

## Immunolocalization of Heparanase in Ameloblastoma and Other Odontogenic Tumors

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### Introduction

Heparan sulphate proteoglycans (HSPG) are the integral molecules of the basement membrane (BM) and the cell membrane. They have certain important functional roles in cellular signaling and the extracellular matrix (ECM) assembly. Their heparan sulphate chains (HS) serve as the extracellular matrix reservoir for a variety of bioactive molecules including growth factors, morphogens and enzymes. Heparanase is the mammalian endo- $\beta$ -glucuronidase enzyme, which selectively cleave HS chains at specific sites. Heparanase is known to be an important determinant in dissemination and invasion of cells associated with inflammation, cancer invasion and metastasis through degradation of BM and ECM and also through the release of HS bound growth factors for cell proliferation. Based on these interesting functions and roles of HSPGs and heparanase, we studied their localization in ameloblastoma and other odontogenic tumors.

### Materials and methods

Formalin-fixed, paraffin-embedded odontogenic tumors were used. Immunohistochemistry for heparanase was done by mouse monoclonal antibody to human heparanase using Mouse IgG Vectorstain Elite ABC kit and DAB staining. In situ hybridization reaction was performed by DIG-labeled RNA probe and anti-DIG-AP Fab fragments and NBT/BCIP staining.

### Results

All ameloblastoma studied show positive cytoplasmic staining for both protein and mRNA and the protein localization reflects the mRNA. No significant difference in staining pattern was noted in the histological subtypes of ameloblastoma. The staining was more prominent in the peripheral ameloblast-like cells although the central stellate cells also stained. Stronger heparanase staining was noted near the invasive and branching areas associated with surrounding loose connective tissue. Heparanase protein and mRNA was specifically concentrated at the basal ends of the peripheral columnar cells of follicular ameloblastoma (Fig.1,A&B). In plexiform type the staining intensity was almost the same within the epithelial strands except for the stronger budding areas (Fig.1,C&D).

In benign epithelial odontogenic tumors such as Adenomatoid odontogenic tumor (Fig.2,A) and Calcifying odontogenic cyst (Fig.2, B) immunostaining for heparanase was observed not only in the cytoplasm of epithelial cells but also in the calcification foci and ghost cells. Malignant odontogenic tumors, Ameloblastic carcinoma (Fig.3,A) and Primary intraosseous carcinoma (Fig.3,B), the tumor cells show very strong cytoplasmic and perinuclear staining. Nuclear staining was also observed in some tumor cells.

In mixed odontogenic tumors, Ameloblastic fibroma (Fig.4,A), heparanase protein was localized in the epithelial cells and also

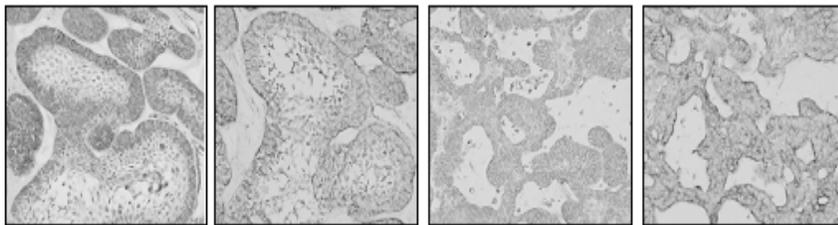


Fig. 1 Ameloblastoma: (A,B) Follicular type, (B,C) Plexiform type

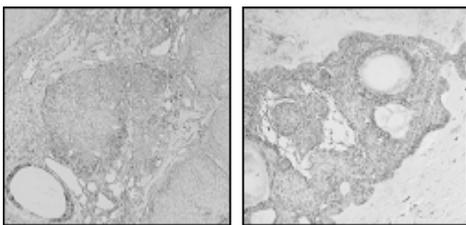


Fig. 2 (A) Adenomatoid odontogenic tumor, (B) Calcifying odontogenic cyst

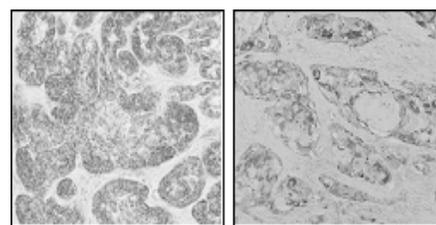
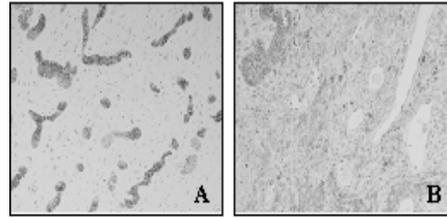


Fig. 3 (A) Ameloblastic carcinoma, (B) Primary intraosseous carcinoma

weakly in the myxoid fibroblastic cells. As for Ameloblastic fibro-odontosarcoma (Fig.4,B), the malignant fibroblastic cells showed diffuse and more intense staining compared to the fibroblastic cells of AF.

### **Conclusions**

Increased heparanase expression detected in all ameloblastoma samples studied with stronger staining in the invasive and branching areas suggest that heparanase is one of the important factors of tumor local invasiveness. Basal columnar cells seemed to be the main producer of heparanase. Immunostaining to heparanase also detected in other benign and malignant odontogenic tumors gave the speculation that it may have other important role related to odontogenic tissue origin.



**Fig. 4** (A) Ameloblastic fibroma, (B) Ameloblastic fibro-odonto sarcoma