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Hot Topics Abstracts

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HT1
Novel Small RNA Function and Delivery *In Vivo*

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Small RNA contains a variety of RNAs of 20–30 nucleotides, which include small inhibitory RNA and microRNA (miRNA), noncoding RNA that negatively regulates gene expression at post-transcription level. Accumulating evidence shows that miRNAs have critical roles in cell growth, differentiation and apoptosis. However, the roles of miRNAs in mechanotransduction and skeletal development are unclear. MiR-365 was the first miRNA identified to be mechanically sensitive from microarray screening of chondrocytes. Its expression is significantly activated by cyclic loading of primary chondrocytes. MiR-365 is both necessary and sufficient for mechanical stimulation of chondrocyte proliferation and differentiation *in vitro*. Furthermore, the effect of miR-365 on chondrocyte proliferation and differentiation is dependent on its suppression of the levels of a target protein histone deacetelyse 4. To test the role of miR-365 *in vivo*, we generated transgenic mice that express miR-365 specifically in cartilage under the Col II Cre. Multiple transgenic lines were established in which the levels of miR-365 activation are similar to those by mechanical activation (6- to 10-fold increase) in a cartilage-specific manner. MiR-365 cartilage-specific transgenic mice were born with normal size, but exhibited premature ossification as early as 1 week postnatally. Both the skeletal size and body weight are reduced in miR-365 transgenic mice after 2 weeks. However, bone mineral density (BMD) is significantly enhanced in miR-365 transgenic mice at 8 weeks. This suggests that miR-365 promotes BMD postnatally. Therefore, cartilage-specific expression of miR-365 *in vivo* affects chondrocyte differentiation, skeletal growth, ossification and BMD. It suggests that the mechanosensitive miR-365 is a regulator of postnatal skeletal development and bone mass *in vivo*.
Small RNAs have strong potential in the development of molecular therapeutics and diagnostics. However, current small RNA delivery systems present significant shortcomings. Inspired by emerging concepts of DNA/RNA nanotechnology, we develop a novel strategy for the intracellular delivery of small RNA through the self-assembly of small RNA with DNA-based rosette nanotubes (RNTs). RNTs and small RNA self-assemble through electrostatic interactions into non-covalent, yet stable nanostructures to achieve intracellular RNA delivery with high efficiency. Such technology works in different cell types and species, both *in vitro* and *in vivo*. This biomimetic approach is highly useful for the intracellular delivery of small RNA therapeutics and diagnostics.

HT2
Targeting Sclerostin for Anabolic Therapy of Bone Disorders

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There are two classes of agents that can be used for the treatment of bone disorders such as osteoporosis: anti-resorptive agents that target osteoclasts and inhibit bone resorption and destruction, and agents that target osteoblasts and stimulate bone formation and building of bone mass and structure. Although there are several agents available for the treatment of bone disorders, there are limitations for each of them such as safety, convenience of administration, compliance and so on. Therefore, there is still a great medical need for agents that have long-term efficacy, ease of administration and minimal safety concerns. In particular, there is a need for agents that can stimulate bone formation, restore bone mass and restore bone structure, leading to rapid and improved reductions in the risk for skeletal fractures and possible improvements in bone healing.
Recent research reveals that the Wingless-type MMTV integration site (Wnt) pathway has an important role in skeletal metabolism, particularly in bone formation and regeneration. For example, knockout of Lrp5/6, co-receptors for Wnts have decreased BMD, while gain-of-function mutation of Lrp5 is associated with increased BMD in rodents and human. Secreted inhibitors of Wnt such as Dickkopf-1 (Dkk-1) and sclerostin (Scl) bind to co-receptors Lrp5/6 and inhibit Wnts from association with Lrp5/6, while secreted Frizzled-related proteins such as cerberus and Wif-1 directly interact with Wnts and Frizzled receptors to interrupt binding of Wnts to Lrp5/6. Scientific evidence has shown that overexpression of Scl induces lower BMD via lower bone formation, while deletion of Scl induces higher BMD via higher bone formation in mice. Higher BMD also occurs in humans lacking Scl caused by the mutation in *SOST* gene. These results support the conclusion that inhibition of Scl promotes bone formation and increases BMD. Therefore, a monoclonal antibody targeting Scl may be

an attractive therapeutic agent for the treatment of skeletal disorders such as osteoporosis and bone healing. We have performed a series of preclinical studies and demonstrated that sclerostin antibody (Scl-Ab) increases bone formation, decreases bone resorption, and increases BMD and bone strength in animal models (mouse, rat and nonhuman primate) of osteoporosis and fracture healing. This presentation will summarize the effects of Scl-Ab in treatment of conditions associated with low bone mass (that is, osteoporosis) and bone regeneration (that is, bone fracture repair).

