Engineered Osteoclasts: Potential Cell Therapy for Ectopic Calcification

Apichai Yavirach, Worakanya Buranaphatthana, Cameron Rementor, Subramanian Dharmarajan, Cecilia M. Giachelli
Department of Oral Health Sciences, School of Dentistry & Department of Bioengineering, University of Washington, Seattle, USA

INTRODUCTION

Ectopic calcification (EC) is the abnormal deposition of mineral in soft tissues such as blood vessels and valves (vascular calcification; VC) as well as joints, tendons, and amputation sites (heterotopic ossification; HO). VC is prevalent and a major risk factor for cardiovascular mortality, with no non-surgical treatment available. Likewise, the incidence of HO increases to over 90% in severe traumatic amputations[1] and current treatments can cause complications and recurrence of HO. Hence, new therapeutic approaches to prevent and/or treat VC and HO are greatly needed.

Osteoclasts (OCs) are multinucleated cells capable of bone resorption. OCs are also able to control bone formation via released factors, but their ability to prevent mineralization through release of inhibitory factors has not been described. We developed **iRANK OCs** by engineering RAW264.7 murine myeloid stem cells with an inducible intracellular receptor activator of nuclear factor kappa-B (iRANK) construct to allow differentiation into OCs under the control of a chemical inducer of dimerization (CID) drug[2] **(Fig.1)**. Utilizing iRANK cells as proposed cell therapy **(Fig.2)** in the present studies, we investigated whether 1.) iRANK OCs could prevent EC *in vitro* and 2.) treat existing EC *in vivo*.

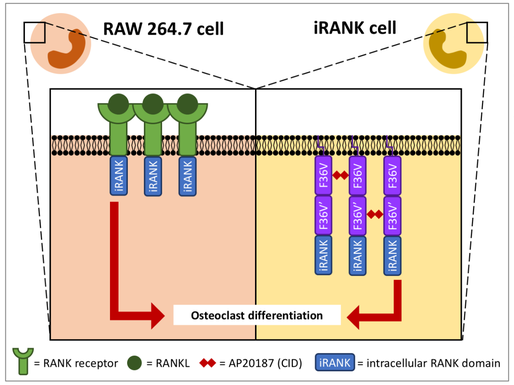

Fig.1. Comparison of RANKL- and CID-induced osteoclast differentiation pathways in RAW 264.7 and iRANK cells respectively. F36V = FKBP12; F36V' = modified FKBP12 (CID binding site).

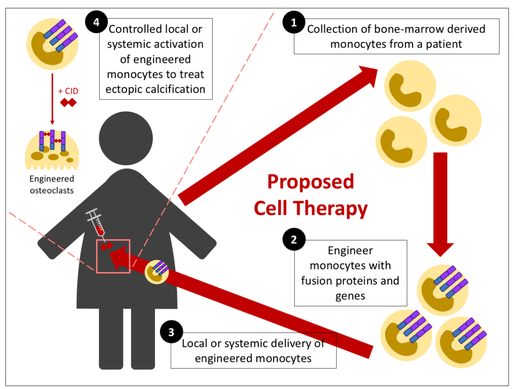

Fig.2. Proposed cell therapy for EC utilizing engineered monocytes

iRANK OCs reduced calcification in both VC and HO models *in vitro*

Methods

***In vitro* VC and HO model:** Bovine valve interstitial cells (BVIC) and C2C12 myoblastic stem cells were used for VC and HO co-culture models respectively. RAW264.7 or iRANK cells were cultured on Transwell insert and induced with either 40 ng/mL hRANKL or 50 nM CID for 4 days to differentiate into OCs. Then, Transwell inserts were transferred to a 6-well plate containing either BVIC or C2C12 cells treated with 2.4 and 3.2 mM Pi respectively, to initiate osteogenic differentiation and mineralization. BVIC and C2C12 cells were collected for calcium measurement on day 7 and day 5 of co-culture respectively.

Results

iRANK OCs reduced calcification in both VC **(Fig.3)** and HO models **(Fig.4)** *in vitro*. This inhibitory effect was comparable to the one observed in RANKL-treated RAW264.7 OCs.

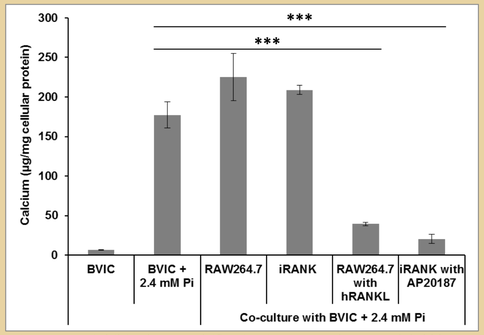

Fig.3. iRANK OCs inhibited BVIC calcification in VC co-culture model (***p<0.001).

