we seeded osteoclasts onto a pre-seeded β-clasts. Our protocol allowed us to passage these cells without cell loss or loss of TRAP staining and functionality in resorption assays proved functional osteo-TCP). This bone model, consisting of differentiated osteogenic cells, is a bone model that mimics glucocorticoid-induced osteoporosis. Our present study indicated that mechanical stress induced upregulation of Glut1, downregulation of SIRT1, and upregulation of Runx2 in osteoblasts, but not in chondrocytes. Since SIRT1 is known to negatively regulate Runx2 activity, mechanical stress-induced downregulation of SIRT1 may lead to upregulation of Runx2, resulting in osteoblast differentiation and bone formation. Incubation with Glut1 inhibitor blocked mechanical stress-induced changes in SIRT1 and Runx2 in osteoblasts. In contrast to osteoblasts, expressions of Glut1, SIRT1 and Runx2 in chondrocytes were decreased by loading. Overloading reduced chondrocyte activity (production of proteoglycan, type II collagen).

Conclusion: Ultimately, we will obtain an in vitro 3D co-culture of osteoblasts/osteoclasts simulating human native bone, which will be treated with dexamethasone to mimic key aspects of GIOP in vitro.

REFERENCES:

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Figure 1. The effect of ORM2 in RANKL-induced osteoclast differentiation and osteoblast differentiation. (A) BM-MNCs transfected with negative control siRNA (siControl) or siRNA targeting ORM2 (siORM2) were cultured with M-CSF (30 ng/mL) and the indicated concentrations of RANKL for TRAP staining. The TRAP-positive MNCs were counted as mature osteoclasts. *P < 0.05 versus siControl group. (B) Mature osteoclasts transfected with siControl and siORM2 were cultured in a hydroxyapatite-coated plate and dentin slice to detect resorption pits. The number of TRAP-positive MNCs and relative resorption areas in the hydroxyapatite-coated plate and dentin slice were quantified. **P < 0.001 versus siControl group. (C and D) Osteoblasts treated with the indicated concentration of recombinant protein were cultured for seven days to perform ALP staining and activity assay. Calcium deposits for matrix mineralization were measured by ARS staining after 21 days of culture and the intensity of staining was quantified with CPC.

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POS0420 GENERATION OF A HUMAN 3D IN VITRO BONE MODEL THAT MIMICS GLUCOCORTICOID-INDUCED OSTEOPOROSIS

Keywords: Bone diseases, Osteoporosis

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Background: Osteoporosis is a bone disease characterized by low bone mass and changes in bone architecture, leading to pain and reduced mobility in patients. Glucocorticoid-induced osteoporosis (GIOP) is the best known form of secondary osteoporosis. Glucocorticoids (GC) are commonly used to treat inflammation, such as in rheumatic diseases. However, GC use can have a negative effect on the skeletal system and lead to osteoporosis.

Objectives: (i) Establishing and characterizing a human in vitro bone model consisting of osteoblasts and osteoclasts embedded in [i-TCP] ("healthy" bone model), (ii) treatment with dexamethasone to induce GIOP ("osteoporotic" bone model) and (iii) using this model as a testing platform for the treatment of osteoporosis.

Methods: Our model includes osteoblasts and osteoclasts, which are mainly responsible for bone remodeling. We defined an osteoclast differentiation protocol using low-attachment plates and cultured the cells for 21 days in xMEM medium, 5% FCS, 5% human AB serum, 2 mmol/L-glutamine, 25 mg/ml M-CSF, and 50 ng/ml RANKL. To provide the basic scaffold for the structure of the "healthy" bone model, mesenchymal stromal cells (MSCs) were seeded on [i-tricalcium phosphate ([i-TCP]). This bone model, consisting of differentiated osteogenic cells, was fully characterized in our previous work [1].

Results: The multinucleity, typical ß-actin ring formation, cellular activity by TRAP staining and functionality in resorption assays proved functional osteoclasts. Our protocol allowed us to passage these cells without cell loss or loss of function. To establish the previously "healthy" (i.e., untreated) bone model, we seeded osteoclasts onto a pre-seeded [i-TCP] construct and cultured the co-culture for 7 days. We then analyzed the supernatant and detected marked secretion of RANKL, MMP-9, and free phosphate. This indicates the functionality of both osteoclasts and osteoblasts in our 3D model. Subsequently, the healthy model will be transferred to the osteoporosis-simulating model where treatment with dexamethasone will be applied. Once established, we plan to use the model we have developed for in vitro preclinical trials to test marketed drugs.

Conclusion: Ultimately, we will obtain an in vitro 3D co-culture of osteoblasts/osteoclasts simulating human native bone, which will be treated with dexamethasone to mimic key aspects of GIOP in vitro.

REFERENCES:

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POS0421 GLUCOSE TRANSPORTER 1 REGULATES MECHANICAL STRESS-ACTIVATED SUBCHONDRAL BONE FORMATION VIA THE SIGNAL TRANSDUCTION NETWORK OF CELLULAR ENERGY SENSOR, NAD+-DEPENDENT DEACETYLASE (SIRTUIN 1) AND OSTEOSTEOGENIC TRANSCRIPTION FACTOR, RUNT-RELATED TRANSCRIPTION FACTOR 2 (RUNX2), IN SUBCHONDRAL BONE TISSUE

Keywords: Cartilage, Cell biology, Osteoarthritis

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Background: Mechanical stress is an important factor affecting bone and cartilage tissue homeostasis in osteoarthritis (OA). We focused on the interaction among mechanical stress, glucose uptake via glucose transporter 1 (Glut1) and the cellular energy sensor sirtuin 1 (SIRT1) in energy metabolism, since it has been recognized that SIRT1, an NAD+-dependent deacetylase, may function as a master regulator of mechanical stress response as well as cellular energy metabolism. Also, it has already been demonstrated that SIRT1 regulates the activity of the osteogenic transcription factor, runt-related transcription factor 2 (Runx2).

Objectives: Purpose of this study was to determine whether mechanical force affects the expression of factors regulating energy metabolism and transcription factors controlling subchondral bone formation and ossification in OA.

Methods: Comparative analyses of the expression of Glut1, SIRT1 and Runx2 in osteoblasts and chondrocytes were performed after mechanical loading of a 3D cell-collagen sponge construct.

Results: Mechanical loading increased osteoblast activity. Mechanical loading significantly increased the expression of Glut1, significantly decreased the expression of SIRT1 and increased the expression of Runx2 in osteoblasts in comparison with non-loaded osteoblasts. Incubation with Glut1 inhibitor blocked mechanical stress-induced changes in SIRT1 and Runx2 in osteoblasts. In contrast to osteoblasts, expressions of Glut1, SIRT1 and Runx2 in chondrocytes were decreased by loading. Overloading reduced chondrocyte activity (production of proteoglycan, type II collagen).

Conclusion: Our present study indicated that mechanical stress induced upregulation of Glut1, downregulation of SIRT1, and upregulation of Runx2 in osteoblasts, but not in chondrocytes. Since SIRT1 is known to negatively regulate Runx2 activity, mechanical stress-induced downregulation of SIRT1 may lead to upregulation of Runx2, resulting in osteoblast differentiation and bone formation. Incubation with Glut1 inhibitor blocked mechanical stress-induced changes in SIRT1 and Runx2 suggesting that Glut1 is necessary to mediate the responses of SIRT1 and Runx2 to mechanical loading. These results suggest that Glut1 regulates mechanical stress-activated subchondral bone formation and ossification via the signal transduction network of cellular energy sensors, sirtuin 1 and Runx2, in OA.

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