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Heparanase expression and localization in different types of human lung cancer☆

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ABSTRACT

Background: Heparanase is the only known mammalian glycosidase capable of cleaving heparan sulfate chains. The expression of this enzyme has been associated with tumor development because of its ability to degrade extracellular matrix and promote cell invasion.
Methods: We analyzed heparanase expression in lung cancer samples to understand lung tumor progression and malignancy from the point of view of heparanase expression. Of the samples from 37 patients, there were 14 adenocarcinomas, 13 squamous cell carcinomas, 5 large cell carcinomas, and 5 small cell carcinomas. Immunohistochemistry was performed to ascertain the expression and localization of heparanase.
Results: All of the tumor types expressed heparanase, which was predominantly localized within the cytoplasm and nucleus. Significant enzyme expression was also observed in cells within the tumor microenvironment, such as fibroblasts, epithelial cells, and inflammatory cells. Adenocarcinomas exhibited the strongest heparanase staining intensity and the most widespread heparanase distribution. Squamous cell carcinomas, large cell carcinomas, and small cell carcinomas had a similar subcellular distribution of heparanase to adenocarcinomas but the distribution was less widespread. Heparanase expression tended to correlate with tumor node metastasis (TNM) staging in non-small cell lung carcinoma.
Conclusion: In this study, we showed that heparanase was localized to the cytoplasm and nucleus of tumor cells and to cells within the microenvironment in different types of lung cancer. This enzyme exhibited a differential distribution based on the type of lung tumor.
General significance: Elucidating the heparanase expression patterns in different types of lung cancer increased our understanding of the crucial role of heparanase in lung cancer biology. This article is part of a Special Issue entitled Matrix-mediated cell behavior and properties.

1. Introduction

Lung cancer is the leading cause of cancer-related death worldwide [1]. This multifactorial disease can be organized into two groups: non-small cell lung cancer (approximately 85% of all lung cancers) and small cell lung cancer (approximately 15%) [2]. Despite recent advances in drug discovery and early detection, lung cancer continues to be associated with a poor prognosis and low survival rates. Therefore, continued research to increase our understanding of the biological features of this disease is strongly encouraged.

Tumor growth occurs in a complex microenvironment composed of stromal cells and extracellular matrix (ECM) [3]. Heparan sulfate

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proteoglycans (HSPGs), located in the ECM and on the cell surface, are composed of a core protein with covalently attached heparan sulfate (HS) chains. HSPGs regulate several aspects of cell physiology and ECM organization. Indeed, HSPGs sequester bioactive molecules, assemble ECM proteins, regulate growth factor-induced cell signaling and mediate cellular interactions with components of the tumor microenvironment [4]. Thus, the balance of HS levels is expected to control cell behavior and might represent a tipping point between health and disease.

Heparanase is the only known mammalian glycosidase capable of cleaving heparan sulfate chains, and consequently it regulates HS function in the ECM and on the cell surface. Heparanase is expressed in different cell types, but there is preferential expression in tumor cells [5]. Indeed, heparanase expression is upregulated in all the human carcinomas that have been studied [6]. Moreover, heparanase activity has been associated with high metastasis rates and poor prognosis in several cancer subsets [7]. This association can be explained by the direct cleavage of HS chains in the ECM and basement membrane by heparanase, which enables cell invasion and dissemination. Additionally, heparanase activity profoundly increases tumor metastasis and vascular density and reduces the postoperative survival rate. Therefore, these data suggest that heparanase is a strong candidate target for cancer treatment.

In this study, we analyzed lung tumor samples of four subtypes: adenocarcinoma, squamous carcinoma, large cell carcinoma, and small cell lung carcinoma. We determined that heparanase was expressed in 90% of the samples. Heparanase staining was detected in several cellular compartments, such as the plasma membrane, nucleus, and cytoplasm, as well as in the extracellular milieu. Regarding tissue distribution, heparanase was expressed in tumor cells as well as in certain epithelial cells and macrophages. Finally, we report the heparanase distribution profile in small cell lung carcinoma.

2. Materials and methods

2.1. Patients

A retrospective survey was conducted with a local population consisting of patients attending the Federal University of Rio de Janeiro Hospital Medical School that were diagnosed with non-small cell lung cancer (NSCLC) or small cell lung cancer (SCLC). The protocol was approved by the Ethics in Research Committee of the Clementino Fraga Filho University Hospital of the Federal University of Rio de Janeiro.

