Introduction and Significance: Osteolysis, erosive arthritis, bone metastasis and other bone pathologies are a constant clinical challenge. Successful intervention for the treatment of such bone loss diseases requires the identification of signaling pathways mediating these diseases. One major phenotype of any bone loss phenomenon is excessive osteoclast recruitment and activity. RANKL and TNF are strong inducers of osteoclastogenesis. Specifically, RANKL primes and facilitates osteoclast differentiation while TNF elevates an osteoclastogenic response typical for pathologic bone loss conditions. Specific mediators of RANKL and TNF activities have been identified. Among these are TNF receptor associated factors (TRAFs), two of which (TRAF2 and TRAF6) have been shown essential for osteoclast development.

Recent studies have shown that RANKL and TNF are strong mediators of osteolysis and other forms of bone pathologies. More importantly, targeted inhibition of these cytokines significantly inhibits cytokine-related functions and dampens osteolysis. Given that both cytokines require TRAFs for their action, targeted inhibition of TRAF2 and TRAF6 signaling might inhibit subsequent osteoclastogenesis. Thus, we investigated the role of TRAF2 and TRAF6 in mediating RANKL and TNF-induced osteoclastogenesis.

Methods: Wild type and dominant-negative forms of TRAF2 and TRAF6 cDNAs were cloned by PCR into pTAT plasmid. Relevant proteins were expressed and purified as TAT-fused proteins to facilitate their cellular transduction for in vitro experiments. Bone marrow macrophages (osteoclast precursors) were purified from mice long bones in the presence of CSF-1. Osteoclasts were formed by incubating osteoclast precursors with 20 ng/ml sRANKL and CSF-1. TRAF proteins and their mutated forms were added to osteoclast cultures at 100nM for one or two days. Cultures were then fixed and stained with tartrate-resistant acid phosphatase (TRAP) to detect osteoclasts. Data are presented as number of multi-nucleated (3 nuclei or more per cell) TRAP-positive cells. DNA-protein interactions were determined using retardation assay (EMSA) with specific radiolabeled probes.

Results and Discussion: Osteoclastogenesis is a pre-requisite for RANKL and TNF-mediated osteolysis. We have used RANKL and TNF signaling molecules, namely TRAF2 and TRAF6, to regulate this process. Abundant expression and cellular transduction of in vitro generated TRAFs and their dominant-negative forms were first tested by immunoblots. Next, we tested the effect of these proteins on osteoclast precursor cells in the absence or presence of RANKL. The data indicate that wild type TRAF2 and TRAF6 significantly increased osteoclastogenesis by RANKL-primed cells (5-6 folds). No such effect was observed in RANKL-depleted cultures. More importantly, dominant-negative (DN)-TRAF2 and DN-TRAF6 inhibited RANKL-induced osteoclastogenesis to base line. TNF strongly induces osteoclastogenesis by RANKL-primed cells and requires TRAFs for its signaling. Thus, we tested the effect of TRAFs on cells exposed to RANKL and TNF. The data indicate that DN-TRAF2 inhibits the TNF osteoclastogenic effect (>90%) while DN-TRAF6 does not. These findings indicate that dominant-negative forms of TRAFs 2 and 6 differentially and directly inhibit osteoclastogenesis by RANKL and TNF. The data demonstrate that, 1) TAT-TRAFs are successfully delivered in osteoclasts and their precursors, 2) wild type forms of TRAF2 and TRAF6 enhance osteoclastogenesis in RANKL-primed cells, and 3) DN-TRAF2 and DN-TRAF6 differentially inhibit RANKL and RANKL/TNF-induced osteoclastogenesis. These observations position TRAF proteins as mediators of RANKL and TNF-induced osteoclastogenesis. Utilizing these proteins to regulate osteoclast recruitment may be useful to investigate the pathogenesis of various bone loss diseases.

Figure 1: RANKL-primed osteoclast precursors (OCP) were treated with wild type (panels B, D) or dominant-negative (panels C, E) forms of TRAF2 or TRAF6 in the presence or absence of TNF (not shown). At the end of incubation (24 hours), cultures were fixed and stained with TRAP (red/purple color) and osteoclasts were counted using light microscope. TRAF2 and TRAF6 increase osteoclast numbers by 5 and 6 fold, respectively (compare panels B and D with A). Dominant-negative TRAF2 (dTRAF2) inhibited RANKL-induce osteoclastogenesis (panel C), while dTRAF6 only have a slight effect on cell shape but did not inhibit RANKL-mediated osteoclast formation.

References: