HEPARANASE EXPRESSION CORRELATES WITH METASTASIS AND POOR SURVIVAL IN OSTEOSARCOMA


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**INTRODUCTION**

Heparan sulfate proteoglycan (HSPG) is the main component of basement membranes and extracellular matrix (ECM). It is composed of a core protein with covalently attached heparan sulfate (HS) chains, the main polysaccharide constituent of the extracellular matrix and basement membrane. HSPG and HS are ubiquitous and play central roles in both cell-cell and cell-matrix interactions (1-2). They also function in signal transduction, angiogenesis or other biological processes binding a multitude of proteins and a wide variety of bioactive molecules (growth factors, chemokines, etc.).

Heparanase is an enzyme that cleaves heparan sulfate and through this activity it is known to promote tumor growth, angiogenesis, invasion, and metastasis in several tumor types (3). Its overexpression has been observed in many human tumors such as head & neck tumors, pancreatic tumors, breast cancer, and correlations between heparanase expression and tumor progression have been found in many types of cancers. Recently the attempts to inhibit the over-expression of heparanase were made to prevent tumor progression for a therapeutic purpose (4).

We studied the expression of heparanase in human osteosarcoma using immunohistochemical methods and evaluated its clinical significance and the possibility as a prognostic marker.

**METHODS**

Tissue microarray method was used to make paraffin blocks for sectioning and immunostaining the tumor tissues. Osteosarcoma tissues in paraffin blocks were deparaffinized and rehydrated. Antigen retrieval technique was used in sodium citrate buffer for improving sensitivity. Immunostaining was performed with a monoclonal antibody against the heparanase (Santa-Cruz Biotechnology, CA, USA). A standard avidin-biotin-peroxidase complex (ABC) technique was used for visualization, with diaminobenzidine as the chromogen (Histostain Plus-kit; Zymed). Counterstaining was performed with Mayer’s hematoxylin.

Semiquantative analysis was used for interpretation according to the immunoreactivity; less than 10% is negative staining, between 10% and 50%, moderate staining, and more than 50%, intense staining. We analyzed the relationships between heparanase expression and various clinopathologic factors such as metastasis, local recurrence, response to chemotherapy and survival periods. Statistical analysis was done using SPSS v.11.5.

**RESULTS**

Overexpression of heparanase was observed in 37 out of 51 patients (72.5%). Heparanase overexpression showed statistically significant correlation with the response to chemotherapy, (p=0.005) and with development of distant metastasis (p=0.029). The correlation between local recurrence rate and heparanase overexpression was marginal (p=0.04) in 46 patients who underwent adequate wide resection. The 5-year-survival rates were higher in patients with heparanase-negative group than in heparanase overexpressed group (p=0.01) (Fig. 1). Multivariate analysis revealed that heparanase expression was an independent predictor of overall survival in osteosarcoma.

**DISCUSSION AND SUMMARY**

Known prognostic factors in osteosarcoma are response to preoperative chemotherapy, metastasis, tumor size, and location (5). Among these prognostic factors, the response to preoperative chemotherapy as well as metastasis (staging) is known to be very important (5). In current study, heparanase showed strong negative correlation with the responses to chemotherapy, although the exact mechanism is still unclear. The possible mechanisms of heparanase in tumor progression are to break and penetrate the ECM barrier, subsequently accelerating tumor cell dissemination by its enzymatic activity (6). Heparanase levels had positive correlation with metastatic potential in metastatic cancer cell lines (3). Heparanase also induce angiogenesis by modulating growth factor activity and bioavailability (7). In this study, immunostaining of heparanase was proved to be a valuable means to predict prognosis. In summary, the current study suggested that heparanase played some role in tumor invasion and distant metastasis as well as the response to the chemotherapy in osteosarcoma resulting in poor prognosis of patients. Further study is required to evaluate the possibility of heparanase as a potential therapeutic target in the treatment of osteosarcoma.

**REFERENCES**