THE SOLUBLE RECEPTOR ACTIVATOR OF NF-κB:Fc FUSION PROTEIN INHIBITS OSTEOLYTIC AND OSTEOSBLASTIC LESIONS INDUCED BY HUMAN PROSTATE CANCER CELLS

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Introduction: The process of osteolysis is thought to play a critical role in various pathologic conditions such as aseptic loosening of joint arthroplasties and tumor metastasis to bone. Receptor activator of nuclear factor-κB (RANK) is a membrane-bound cell surface protein similar in structure to the tumor necrosis factor receptor that has been identified as an essential component of osteolytic bone resorption. The interaction between RANK and its ligand, RANKL, stimulates a signal transduction cascade that ultimately results in the differentiation and activation of osteoclasts. A recombinant soluble fusion protein consisting of the extracellular domain of RANK coupled with the Fc domain of human IgG (RANK:Fc) has been shown to inhibit osteolysis by blocking RANK-mediated osteoclastogenesis. Prostate cancer metastasis to bone is typically characterized by the formation of osteoblastic lesions. However, at this time it has not been determined whether the resorptive activity of osteoclasts is necessary for the development of osteoblastic bone lesions. The purpose of this study is to evaluate the efficacy of a soluble RANK:Fc fusion protein in limiting the growth of osteolytic and osteoblastic bone lesions induced by human prostate cancer cells using a bone metastasis model.

Materials: Human prostate cancer cells lines that produce either osteolytic lesions (PC-3) or osteoblastic lesions (LAPC-9) were used in this study. Suspensions containing 1x10⁶ cells were isolated from PC-3 and LAPC-9 tumors and implanted in the tibias of SCID mice. These animals were divided into the following groups: Group I: PC-3 Treatment (4 weeks of treatment after tibial implantation, n=7); Group II: PC-3 Delayed Treatment (4 weeks of treatment beginning 4 weeks after tibial implantation, n=7); Group IV: LAPC-9 Pretreatment (1 week of treatment prior to tibial implantation followed by an additional 4 weeks of treatment, n=8); Group V: LAPC-9 Treatment (n=8); and Group VI: LAPC-9 Delayed Treatment (n=8). All of these groups received 350 μg of RANK:Fc protein (Immunex, Inc., Seattle, WA) administered subcutaneously twice a week. Tumor-bearing animals injected with saline served as the control groups for each cell line (Group III: PC-3 Controls and Group VII: LAPC-9 Controls). All animals were sacrificed 8 weeks after tibial implantation, at which time hindlimb tumor diameters were measured and radiographic analysis was performed.

Results: Radiographic scoring of the PC-3 groups was performed in a blinded fashion according to a grading scale based upon the extent of cortical disruption (0 = normal; 1 = endosteal scalloping; 2 = cortex disrupted; 3 = 2 cortices disrupted; 4 = complete destruction of the proximal tibia). Group III (PC-3 Controls) demonstrated extensive osteolytic lesions with obliteration of the entire proximal tibia and large tumor masses (mean radiographic score = 4.0, mean tumor diameter = 12.8 mm). However, in Group I (PC-3 Treatment), there was almost complete preservation of normal bony anatomy with minimal cortical disruption (see Figure 1; mean radiographic score = 0.7). In addition, the mean tumor diameter in this group was only 7.3 mm (43% smaller than Controls). Although Group II (PC-3 Delayed Treatment) exhibited less cortical destruction than Controls (mean radiographic score = 3.0), the mean tumor diameter was nearly identical (12.7 mm).

Radiographic analysis of the LAPC-9 groups was also performed in a blinded fashion using a grading scale based on the anatomic location of the osteoblastic lesion within the tibia (0 = normal; 1 = metaphyseal involvement; 2 = diaphyseal extension; 3 = diaphyseal extension with extracortical involvement). Group VII (LAPC-9 Controls) and Group VI (LAPC-9 Delayed Treatment) exhibited diffuse osteoblastic lesions involving the entire tibia with extension outside the cortices (see Figure 2; mean radiographic score = 3.0). In contrast to these groups, Group IV (LAPC-9 Pretreatment) and Group V (LAPC-9 Treatment) showed marked improvement in the radiographic appearance of osteoblastic lesions with more limited involvement of the tibia (mean radiographic score = 1.4 and 2.1, respectively). The mean tumor diameter of the RANK:Fc-treated LAPC-9 groups also decreased in a dose-dependent fashion (Group VII (LAPC-9 Controls) – 14.3 mm, Group IV (LAPC-9 Pretreatment) – 6.3 mm, Group V (LAPC-9 Treatment) – 10.7 mm, Group VI (LAPC-9 Delayed Treatment) – 11.9 mm).

Discussion: The results of this study indicate that a soluble RANK:Fc fusion protein that effectively blocks osteoclast function may prevent the formation of prostate cancer-induced osteolytic lesions in bone, similar to other osteoclast inhibitors such as the bisphosphonates. In this study, administration of RANK:Fc protein also appeared to suppress osteoblastic prostate cancer lesions, suggesting that osteoclast-mediated osteolysis may play an integral role in the development of tumor-associated blastic bone lesions, at least in the early stages of the metastatic process. It is conceivable that the bone resorption attributable to osteoclast activity may promote metastasis by facilitating the invasion of tumor cells into bone as well as by liberating osteogenic growth factors and cytokines that promote the growth of blastic lesions. Although the mechanism by which malignant cells stimulate bone production is not completely understood, these findings support the use of osteoclast inhibitors such as RANK:Fc protein as a novel treatment for prostate cancer metastases to bone.