HT3
Bone Adaptation Induced by Mechano-intervention—from Phenomenon to Mechanism

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Mechanotransduction has demonstrated potentials for tissue adaptation *in vivo* and *in vitro*. It is well documented that ultrasound, as a mechanical signal, can produce a wide variety of biological effects *in vitro* and *in vivo*.[1] As an example, acoustic-induced radiation force, for example, pulsed ultrasound, can be used to accelerate the rate of bone fracture healing noninvasively. Although a wide range of studies have been done, mechanism for this mechanical effect on bone regeneration is unknown and still under active investigation. A potential mechanism by which bone cells may sense ultrasound is through deformation and acoustic streaming of bone cells and their surface structures, which can be visualized in osteoblasts.[2] The purpose of this study was to (1) develop a methodology to allow for *in-vitro* manipulating osteoblastic cells using acoustic radiation force generated by ultrasound, (2) use this methodology to determine the morphological and biological responses of bone cells to ultrasound and (3) mitigate bone loss under estrogen-deficient osteopenia.

The developed methodology allowed manipulation of MC3T3-E1 cells by acoustic radiation force. The most contribution of acoustic streaming could be easily blocked by the cone coupled with transducer. The acoustic radiation force has a linear relationship with the power of ultrasound in this spherical transducer according to the formula, $F=W\times\cos\alpha/C$ (F, radiation force; W, power; C, speed of ultrasound in medium; α is half of the internal hole aperture angle of the spherical zone transducer). The deformation of cell membranes was observed by the US manipulation, which appeared after 15 s treatment of pulsed ultrasound in 6 W. We also imaged the movement of primary cilia, which had a relatively simple linear morphology and less than 10 μm length, attaching to the cell membrane. Cilia showed corresponding movement when subjected to pulsed ultrasound.

Ultrasound treatment also significantly increased BVF compared with OVX controls for the 100 mW cm^{-2} treated group. Additionally, SMI and Tb.N showed significant improvements compared with OVX for the 100 mW cm^{-2} treated group, and Tb.Th was significantly improved in the 30 and 100 mW cm^{-2} treated groups. Moreover, improvements in bone's microstructure with 100 mW cm^{-2} US translated into significant improvements in apparent Elastic Modulus.

These findings support the hypothesis that dynamic acoustic signals can inhibit bone loss and preserve bone strength under conditions of estrogen-deficient osteopenia. This study also suggests that there exists a minimum intensity threshold below which the stimulation is less effective at maintaining bone's microstructural and mechanical characteristics.

References

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HT4
Glucocorticoids and Bone, Few Surprise Findings

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Glucocorticoids (GCs) are widely used for their potent immunomodulatory actions, but diabetes, obesity and osteoporosis are serious side effects of long-term exogenous GC therapy. The catabolic effects of supraphysiological levels of GC on bone are in direct contrast to anabolic actions of endogenous GC at physiological levels.

Using a transgenic model (Col2.3-11βHSD2-tg) in which GC signaling disrupted exclusively in osteoblast/osteocyte, we found that endogenous GC act through the osteoblast/osteocyte to direct mesenchymal lineage commitment away from adipogenesis or cartilage toward osteoblastogenesis. Of note, GCs appear to regulate osteoblastic Wnt expression in a concentration-dependent biphasic mode, which may in part explain the apparently paradox anabolic and catabolic effects of GC on bone.

Surprisingly, we found that endogenous GCs, through the osteoblast, promote inflammation in a mouse model of autoimmune arthritis, a finding that challenges our traditional concept of the ubiquitous anti-inflammatory actions of GC.

Treatment of Col2.3-11βHSD2-tg mice with pharmacological doses of GC revealed that the bone loss effects of exogenous GC are mediated via the osteoblast.

Taken together, endogenous and exogenous GCs often act in contrasting ways, depending on their concentrations and the target cell. In bone, osteoblasts are the primary target for both the catabolic and anabolic effects of endogenous and exogenous GCs.

HT5
Stem Cells Research and Tissue Engineering Applications

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Stem cell research contributes to a fundamental understanding of how organisms develop and grow, and how tissues are maintained throughout adult life. This is the knowledge that is required to work out what goes wrong during disease and injury and ultimately how these conditions might be treated. The development of a range of human tissue-specific and

embryonic stem cell lines will provide researchers with the tools to model disease, test drugs and develop increasingly effective therapies.

Replacing diseased cells with healthy cells, a process called cell therapy, is a promising use of stem cells in the treatment of disease. Currently, researchers are investigating the use of adult, fetal and embryonic stem cells as a resource for various, specialized cell types, such as nerve cells, muscle cells, blood cells and skin cells that can be used to treat various diseases. Mesenchymal stem cells (MSCs) combined with biomaterials to replace or regenerate damaged or degenerative tissues are termed as tissue engineering.

The issues of how to select MSCs source; what are the differences of MSCs from difference sources; how to expend MSCs in culture and bioreactors; the use of allogenic MSCs; how to deliver MSCs (local vs systemic) and tissue engineering applications will be discussed. Stem cell applications that extended to gene therapy and targeted gene delivery for treating specific diseases such as cancer and immune diseases will also be discussed.