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Orthopedic gene therapy

# Building bones

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Promising animal studies have raised hopes of an effective gene therapy to enhance bone regeneration, but clinical trials using recombinat proteins have been less impressive. However, in last September's *Journal of Clinical Investigation*, Hairong Peng and his co-workers reported exciting new results that could pave the way to success in the clinic.

Each year approximately 2 million patients worldwide undergo bone graft operations to repair skeletal defects resulting from trauma, tumor resection or to enhance the healing of a spinal fusion.[1] Bone grafting, however, has its problems. Patients can lose blood and experience pain. They also risk infection, gait disturbance and nerve or arterial injury.[2] Thus, strategies to enhance bone regeneration without having to borrow the patient's own tissue is an important goal of modern-day skeletal research.

Like blood and liver, bone has true regenerative capacity: when bone is injured or lost, it heals with the formation of new bone unlike most other tissues which simply form scar. Bone morphogenetic protein (BMP), a growth factor in the extracellular matrix of bone that can induce bone formation, is likely to be a key effector in the regeneration process.[3]

BMP comprises a family of isoforms, many of which have been sequenced, cloned and shown to enhance skeletal repair when implanted or expressed in animals.[4–6] Yet while the findings in animal studies have been impressive, clinical trials, although promising, have not achieved comparable results.[7,8] Reasons for this are unclear but may relate to the need for improved methods of growth factor delivery, a requirement for multiple factors introduced as 'cocktails' with simultaneous or sequential activity, or the lack of sufficient numbers of responding cells at the site of implantation in the patient.

Peng *et al*'s[9] new study begins to address these concerns. Their model system was a six-millimeter diameter defect in the parietal bone of mice. They monitored healing in this defect with both radiology and histology. Their approach was to use *ex vivo* gene therapy with muscle-derived stem cells genetically engineered to express human BMP-4 and vascular endothelial growth factor (VEGF). They showed that bone formation elicited by BMP-4 was significantly enhanced when combined with VEGF.

As with all other studies in which BMPs have been used to enhance skeletal repair, endochondral ossification played a key role. In this process, undifferentiated mesenchymal cells are recruited to the site of the defect; cartilage forms and gradually is calcified. Cartilage is then removed by resorption and replaced with bone. The newly deposited bone is then remodelled to form a structure capable of supporting mechanical loads.

Peng and co-workers showed that VEGF and BMP-4 act synergistically to enhance both bone formation and healing. In addition to its effects on enhancing angiogenesis and accelerating cartilage resorption, VEGF in the presence of BMP-4 promoted recruitment of mesenchymal stem cells to sites of bone formation and enhanced cell survival. Notably, these results were critically dependent on the ratio of VEGF to BMP-4, as improper proportions led to detrimental effects on healing, and VEGF alone did not enhance bone formation. Moreover, they showed that expression of Flt1 (the soluble antagonist of VEGF), inhibited bone formation elicited by BMP-4.

This study has important implications for the genetic engineering of bone. It points the way to possible strategies for addressing limitations of the use of BMPs and other growth factors in the restoration of human skeletal defects. For example, the ability to incorporate multiple genes expressing a variety of factors in varying concentrations opens numerous therapeutic possibilities. The new work also opens up the possibility that healing responses to growth factor treatment could be enhanced by titrating optimum ratios of expression. To do this using recombinant proteins would involve multiple hit or miss experiments, and the production of these proteins would be laborious, time-consuming and expensive.

Finally, the enhanced mesenchymal stem cell recruitment and cell survival that Peng and co-workers found suggest that it may be possible to prime the host environment by increasing the number of cells available to give a robust bone formation response. Such a strategy could address one of the major limitations of moving from lower mammals to humans. Other intriguing aspects of this report include the effects of combined VEGF and BMP-4 expression on skeletal tissue resorption. The removal of cartilage and its replacement by bone may be an important restriction point during the process of endochondral bone formation. Therefore, the enhancement of this transition may promote and accelerate healing.

This work is exciting and offers new possibilities for regenerative musculoskeletal medicine. However, there are limitations and while the list may not be long, they are very familiar to most investigators in the field. The most obvious, of course, is that these studies were conducted in mice. The history of bone regeneration research has shown that exciting results in animals become somewhat less exciting as they climb the phylogenetic tree. Then, of course, there are all of the concerns associated with the safety of gene therapy. Indeed, while risk–benefit ratios may make sense when applied to life-threatening genetic diseases, skeletal defects, while often-times limb threatening, may not carry the weight necessary to tip the balance in the beneficial direction.

These limitations aside, this work advances our understanding of skeletal regeneration and should lead to new experiments to test these and other combinations of growth factors. Other BMPs such as BMPs-2, 3, 6, 7 and 9 may be as, or even more osteogenic than BMP-4.[10] Moreover, new vectors for gene therapy which are both safe and effective in humans may bring this technology to the front of the musculoskeletal research agenda. In an aging society that is increasingly more active and prone to skeletal injuries, it is becoming even more important that we get novel approaches like these out of the lab and into the clinic. ■

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