Statement of Purpose: Exosomes derived from mesenchymal stem cells (MSCs) are an active area of research due to their therapeutic potential in treating osteoporosis. To further harness their therapeutic performance in osteoporotic conditions, we have equipped exosomes with 1) osteoclast-targeting moieties on their surface and loaded 2) chemokine receptor antagonists that successfully reversed osteoporotic bone loss in an ovariectomized mice model.

1) Osteoclast-targeting exosome mimetics: Apoptotic cells present phosphatidylserine (PS) at their external surface as "eat-me" signals, recognized by macrophages to go through effective phagocytosis. PS lipids are also known to exert anti-inflammatory effects in macrophages, mediating M1 (inflammatory)-to-M2 (anti-inflammatory) polarization of macrophages. We noted the previous finding that PS receptors are highly expressed on the surface of macrophages, osteoclast precursors and bone-resorbing osteoclasts over the course of osteoclastogenesis. Our approach was to incorporate PS lipids in the membrane of exosome mimetics (EMs) through physical fusion, for the purpose of mimicking apoptotic cells and therefore achieving a marked affinity for osteoclast precursors and potential anti-resorptive effects.

2) Blockade of osteoclast recruitment: Recently, the chemokine ligand CXCL9 secreted by osteoblasts and receptor CXCR3 expressed on osteoclast precursors were identified as an essential axis to control osteoclast precursor recruitment and differentiation at bone resorption sites in osteoporotic conditions. The chemical antagonist of CXCR3, AMG487, has been suggested as a potential small molecule drug to suppress the migration and activation of osteoclast precursors to bone matrix. While AMG487 has been tested in preclinical and clinical trials in psoriasis, it has not been used as targets for osteoporosis. Herein we encapsulated AMG487 in the PS-incorporated EMs (PS-EMs) to block osteoclast recruitment and mitigate osteoporotic effects.

Methods: EMs were generated by extruding mouse bone marrow-derived MSCs through pore membranes. PS-EMs were prepared by lipid thin film hydration followed by a membrane extrusion method. The zeta-potential and size distribution were characterized by dynamic light scattering and nanoparticle tracking analysis (NTA), respectively. To ensure successful incorporation of PS lipids into the membrane of EMs, fluorescence resonance energy transfer (FRET) study was performed. Cellular internalization of PS-EMs was investigated using confocal light scanning microscopy in the co-culture system of macrophages and MSCs. The inhibitory effect of PS-EMs on osteoclast differentiation was studied using Tartrate-resistant acid phosphatase (TRAP) staining. The inhibitory mechanism was further investigated using qPCR analysis and transfection and luciferase reporter gene assays. In-vitro transwell migration assay was performed to evaluate the effect of AMG-487 in hindering macrophage migration under exposure to CXCL9. Ovariectomized (OVX) mice model was used as an in-vivo model to investigate the effects of PS-EMs in preventing osteoporosis. In-vivo biodistribution of PS-EMs was analyzed using PET scanning with radiolabeled PS-EMs. After 8 weeks after injection (once a week), femur bone tissues were harvested for μCT scanning and histological analysis.

Results: FRET assay showed that PS lipids were successfully incorporated into the membrane of EMs in a concentration-dependent manner. NTA of PS-EMs displayed a size distribution with a peak diameter of around 100 – 150 nm. Confocal imaging demonstrated that PS-EMs were preferentially internalized by macrophage cells (RAW 264.7) over hMSCs compared to control EMs incorporated with phosphatidylcholine (PC), indicating a role of PS in targeting macrophages. Moreover, PS-EMs exhibited enhanced inhibitory effects on the formation of TRAP-positive multinuclear cells in RAW 264.7 cells cultures compared with control EMs as shown below.

Increased mass ratio of PS to EM resulted in enhanced osteoclastogenesis inhibition effects in sets of PS-EMs. The role of PS-EM in inhibiting osteoclast differentiation was further explored via mechanistic studies where PS-EMs inhibited RANKL-induced osteoclast differentiation in RAW264.7 cells by suppressing the activation of NF-κB and NFATc1. In chemotaxis assays, PS-EMs loaded with AMG-487 were highly effective in blocking migration of osteoclast precursors in the presence of CXCL9. We found that intraperitoneal administration of PS-EMs attenuated bone loss in osteoporosis mice from the quantification of bone parameters in left femurs (increase in bone mineral density, bone volume fraction, trabecular thickness but decreased trabecular separation). PS-EMs reached the bone tissue after they were injected intraperitoneally. Our findings demonstrate the great promise of PS-EMs as cell-free anti-resorptive nanocarriers for alleviating osteoporosis.

References: