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Synthetic Biology Enabled Modification of Bone Cells and the Impact on Bone Mineral Formation

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Purpose of Study

Modification of inorganic biomaterial by engineering bone producing osteoblasts to increase levels of the signaling molecule, osteoprotegerin (OPG).

Impact: If successful, this is a first step toward tuning bone cells to produce bone material to desired properties in- vitro.

Introduction

- Bone is a hybrid inorganic material composed of the mineral hydroxyapatite and collagen.
- Bone tissue is composed of osteoblasts (form bone), osteocytes (sense mechanical stress), osteoclasts (remove bone)
- In vivo, bone regenerates in response to mechanical stress and transduces to biochemical response, specifically modifies the ratio of OPG and Receptor Activator of Nuclear factor Kappa-B Ligand (RANKL) which makes it attractive for advanced materials applications
- K7M2 bone cells are osteoblasts which secrete OPG and a high ratio of RANKL/OPG enhances the differentiation of pre-osteoclasts to mature osteoclasts while a low ratio of RANKL/OPG inhibits this differentiation of pre-osteoclasts such as RAW cells (monocytes).

Study Overview

Engineered K7M2 cells to overexpress OPG signaling molecule in the RANKL/RANK/OPG pathway and then assessed the effect on osteoclast formation and activity with a resorption pit assay using calcium phosphate as a synthetic biomimetic material. K7M2 is an osteosarcoma cell line that promotes osteoclastogenesis. In this study, we questioned whether engineering OPG is sufficient to alter the osteoclastogenic capacity of K7M2 cells.

Findings

- OCs in UCM had more than 8 nuclei and OCs in CM generally had less than 3 nuclei
- There is a reduction in number of pits formed in CM from K7M2 cells

Conclusions

- Successfully engineered and characterized K7M2 cell line.
- The OCs in CM did not generally differentiate into mature OCs compared to OCs induced in UCM.
- Induction in K7M2 CM needs to be optimized.

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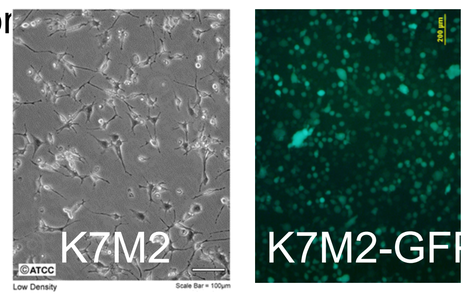
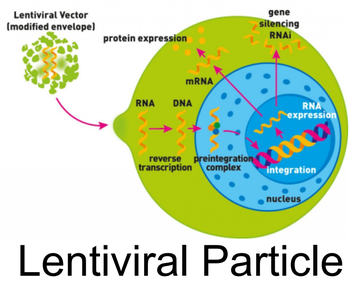
Experimental Details & Results

Genetic Engineering of K7M2

K7M2 cells were incubated with LVP's from Origene which introduced OPG gene into K7M2 chromosor





Lentiviral Particle (LVP)

Verification of Increased OPG Expression

- K7M2-OPG modified cell line was cultured in DMEM medium for 48 hours.
- Medium was assayed using Enzyme-linked immunosorbent assay (ELISA) to measure OPG protein concentration compared to cells modified with a control LVP which should not increase OPG (K7M2-Myc Tag), and the parent cell line (K7M2) (Fig.1a)
- Polymerase Chain Reaction (PCR)was used to assess gene expression. (Fig.1b)

Fig.1a

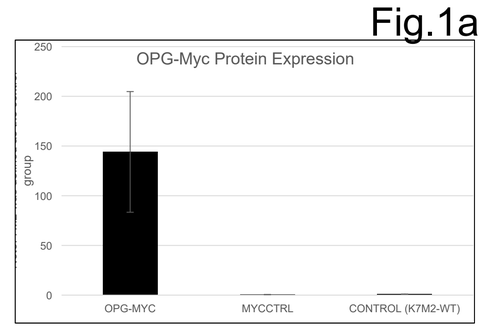


Fig.1b

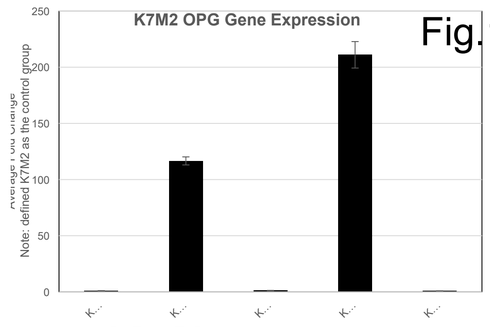
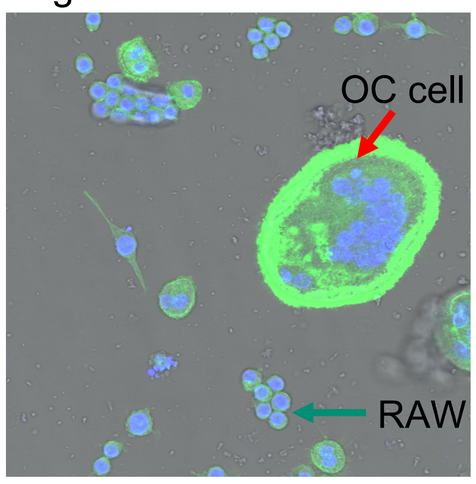


Fig. 2 **Differentiation of RAW cells to Osteoclasts (OC)**



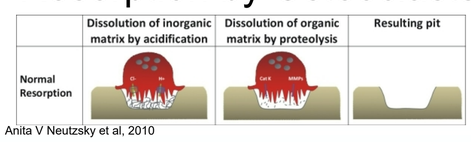
- RAW cells (Pre-osteoclasts) were seeded on 6 well plates or IBIDI chambers and allowed to incubate for a day.
- The next day, 25ng/mL of RANKL was introduced to induce OC formation. Media was changed every 2 days for a total of 6 Days.
- OCs have multiple nuclei and a F-actin ring making them visually distinct from RAW cells. An example confocal fluorescence image is shown on Fig..2 Nuclei stained with Hoechst and 488 phalloidin was used to stain F-actin ring,

Fig 3.Resorption Pit Assay

Effect of Increased OPG on Osteoclast Differentiation

K7M2-OPG cells were grown overnight in DMEM medium. The following day, this conditioned medium (CM) was added to RAW cells. A resorption pit assay and quantitative confocal microscopy was used to determine the effect.

Resorption by Osteoclasts



Anita V Neutzsky et al, 2010

Resorption Pit Assay

6 well plates were coated with simulating body fluid for 3 days and then 1 day with $CaPO_4$. RAW cells were seeded on these plates and induced with RANKL with conditioned medium from K7M2-OPG cells (Fig.3 c) or K7M2-Myc ctrl cells (Fig.3b) and as a positive control, RAW cells were seeded with unconditioned medium (UCM) (Fig. 3 a). After 6-7 days, plates were stained with 5% Silver Nitrate and treated with UV for an hour and then imaged with inverted microscope to visualize pits.

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