



Understanding Osteoporosis: Identifying and Characterizing the Binding Partners of RGS12 in Osteoclasts

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INTRODUCTION

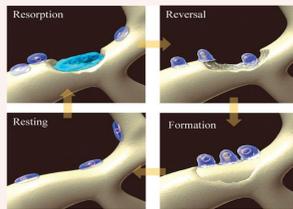
What is the **osteoporosis**?

- ❖ A pathological condition characterized by **low bone density**, which leads to increased risk and incidence of **bone fracture**.

How does osteoporosis **occur**?

- ❖ The human skeleton continuously undergoes **remodeling**, which is the cyclical process of controlled bone **degradation** (resorption) followed by bone **formation**.
- ❖ Two types of cells responsible for the process of bone remodeling.
 - **Osteoblasts**: Bone **formation**.
 - **Osteoclasts**: Bone **resorption**.

- ❖ Osteoporosis occurs when the body is not able to form adequate amounts of new bone or reabsorbed excessive existing bone.



How can this research be **beneficial**?

- ❖ The long-term usage of osteoporosis medications have critical **safety concerns** related to **side effects**.
- ❖ Understanding specific mechanism that targets bone remodeling will help to find **better treatment strategies**.

How does **RGS12** affect the bone loss?

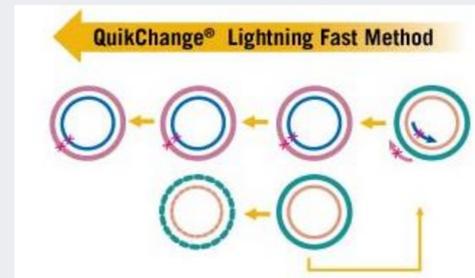
- ❖ **Regulator of G-protein Signaling 12 (RGS 12)**
 - Members of proteins that play a role in controlling the signaling cascade inside the cell.
 - Responsible for **inactivating G proteins** and subsequent **signaling events**.
- ❖ Mutated mice with **RGS12 deficiency** did **not** develop a **pathologic bone loss** through inhibiting the development of osteoclasts.



MATERIALS & METHODS

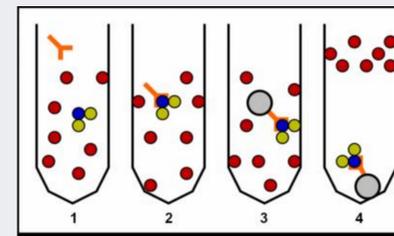
Phase I. Site-directed mutagenesis

- ❖ Materials: QuikChange Lightning Site-Directed Mutagenesis (SDM) kit, Custom Order Primers & Lipofectamine 3000 Transfection Reagent
- ❖ Perform **site-directed mutagenesis (SDM)** to systematically mutate and disrupt each RGS12 domain.
- ❖ Transfect osteoclast precursors with wild-type or mutant RGS12 and allow cells to express either the normal or disrupted RGS12 protein.
- ❖ Culture cells and wait for osteoclast differentiation (1-2 weeks).
- ❖ Compare wild-type/mutant osteoclasts precursors' ability to differentiate into mature and active osteoclasts. Identify which RGS12 domains are related to osteoclast differentiation.



Phase II. Co-Immunoprecipitation

- ❖ Materials: Anti-FLAG M2 Magnetic Beads & Anti-c-Myc Magnetic Beads
- ❖ Harvest cells and extract total protein.
- ❖ Perform co-immunoprecipitation (co-IP) to **purify RGS12** and its **binding partners**.
 - Notes: Antibodies that selectively bind either FLAG or c-Myc tags on RGS12 are conjugated to magnetic beads that allow them to be separated from all other proteins in the cell. RGS12 interacting partners, via binding with RGS12, is also "pulled down" from the mixture.
- ❖ **Compare** the RGS12 binding proteins between wild-type and mutant RGS12. Domain mutation should result in disrupted binding to partner proteins.

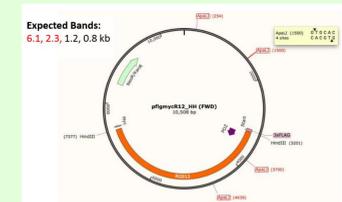


Phase III. Mass Spectrometry

- ❖ Materials: Orbitrap Fusion Tribrid Mass Spectrometer (LC/MS) & Ingenuity Pathway Analysis (IPA) Software
- ❖ Perform mass spectrometry (MS) **analysis** on the co-IP purified sample to discern the identity of RGS12 binding proteins.
- ❖ Use Ingenuity Pathway Analysis (IPA) bioinformatics software to organize RGS12 binding proteins into signaling networks.



PROGRESSES & DISCUSSION



- ❖ Six mutated RGS12 were obtained through SDM; working to make additional amount.
- ❖ Successfully transfected RAW264.7 cells (mouse osteoclasts precursor cells) with the wild type RGS12.
- ❖ Pulldown with anti-FLAG and anti-myc against recombinant RGS12 was not successful.
 - Problem?
 - cDNA sequence was not in frame with protein tags; results in a truncated product.
 - Solution?
 - Use site-directed mutagenesis to delete two nucleotides ($\Delta 1027/5096$) in order to correct the open reading frame (ORF)
 - Result
 - Proteins with corrected sequence were successfully fused to a FLAG protein tag on the left and a c-Myc protein tag on the right.
- ❖ After necessary parts are obtained, LC/MS will be used to address the hypothesis
 - RGS12 is a central regulatory node where multiple pathways converge.

ACKNOWLEDGE

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