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

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Bone is a hybrid inorganic-organic material composed primarily of the mineral hydroxyapatite and the protein collagen. The ability of bone to remodel and regenerate makes it attractive for advanced material applications. Bone tissue is composed of three types of cells; osteoblasts (form bone), osteocytes (sense mechanical stress) and osteoclasts (remove bone). In vivo, bone continuously remodels in response to RANKL/RANK/OPG signaling between osteoclasts and osteoblasts. Abnormal signaling in bone results in bone metabolic diseases caused by the imbalance between bone formation and bone resorption. For example, osteosarcoma can produce osteolytic lesions, resulting in excessive destruction of bone. In this study we engineered osteoblasts to alter the levels of signaling molecules in the RANKL/RANK/OPG pathway and assessed the effect on osteoclast induction and resorption. K7M2, osteoblast cells were genetically modified to overexpress osteoprotegerin (OPG) using lentivirus transduction. OPG overexpression was confirmed at the gene and protein level with Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunoassay (ELISA) assays respectively. RAW 264.7, monocyte cells were induced with K7M2-OPG conditioned medium with RANKL and confocal fluorescence microscopy was used to confirm the formation of osteoclasts. Osteoclast functionality was confirmed with a resorption pit assay using calcium phosphate as synthetic biomimetic material. Preliminary results show that engineering OPG is sufficient to alter the osteoclastogenic capacity of K7M2 cells. This work represents an important initial step in utilizing synthetic biology to elucidate the mechanisms that inhibit osteoclastogenesis in osteosarcoma in-vitro.

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