**Introduction**

- SIT identified as potential bone mass regulator
  - SIT (SHP2-interacting transmembrane adaptor) phosphorylated by c-src family kinases
  - Previously known to be involved with function of T-cells
  - Mice in which gene had been deleted (knockouts) have increased bone volume trabecular number and connectivity

**Bone cells in culture useful in further study**

**CRISPR system used to delete SIT in rat osteosarcoma (ROS) cells**

**SIT RNA levels higher in cells in which SIT was deleted**

**Further testing to be done on cells in culture**

**Rate growth, differentiation markers, SIT protein levels**

The SIT gene editing system was used to delete the gene in rat osteosarcoma (ROS) cells in culture. A guide RNA for rat SIT predicted to have the best balance between cell kill and lack of off-target effects was chosen. Its coding DNA was cloned into an all-in-one CRISPR plasmid (PX458; both guide RNA cloning and Cas9) at the BbsI site. This plasmid was introduced into the rat osteosarcoma cell line ROS 17.2.8 and transfected cells were selected for G418 resistance. Interestingly, we were unable to clone individual cell sublines. This suggests some critical role for SIT in regulating growth in response to cell density. In the G418-resistant SIT CRISPR pool, the levels of SIT RNA were found to be higher than in cells transfected with the empty vector. We are determining the levels of SIT protein by Western blot as well as analyzing how Cas9 disrupted the SIT gene. The growth and expression of osteoblastic differentiation markers such as alkaline phosphatase, osteocalcin and matrix Gla protein genes are also determined in these cells as compared to the control cells.

**Deleting SIT Gene in Bone Cells in Culture**

**CRISPR–Cas9 Mechanism and Generation of Plasmid**

- Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)
- CRISPR associated genes (Cas)
- Type 2 CRISPR based on mechanism of homology
- Double-stranded breaks at target locus
- Cas9 directs small RNAs to target
- Cell initiates Non-Homologous End Joining
- Error prone – frameshift mutation

**Future Directions**

- The levels of SIT RNA and protein expression in clonal ROS sublines will be compared to those of cells transfected with the empty vector.
- ROS sublines where SIT expression is very low will be analyzed for markers of osteoblastic differentiation over a 12 day time course. These markers include cell proliferation, alkaline phosphatase enzyme activity, and the expression of the osteocalcin and matrix Gla protein genes.
- Another group of students is validating CRISPR reagents for deleting the c-src gene. We will use these reagents to delete c-src in ROS sublines that have reduced SIT expression to identify additive effects on osteoblast markers.

**Ethical Considerations of Research and Drug Development**

$2.55$ billion to develop a new drug (Source: *E. al. 2016*)

Why is it worth investigating the mechanisms behind bone loss when treatments already exist? **Four Pillars of Medical Ethics**

1. Autonomy – Give patients more choice and information
2. Beneficence – Increase quality of life
3. Non-Maleficence – Lack of need for discordant neglect, omission of better care
4. Justice – Equitable treatment options. Cost must be shared, benefits distributed according to need. (Children and Beauchamp 1978)

Osteoporosis is increasingly prevalent in our aging population. Today, it is the second most disruptive affliction according to the Institute of Bone Health. Research and a potential new treatment will give patients more options and information, better quality of life, and commit to reducing harm of bone loss. The distribution of the advancements will be just as fair given according to need.