PATHOGENESIS OF OSTEOCLASTIC BONE DESTRUCTION OF GIANT CELL TUMOR OF BONE – POSSIBLE INVOLVEMENT OF VEGF-FLT-1-FAK PATHWAY

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Introduction

Giant cell tumors of bone (GCT) is a rare primary skeletal neoplasm occurring in young adults. GCT is histopathologically characterized by multinucleated giant cells (MNCs) scattered among a mass of mononuclear cells. The current hypothesis for the cellular origin of GCT is that the majority of mononuclear cells are tumor cells, whereas other population of mononuclear cells are reactive macrophages and/or osteoclast precursor cells (pOCs). MNCs in the GCT also have been considered to be reactive osteoclasts.

We previously reported the possible involvement of Vascular Endothelial Growth Factor (VEGF)-Flt-1 (type-1 VEGF receptor)-Focal adhesion kinase (FAK) pathway in chemotaxis and cell proliferation of pOCs in arthritic joint destruction. Furthermore, it has been shown that overexpression of VEGF in GCT is associated with the oncological activities. Therefore, we hypothesized that the GCT cells, both tumor cells and reactive cells, might produce VEGF that attract pOCs to the neoplastic lesions.

In this study, using clinical samples of GCT, we investigated the possible involvement of VEGF-Flt-1-FAK pathway in the pathogenesis of bone destruction of GCT.

Material and methods

Immunohistochemistry and TRAP-staining were carried out using surgical specimens from 14 patients with GCT. Tumor tissue was freshly chopped and cultured on collagen coated dishes. The conditioned medium was collected from cultured GCT (GCT-CM) and filtered. The primary cultured cells were lysed and subjected to immunoblotting. For immunofluorescence examination, the primary culture of GCT cells was plated on cover slips and incubated with primary antibodies (Abs), followed with FITC or Cy3 conjugated secondary Abs. Then, the samples were examined using confocal laser scanning microscopy.

Mouse myeloid cell line Raw 264.7 (Raw cells) were used as the model of pOCs. Chemotaxis assay was carried out using modified Boyden chambers. Cell proliferation was analyzed by BrdU incorporation assay.

Results

Initially, we analyzed the expression profiles of VEGF and VEGF receptors in the clinical samples. All samples showed the accumulation of mononuclear cells and MNCs. Expression of VEGF and Flt-1 were observed in TRAP-positive MNCs and mononuclear cells. Tyrosine-phosphorylated Flt-1 (activated Flt-1) were also observed in the same samples. (Fig.1) Immunofluorescence of primary culture of GCT indicated the co-localization of CD68 (a specific marker of monocyte/macrophage lineage) and Flt-1. Secretion of VEGF into GCT-CM was confirmed by ELISA. GCT-CM enhanced the chemotaxis of Raw cells. Moreover, GCT-CM stimulated the cell proliferation of serum-starved Raw cells. In the primary culture of GCT, pY397-FAK was mainly co-localized in CD68 positive mononuclear cells, supposed to be pOCs and/or macrophages. Ten samples out of 14 samples of GCT expressed pY397 FAK. Recurrent tumors (3 cases) showed strong expression of pY397-FAK (Fig.2) compared to corresponding primary tumors.

Discussion

Although many studies have suggested that reactive host cells, including macrophages may be cytotoxic to tumor cells both in vivo and in vitro, the exact biological role of these reactive components in GCT is unknown. Previous studies have shown that mononuclear cells in GCT may contribute to tumor spread by producing urokinase-plasminogen activators. Others have found that the mononuclear cells produce IL-1, IL-6, TNF and MCP-1 that stimulate bone resorption. Thus, the infiltration of mononuclear cells might play significant roles in progression of GCT.

VEGF has been detected in many solid tumors associated with tumoral angiogenesis. Furthermore, VEGF-induced vascularity during bone development is shown to be critical for the formation of the osteoclasts. In other words, VEGF may have an important role in both angiogenesis and osteoclastogenesis.

In this study, VEGF expression was clearly found in stroma-like tumor cells and mononuclear cells and MNCs in GCT. We also demonstrated that CD-68 positive cell, including pOCs in GCT expressed the active, tyrosine-phosphorylated Flt-1. Since FAK was tyrosine-phosphorylated by activated Flt-1, the expression of pY397-FAK suggested that the VEGF-Flt-1-FAK pathway may exist in the site of bone destruction in GCT. We confirmed that GCT-CM contained VEGF have chemotactic and mitogenic activities for Raw cells. Thus, it is possible that the Flt-1 positive pOCs-like cells in GCT might be attracted by tumor-derived VEGF and that these cells have the capability of further differentiation into osteoclasts in the tumor.

In conclusion, our results suggest the possible involvement of VEGF-Flt-1-FAK pathway in the recruitment of pOCs in GCT. The pathway could be an attractive therapeutic target of the treatment of the GCT.