RING Finger Protein RNF114 Inhibits RANKL-induced Osteoclast Maturation

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Introduction: Normal bone remodeling is a continuous process of bone turnover (conducted by osteoclasts) and bone formation (conducted by osteoblasts). Any imbalance in the regulation of these two cell types can result in metabolic bone diseases (1). In multiple myeloma patients, tumor cells accumulate in the bone marrow and augment expression of RANKL, a major cytokine responsible for osteoclast differentiation and maturation. Augmented RANKL expression accelerates osteolytic resorption and leads to bone destruction (2). Here, we investigated a role for RNF114, previously identified as a psoriasis susceptibility gene (3), in controlling RANKL/RANK/TRAF6 signaling (4).

Methods: HEK293 cells were co-transfected with a NF-κB-dependent luciferase reporter, TRAF6 and/or RNF114, lysates harvested after 24 h, and luciferase activity measured to assess the impact of RNF114 on TRAF6 induction of the RANKL/RANK pathway. To assess regulation of the osteoclast marker cathepsin K by RANKL, preosteoclast RAW 264.7 cells were transiently transfected with RNF114, then treated with RANKL (50 ng/ml, 72 h) followed by real-time RT PCR analysis of cathepsin K mRNA levels. RAW 264.7 cells were also used to assess the impact of RANKL on endogenous RNF114 expression. To extend these observations to an in vivo model, primary bone marrow cells from RNF114 KO and wild-type mice were treated with RANKL (72 h) to assess cathepsin K and osteocalcin induction by real-time RT PCR. Bone marrow cells harvested from mice receiving adoptive transfer of 10^6 myeloma cells after 4 weeks were similarly assessed for osteocalcin and cathepsin K expression.

Results: RNF114 co-transfection inhibited TRAF6-induced activation of the NF-κB luciferase plasmid (Fig 1). Overexpressing RNF114 similarly attenuated RANKL-mediated osteoclast differentiation of RAW 264.7 cells, as shown by the decreased cathepsin K mRNA induction as compared to cells transfected with an empty vector. RANKL treatment induced transient RNF114 expression that peaked at 6 h and then decreased to below basal levels at 24 and 72 h post-treatment. RANKL induced significantly higher cathepsin K mRNA expression in RNF114 KO bone marrow cells as compared to wild-type cells (Fig 2). To assess the potential role in a bone disease, tumor-bearing animals were generated by injecting myeloma cells into RNF114 KO or wild-type mice. In the RNF114 KO animals, myeloma-induced cathepsin K mRNA levels were augmented as compared to control animals, whereas the bone formation marker osteocalcin was decreased significantly in the knockout mice (Fig 3).

Discussion: Our data demonstrate that RNF114 may play a previously unrecognized role in regulating bone metabolism. When preosteoclast and primary bone marrow cells were induced to differentiate by RANKL, RNF114 expression inhibited the process. RNF114 also inhibited TRAF6 signaling, perhaps providing a mechanism by which it perturbed RANKL signaling. In a multiple myeloma mouse model, RNF114 loss significantly augmented bone resorption markers. Together, these data suggest that RNF114 can be a potential target in studies of osteoclast-related bone diseases.

Significance: There is growing interest in understanding the crosstalk between bone regeneration and immune/inflammatory responses. We have identified RNF114 as a novel regulator of
osteoclastogenesis. Previously associated with immune signaling, this protein may be another link between immune signaling and bone metabolism. Further studies on this protein and its actions may help to identify additional signaling network components responsible for bone resorption and formation, especially in cancer-induced bone diseases.

Fig 1. RNF114 inhibited TRAF6-induced NF-kB activation.
Fig 2. Silence of RNF114 expression augmented RANKL-mediated osteoclast maturation (left: cathepsin K; right: osteocalcin).

Fig 3. Silence of RNF114 expression augmented osteolytic activity in multiple myeloma bone marrow (left: cathepsin K; right: osteocalcin).

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