

Number & Title: 10918 - Protein Expression Profiling Of Giant Cell Tumors Of Bone Treated With Denosumab
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Background: Giant cell tumor of bone (GCTB) is a rare benign, but locally aggressive osteolytic bone tumor. The rate of local recurrence following surgical resection is relatively high. Rarely, metastasis of GCTB to lung can develop and malignant change in GCTB also occurs. Histologically, GCTB is mainly composed of mononuclear stromal cells highly expressing receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoclast-like multinucleated giant cells expressing receptor activator of nuclear factor kappa-B (RANK). Recently, some studies have reported that the RANKL inhibitor, denosumab, is a novel and effective treatment option for cases of aggressive GCTB. Direct inhibitory mechanism of denosumab through RANK/RANKL pathway alone is not enough to understand the potent effect against GCTB. However, further indirect effects underlying biological process of GCTB treated with denosumab are still unknown.

Purposes: The aim of this study is to gain a deeper understanding of the protein expression pattern involved in indirect effect of denosumab within the framework of the RANK/RANKL pathway.

Patients and Methods: Clinical samples were obtained from two patients with primary GCTB who were treated in our institution. These samples were included two pairs of surgically resected tumor tissues in pre- and post-denosumab treatment. To identify the proteomic change of GCTB associated with denosumab treatment, we performed comparative proteomic analyses by i-TRAQ method between pre- and post-denosumab treatment samples. Each expression profile was analyzed by Ingenuity Pathways Analysis (IPA) system to further understand the affected biological network. Identified proteins were additionally assessed by gelatin zymography and immunohistochemical analysis in vitro.

Results: The proteomic analyses identified approximately 1,600-2,200 proteins in each sample. We analyzed the two pairs of profiles to identify proteins that were similarly altered in both sample sets, and found 37 consistently upregulated and 32 consistently downregulated proteins. In these data sets, IPA analysis provided several subpathways and molecular interaction between most differentially expressed proteins and RANK/RANKL pathway. This molecular network indicated that identified protein play a critical functional role in the treatment with denosumab in GCTB. We confirmed the localized expression of identified proteins in tumor

cells by immunohistochemical analysis. Furthermore, consistent with our proteomic data, the gelatinolytic activity of identified proteins was significantly decreased in post-treatment state ($P < 0.05$).

Conclusions: Our proteomic finding is the first report regarding the proteomic changes of GCTB treated with denosumab. We successfully identified several proteins associated with denosumab treatment in GCTB using clinical samples. We believe that the protein expression pattern and the results of the network analysis will help to understand the effects of denosumab treatment on GCTB.