Novel therapeutics to promote bone formation in skeletal diseases

Professor Jae-Hyuck Shim is based at the University of Massachusetts Medical School, where his research focuses on novel molecular pathways that regulate bone turnover and, ultimately, how we can harness these to combat diseases such as osteoporosis and inflammation-induced bone loss. A major interest of the laboratory is how pro-inflammatory cytokines or osteoblast/osteoclast coupling factors can influence skeletal development, homeostasis and remodeling in pathological conditions.

Bone is constantly being destroyed and re-formed by cells called osteoclasts and osteoblasts, respectively. This continuous renewal process allows mechanical imperfections to be repaired and calcium homeostasis maintained. It is this process, called remodeling, which is the focus of Professor Jae-Hyuck Shim’s research. The group of cells responsible for bone remodeling are referred to as a basic multicellular unit (BMU). Osteoclasts and osteoblasts, however, are not simultaneously present on the bone surface. The BMU exists in different forms at the same location in human bone over approximately six months. There are four main classes of osteoclast-derived factors that may promote bone formation in the BMU. These factors provide potential targets for research groups such as Professor Jae-Hyuck Shim’s. The first are bone matrix derived signals which are released during bone resorption, the second are factors released by the mature osteoclasts themselves. Thirdly, factors may be expressed on the osteoclast membrane and finally, topographical changes effected by the osteoclast on the surface of the bone. Due to the close relationship between osteoclasts and osteoblasts, the coupling activity can make it hard to target one cell type without affecting the other.

The balance between bone degradation and replacement is crucial to maintain skeletal mass. When this balance is lost, low bone mass can occur, as seen in aging and chronic inflammation. It can also lead to the development of diseases such as osteoporosis and inflammation-induced bone loss. Osteoporosis affects approximately ten million people in the US over the age of 50, with around 1.5 million suffering from osteoporosis-related fractures each year. Unfortunately, the prognosis for patients who suffer these kinds of fractures can be poor; approximately 20% of patients with osteoporosis who suffer a hip fracture will die within a year. Similarly, patients with inflammatory arthritis develop focal articular erosions and systemic bone loss, resulting in osteopenia/osteoporosis. New approaches are needed to address the bone manifestations of inflammatory arthritis for approximately 1.3 million Americans with rheumatoid arthritis because disease modifying agents are inadequate to fully prevent systemic bone loss. Whilst there are some therapeutic options already available, it is crucial that further novel therapeutic strategies are developed. Some existing options focus on inhibiting bone resorption, however many are associated with a variety of undesirable side effects. For example, some therapies that increase bone formation,
such as teriparatide, also increase bone resorption and a risk in bone tumour, and treatments that block bone resorption such as bisphosphonates, arrest new bone formation along with atypical fractures and osteonecrosis of the jaw.

**NOVEL GENE THERAPIES**

Extracellular cues are crucial for the body to adjust the rate of bone formation. An example of one such cue is the WNT/T-cell cadherin pathway, which is well established as a positive regulator of osteoblast differentiation. A possible novel regulator involved in bone formation is adaptor protein Schnurri-3 (SHN3). Previous work done by Professor Shim has shown that deletion of SHN3 in adult mice resulted in a high-bone phenotype, resulting from increased osteoblast activity. He proposes that the mechanism by which SHN3 achieves this is through downstream effects on WNT signalling. SHN3 may therefore offer a promising therapeutic through promotion of bone formation. Small molecules with the ability to reduce SHN3 gene expression, or to inhibit its activity may have the potential to increase bone mass by increasing anabolic bone formation.

On the opposite side, charged multivalent β5 (CHMP5) acts on osteoclasts to dampen the signals that lead to bone resorption, while also controlling bone formation by osteoblasts. Osteoclast-specific deletion of CHMP5 may allow increased bone formation, providing an additional option for treating osteoporosis via a slightly different pathway. Earlier studies done by Professor Shim showed that, aside from its bone remodelling association, CHMP5 is also important for lysosomal biogenesis and regulation of receptor signalling during embryonic development.