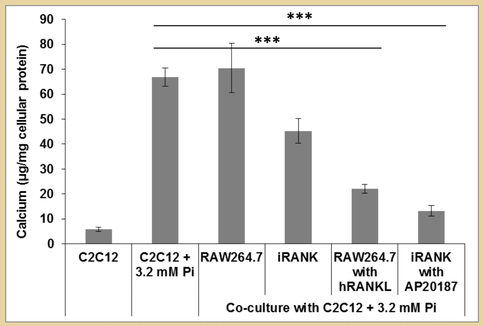

Fig.4. iRANK OCs inhibited C2C12 calcification in HO co-culture model (***p<0.001).

iRANK OCs reduced existing EC *in vivo*

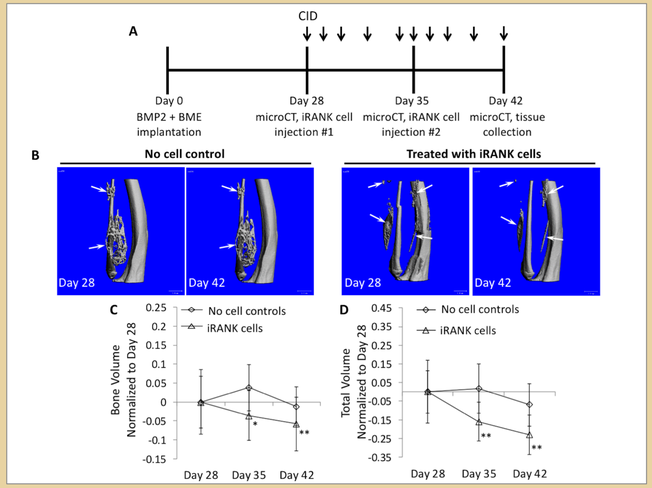

Fig. 5. HO lesions were resorbed overtime in mice treated with iRANK cells **(A)** *In vivo* experiment timeline **(B)** micro-CT images of day 28 and 42 (white arrows = HO lesions) **(C)** Quantitation of bone volume **(D)** Quantitation of total volume (*p < 0.05, **p < 0.01)

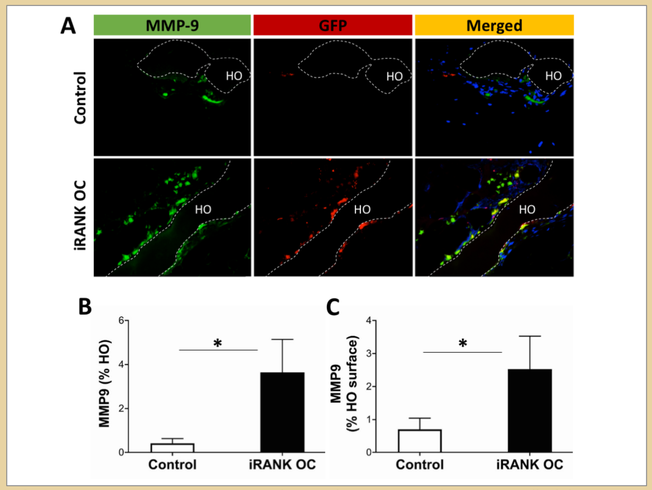

Fig. 6. MMP-9+GFP+ OCs were observed in mice with iRANK cell delivery **(A)** immunofluorescence images of OCs **(B)** Percentage of MMP-9+ OCs to the total HO area (% #Ocs/mm^2) **(C)** Percentage of MMP-9+ OCs to the total HO surface (% #Ocs/mm) (*p < 0.05)

Methods

***In vivo* HO model:** Eleven nude mice were anesthetized and the mid-belly of the gastrocnemius muscle was injected with a mixture of BMP-2 and Cultrex Basement Membrane Extract (BME) (2.5 μg BMP-2/20 μL BME) to induce HO formation. On day 28, micro-CT (Scanco vivaCT 40; 21-μm voxel resolution 55 kVP, 145 μA) was used to verify HO formation and quantitate the lesion volume.

iRANK cell delivery: On day 28, 5x10^6 iRANK cells in 0.5 mg/mL neutralized collagen hydrogel were injected to six mice; the rest did not receive cells as a control group. For the cell delivery group, CID was administered intraperitoneally daily for three days and then every other day throughout the whole experiment to induce OC differentiation. On day 35, micro-CT scan was performed in all mice and iRANK cells were delivered again as explained above in the cell deliver group. On day 42, micro-CT scan was performed, and the entire calf was collected in all mice for immunohistochemistry.

Results

- HO lesions in the iRANK cells + CID treated mice were decreased significantly in both bone volume (BV) and total volume (TV) compared to the baseline. On the other hand, the control group had relatively stable HO lesions in both BV and TV compared to the baseline **(Fig. 5)**.
- The numbers of MMP-9+ OCs were significantly higher in cell delivery group compared to control group. These OCs were also GFP+ indicating that they differentiated from the delivered iRANK cells **(Fig. 6)**.

ACKNOWLEDGMENTS

This work was supported by United States Department of Defense Peer Reviewed Orthopaedic Research Program Award [OR120074] and NIH R35 HL139602 to CG and Fulbright Scholarship to AY.

REFERENCES

1. Meyers C et al. JBMR plus vol. 3,4 e10172, 2019.
2. Rementer CW et al. PloS one vol. 8,12 e84465, 2013.