2.2. Histological analysis

Samples of normal or tumor pulmonary tissue were obtained surgically or via biopsy. The specimens were fixed in 40 g/l formaldehyde saline and embedded in paraffin for the histological analysis. Formalin-fixed 5-μm-thick lung sections were stained with hematoxylin and eosin to determine the tumor type. All of the histological analyses were performed by three independent pathologists. For the immunohistochemical study, 5-μm-thick sections were mounted on poly-L-lysine-coated slides.

2.3. Immunohistochemistry

Paraffin sections were cut and placed on slides that had been pretreated with poly-lysine; these slides were subsequently deparaffinized with xylene and were used to characterize heparanase expression using the indirect immunoperoxidase technique. Immunohistochemical staining was performed applying the rabbit polyclonal antibody HPA1 H-80 (catalog number sc-25825, Santa Cruz Biotechnology, Dallas, TX, USA) to paraffin-embedded sections following antigen retrieval. A total of 37 samples were analyzed, including 14 adenocarcinoma samples, 13 squamous cell carcinoma samples, 5 large cell carcinoma samples, and 5 small cell carcinoma samples. Subsequently, the tissue sample slides were immersed in 3% hydrogen peroxide in methanol for 10 min to block endogenous peroxidase activity. After being rinsed in phosphate-buffered saline (PBS) containing 0.5% Tween 20 for 10 min, the tissue sections were incubated with non-immune horse serum for 30 min and then with the respective monoclonal antibody in a humidified chamber overnight at 4 °C. Two sections from each sample were incubated with either PBS alone or mouse monoclonal IgG1 (concentration-matched) (Dako A/S, Glostrup, Denmark) to serve as the negative controls. After a 10-min wash with PBS, all of the tissue sections were incubated for 30 min with the LSAB + system HRP kit reagents (Dako, Glostrup, Denmark). After washing with PBS, all the sections were developed using a solution containing hydrogen peroxide and diaminobenzidine. The preparations were lightly counterstained with Harris's hematoxylin, dehydrated, and mounted in Permount (Fisher Scientific, Pittsburgh, PA, USA). Heparanase-immunostained lung sections were analyzed by three independent pathologists who were blinded to the clinical data, including the tumor node metastasis (TNM) classification and the smoking history. The pathologists quantified the heparanase staining in the tumor samples using the following scores: 0, no staining; 1, weak staining; and 2, moderate or strong staining. The pathologists also examined the heparanase-positive tumor stroma cells to determine their identity based on morphology.

2.4. Immunofluorescence and confocal laser scanning microscopy

In a double indirect immunofluorescence study, sections were incubated overnight at 4 °C with blocking buffer (2.5% bovine serum albumin (BSA), 2.0% skim milk, and 8.0% fetal bovine serum (FBS)) with shaking. The slides were rinsed once with PBS and 0.05% Tween 20 and then incubated with the primary antibodies diluted in PBS. The tissue sections were incubated with the rabbit anti-human polyclonal HPA1 H-80 antibody (catalog number sc-25825, Santa Cruz Biotechnology, USA) and the mouse monoclonal anti-human CD68 fluorescein isothiocyanate (FITC) antibody or the mouse monoclonal anti-human CD3 FITC antibody (both from Dako Glostrup, Denmark) overnight at 4 °C. After incubation, the slides were rinsed three times and were incubated with the Alexa Fluor® 546-conjugated anti-rabbit IgG antibody (Molecular Probes, Eugene, OR) for 1 h at room temperature. Sections

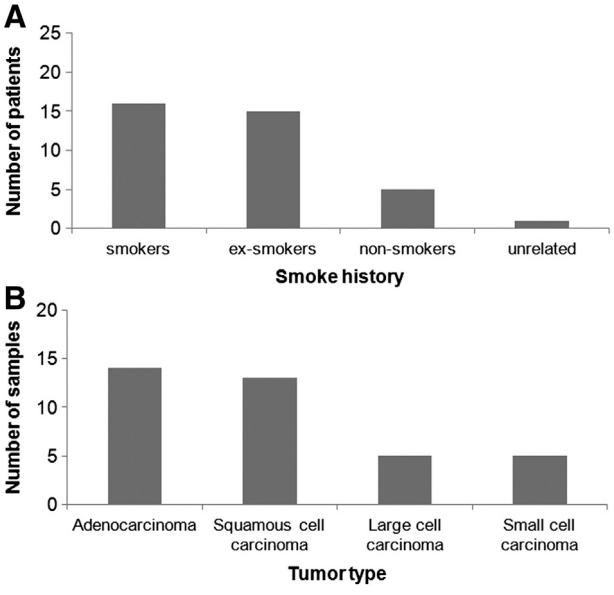

Fig. 1. Sample data. Patient information: (A) smoking history and (B) tumor type.

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