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**How did you first become interested in bone remodelling?**

During my MS and PhD degrees, I studied the molecular pathways that control innate and adaptive immune responses. However, during my postdoctoral training in Dr Laurie Glimcher’s laboratory at Harvard University, I took advantage of the premise that tissues emerging from similar points during vertebrate evolution may share common intracellular signalling networks to guide their activity. I sought to leverage our knowledge obtained from the immune system to understand how bone cells are regulated. Since then, my research has focused on understanding the cross-talk between skeletal system and immune responses.

**Given the obvious physiological differences between humans and mice, how translatable do you think these findings are?**

If the regulators that we’ve identified play crucial roles in both mouse and human bone cells and their functions are conserved in both species (highly likely), then additional work may be potential targets for gene therapy. Gene therapy can correct the genes with noxious mutations (gene editing), silence expression of undesirable genes (gene silencing), or express therapeutic materials (gene addition) as currently used in human clinical trials. Additionally, the high efficiency of transduction, persistent gene expression, and lack of post-infection pathogenicity make aden-associated viruses (AAVs) attractive viral vectors for gene therapy. To date, AAV vectors have been evaluated in over 100 clinical trials worldwide. Nano-gold particles are also attractive carriers for gene therapeutics due to their low toxicity and high efficiency to tissue-targeting.

**What steps must be taken before therapies such as these can be trialled in patients?**

Once the ability of our therapeutics to promote bone formation is validated in mouse models of osteoporosis or inflammation-induced bone loss, we will examine whether they have the same functional efficacy in human bone cells, including osteoblasts and osteoclasts. Additionally, preclinical proof-of-concept studies will be conducted in large animals (i.e., nonhuman primates). To allow for translation of the effect of our therapeutics on mice to human subjects in gene therapy clinical trials, a large animal model closer to human is required for extrapolation of more relevant doses per injection site and per kg body weight.

Both SHN3 (also known as human immunodeficiency virus type 1 enhancer binding protein 3) and CHMP5 are well known to play a role in immune inflammation. Are there any effects on the immune systems of the SHN3 or CHMP5 deficient mice? As I mentioned previously, SHN3 was originally identified as a suppressor of pro-inflammatory (TNF)-induced pathway in immune cells (macrophages), and a follow-up study showed that SHN3 deletion in vitro leads to a positive role in CD4+ T cell activation. However, in vivo studies using germeline knock out (KO) and conditional KO mice demonstrated that SHN3 deletion is dispensable of these pathways, but augments osteoblast differentiation. These data implicate that in vivo function of SHN3 is intrinsic to osteoblasts. In contrast, CHMP5 plays critical roles in immune cells. Using CHMP5 conditional KO mice crossed with CD4-cre mice (T cell-deletion) or CD19-cre mice (B cell-deletion), we demonstrated that CHMP5 deletion in T cell precursors or in B cell precursors impaired the development of T cell or B cell, respectively. These data suggest that CHMP5 is important for both skeletal and immune systems.

**What is next for your research?**

Our laboratory has been developing a platform that translates basic scientific findings to drug development in order to treat human diseases with low bone mass, particularly osteoporosis and inflammation-induced bone loss. To this end, first, using a high-throughput screening and searching for key regulators with shared functions in skeletal and immune systems, we will identify novel key regulators important for bone remodelling, and test whether manipulation of their expression can promote bone formation or mouse models of osteoporosis or inflammation-induced bone loss. Second, since AAVs are already in use in human clinical trials, once proof of concept studies of the genes that we identified are validated, they will be applied to human bone cells, including osteoblasts and osteoclasts. Finally, if proof-of-concept studies of these gene therapeutics will be performed in mice and large animals (i.e., nonhuman primates).

**Do treatments that block bone resorption have a potent therapeutic effect on neuronal diseases and on the degenerative heart?**

The effects of treatments that block bone resorption are mainly on bone mass. However, treatments that block bone resorption may also have other therapeutic effects. For example, treatments that block bone resorption may have beneficial effects on the degenerative heart. Use of the therapeutic approaches described above can be used as a therapeutic approach for patients suffering with low bone density disorders.