Authentication of Key Resources Plan

Most of the proposed studies in this application involve the use of various mouse models such as conditional knockout mice in which PPARγ is selectively deleted in osteoclast precursors and the use of primary bone marrow macrophages. We do not expect that these models and primary cells vary. However, the following reagents and cell lines need to be validated to ensure rigor and reproducibility of the proposed research.

Recombinant RANKL

We prepare our own recombinant RANKL for our in vitro osteoclast formation assays. The construct was initially generated by the PI in collaboration with Dr. Nori Namba in the laboratory of Dr. Steve Teitelbaum at Washington University when the PI was a postdoc there. The recombinant RANKL prepared with this construct is very potent in promoting osteoclast formation from primary bone marrow macrophages and RAW264.7 cells. The recombinant RANKL has been used in both PI's lab and Dr. Teitelbaum' lab since 1999. Moreover, the construct has been provided to numerous investigators at and outside of the University of Alabama at Birmingham. We always ensure the quality of the recombinant RANKL by testing its ability to promote osteoclast formation.

RANKL Neutralizing Antibody

We propose to use a commercially available anti-mouse RANKL neutralizing antibody for our mouse model studies (Study 2.2). This RANKL neutralizing antibody (mAb, clone IK22/5) has been used by a number of investigators for mouse model studies and can be purchased in large quantities from Bio X Cell (West Lebanon, NH). After I purchase the antibody, we will validate its ability to neutralize the function of RANKL by performing in vitro osteoclast assays.

RAW 264.7 Cells

This cell line will be only used for promote assays in Study 3.3 since primary bone marrow macrophages are not transfectable. This cell line is only mouse macrophage-like cell line which can differentiate into osteoclasts in response to RANKL treatment. We have obtained this line from ATCC many years ago. However, after a number of passages, we always evaluate the ability of cells to form osteoclasts in vitro in response to RANKL treatment. As long as the cells can form osteoclasts in the presence of RANKL, the cells continue to share high similarity to primary bone marrow macrophages. Thus, the data obtained will be more likely applicable to authentic osteoclast precursors and therefore are very likely reproducible.