow specific identification of the tumour cells in OM.

Collagen type VI which was found to be a major component in areas of high cellularity and at the periphery of epithelial islands in ameloblastic fibroma [14] was rarely found in OM. Intracellular staining was not present. These findings underline that the individual types of odontogenic tumours are characterised by a specific distribution and organisation of ECM proteins.

Fibronectin and undulin showed a staining pattern much weaker than in normal oral mucosa. Due to undulin's association with major collagen fibrils [17], the effective lack of these proteins in OM might explain the weaker staining reaction for undulin. Tenascin expression has been strongly linked to epithelial–mesenchymal interactions as well as to processes such as proliferation, migration and organogenesis [19]. It plays a role in physiological tooth development [9] and is found in the tumour stroma of ameloblastomas and ameloblastic fibromas [16]. The lack of epithelial cells in the myxomas of this study might explain the weak staining reaction for tenascin.

The ECM of OM was believed to be similar to that formed in the embryonic dental follicle between the cells of the primitive pulp as studied by conventional histology [4]. Preliminary data in monkeys displayed a pronounced reaction for collagen type VI and a structural organisation of the ECM in the primitive ECM which is quite distinct compared to that found in OM (Becker *et al.*, unpublished results).

In summary, our results demonstrate that OM is characterised by an as yet unobserved structural organisation of ECM proteins, a secretion defect of fibrillar collagens type I and III with no resemblance to physiological tooth development.

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