Heparanase mRNA Gene and Protein Expression in Oral Cancer Development and Progression

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Abstract: The human heparanase gene, an endo-beta-D-glucuronidase, has recently been cloned. It functions as an extracellular degradative enzyme that cleaves heparan sulfate proteoglycans, and acts as a critical modulator of tumor metastasis and angiogenesis. Heparanase RNA probe and monoclonal anti-heparanase antibodies were used to examine the expression of heparanase mRNA gene and protein in epithelial dysplasia, carcinoma-in-situ and/or microinvasive carcinoma and oral squamous cell carcinoma. Strong signal and expression of both gene and protein were detected in epithelium progressing from dysplasia to invasive carcinoma. Carcinomatous cells at the tumor invasive front showed the highest levels of heparanase mRNA gene and protein. These results suggest that heparanase plays an important role during oral cancer development and progression, and this may have both prognostic and therapeutic implications.

Key Words: Heparanase, Oral cancer, Squamous cell carcinoma, Gene

Introduction

Oral squamous cell carcinoma and its precursor lesions i.e. potentially malignant epithelial lesions and conditions, are an important health concern in many countries. It is now believed that oral carcinogenesis and progression is a multistep process involving both genetic insults to epithelial cells and changes in epithelial–stromal interactions. However the precise nature of the genetic alterations occurring at each step is still unclear.

Heparanase is an endo-beta-D-glucuronidase that specifically cleaves the heparan sulphate chain of heparin sulfate proteoglycans (HSPGs). Degradation of HSPGs - which are the main components of the extracellular matrix (ECM) - is critical for the regulation of biological functions of heparin sulfate-binding proteins namely growth factors, ECM molecules, cell-attachment proteins, enzymes and enzyme inhibitors. Heparanase is expressed on the cell surface of a wide range of benign and neoplastic cells and tissues including inflammatory cells, fibroblasts, eosophageal carcinoma, gastric carcinoma. Within the neoplastic environment, HSPGs bind to growth factors and cytokines that are involved in the modulation of tumour cell growth. Their subsequent degradation by heparanase has been demonstrated to play an important role in signal transduction resulting in the inhibition of tumour cell growth. However to date, the role of heparanase in human oral cancer development has not been clarified.

In this study, we examined the normal, dysplastic and neoplastic oral mucosa and its stromal components for heparanase mRNA by *in situ* hybridization technique, and for heparanase protein by immunohistochemical procedure in an attempt to gain insights into the role of this enzyme during oral cancer development and progression.

Materials and methods

Tissue samples

The source of the sample used in this study was from the surgical pathology files of the Department of Oral Pathology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University. Archival formalin-fixed, paraffin embedded tissue blocks of normal mucosa (n=5), epithelial dysplasia (n=15), specimens with areas of carcinoma in situ contiguous with microinvasive carcinoma (n=10), and oral squamous cell carcinoma (n=40) were retrieved. New 4 micron-thick sections prepared for routine staining with hematoxylin-eosin, immunohistochemistry and *in situ* hybridization. All these selected cases were histologically verified and diagnosed according to the *Histological Typing of Cancer and Precancer of the Oral Mucosa* and other established criteria in current use.

Monoclonal antibodies

The primary antibody used for immunohistochemical analysis was mouse monoclonal antibody to human heparanase raised against recombinant human heparanase.

Tissue and probe preparation for in situ hybridization

Digoxigenin-11-UTP-labeled single-strand RNA probes were prepared using a DIG Labeling Kit (Roche Diagnostics GmbH, Penzberg, Germany) according to manufacturer's instructions. For generation of the heparanase probe, a 571-bp fragment of human heparanase cDNA (base 261-832 of the total cDNA) [Gene Bank Accession No. AF144325] was obtained by RT-PCR and subcloned into pCR21 (Invitrogen).

Results and Discussion

Expression pattern of the heparanase protein

In all specimens with epithelial dysplasia a faint diffuse positivity for heparanase protein was observed throughout the entire epithelium with strong labeling of the cells in the parabasal layer and upper third of the prickle cell layer(Fig. 1a).. Specimens of carcinoma in situ contiguous with areas of microinvasive

carcinoma were available for investigation in this study. In all these specimens, heparanase was widely expressed by the dysplastic cells from the prickle to the basal cell layers in carcinoma in situ areas, extending to include the tumor nests at the invasive front of microinvasive carcinoma.

Forty cases of oral squamous cell carcinoma specimens yielded a similar pattern of heparanase expression, differing only in their staining intensity. Areas of keratinizing pearls and well-differentiated tumor nests showed weak to moderate stain (Fig. 2a), whereas poorly-differentiated tumor sites expressed stronger and more abundant staining.

Expression pattern of the heparanase mRNA

In all samples of epithelial dysplasia investigated, heparanase mRNA signals were observed in the parabasal and prickle cell layers (Fig.1b), correlating to those areas expressing the heparanase protein. For all specimens of carcinoma in situ with contiguous areas of microinvasive carcinoma examined, heparanase mRNA signals were observed in the parabasal and prickle cell layers, correlating to those areas expressing the heparanase protein. All oral squamous cell carcinoma specimens investigated showed co-localization of the heparanase protein and mRNA gene. The signals were moderate to strong in well-differentiated tumor areas, but weak to absent within keratinizing pearls (Fig. 2b). In poorly-differentiated tumor areas, the labeling was stronger and more diffuse.

Heparanase has been well-recognized as a key enzyme in cancer cell invasion and metastasis. Its gene and protein has been identified in various cancer cells, and their overexpression in tumor cells has been reported to correlate with the metastatic potential and prognosis of cancers involving the gastrointestinal system, pancreas, and breast. This study investigated heparanase mRNA gene and protein expression in normal, dysplastic and cancerous oral epithelial tissues by immunohistochemical and in situ hybridization techniques in order to clarify the functional significance of this enzyme during oral carcinogenesis. Though the functional roles of heparanase in oral cancer development and progression were not precisely clarified by the present morphological study, the enhanced heparanase activity in the early phase of epithelial dysplasia suggested the participation of this enzyme occurs as an early event during oral cancer development. The subsequent identification of increasing heparanase actvitiy at sites corresponding to areas of severe dysplasia or carcinoma in situ, along the tumor invasive front and in poorly differentiated carcinomatous lesions led us to conclude that oral cancer progression is also governed by this enzyme. In short, heparanase enzyme plays a significant role during oral cancer development and progression.

References

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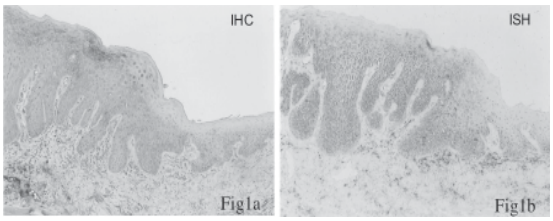

Fig. 1. Immunohistochemical staining and *in situ* hybridization study of heparanase protein and mRNA gene expression in dysplastic oral mucosa.

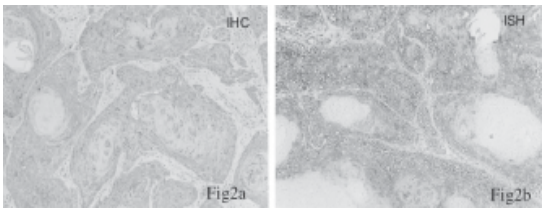

Fig. 2. Immunohistochemical staining and *in situ* hybridization study of heparanase protein and mRNA gene expression in squamous cell carcinoma.