

# Gene transfer, immunomodulation, and bone healing

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## Chapter 8

### **Impact**

## **Main objectives and results**

When a person breaks a bone they normally expect it to heal by itself. Indeed, this is the usual outcome. But under certain conditions bones fail to heal, which creates major problems for both the patient and the doctors who treat them. The main objective of the work described in this thesis was to undertake research into bone healing in rats, in order to develop new techniques that could be used in the future to improve bone healing in humans.

The research centered on a protein called bone morphogenetic protein-2 (BMP-2). This protein has the ability to promote bone regeneration and is already used by orthopedic surgeons to heal broken bones. However, its potency is weak; it is also very expensive and it is used in a way that causes side effects. Some of these side effects are serious. It is likely that these disadvantages reflect the way in which BMP-2 is administered to the patient, particularly the very high doses that are placed into the fracture site.

Like other proteins in the body, BMP-2 is made by cells using a specific gene that directs its synthesis. Instead of placing BMP-2 protein into the fractured bone, this thesis explored the alternative approach of placing the gene that instructs the cells to make BMP-2 for themselves. In this way the broken bone makes its own medicine in a natural fashion that is likely to be more effective and cause less side effects. This is an example of how gene therapy can be used to heal bones.

Gene therapy often uses viruses to transfer genes into cells. For many of the experiments described in this thesis, we used an adenovirus to do this. This virus was used in conjunction with stem cells that are able to become bone cells under the influence of BMP-2. In one set of experiments the virus was used to transfer the BMP-2 gene into pieces of muscle, which has many stem cells. In another set of experiments, the virus was used to transfer the gene into stem cells derived from fat. In both cases, the effectiveness of the approach was tested in rats where a piece of bone had been removed from the thigh bone, creating a defect that will not heal by itself. Both of these approaches showed promise but were not completely effective. We speculated that the rat's immune system was limiting the success of these procedures. So we repeated them, this time including a drug called FK506 that is already used clinically to prevent the rejection of transplanted organs. Indeed, FK506 greatly improved the ability of the gene therapy to induce bone healing. In a further approach to improving the effectiveness of our gene therapy, we inhibited a molecule called BRD4 to enhance the potency of the adenovirus we used and indeed found that it enabled the cells to make even more BMP-2.

While performing these experiments we noticed something strange; the best healing results were not associated with the highest level and longest duration of BMP-2 gene activity, as we had expected. Instead

we observed efficient healing when only a small amount of BMP-2 was made by the genetically-modified cells for a few days. This led to a major technological advance.

When a gene directs a cell to make a particular protein it does so via an intermediary known as messenger RNA (mRNA). When mRNA is introduced into cells they make the relevant protein, in this case BMP-2, very efficiently for a short period of time. Based on our previous data, it seemed that mRNA- directed BMP-2 production should be able to heal bones effectively without all the complexities of gene therapy using viruses. We tested this in our rat model using BMP-2 mRNA that had been chemically modified to enhance its stability, improve its efficiency and make it less inflammatory. This material proved extremely effective in healing rat bones, setting the stage for its further development towards human clinical trials.

### **Relevance to the scientific and social sectors**

Gene therapy generated much scientific interest in the second half of the twentieth century, continuing into present times. The potential of introducing DNA molecules as a cure for genetic conditions with a missing or faulty single gene was a groundbreaking concept. The field has expanded subsequently to include the use of gene therapy techniques for treating non-genetic conditions. Efficacy and safety are, possibly, the main concerns driving the field of gene therapy. This was put in evidence after the first death of a patient receiving a gene therapy therapeutic. The death of Jesse Gelsinger in 1999 was a pivoting point for gene therapy, where safety became fundamental to every project.

As it happens, this first death occurred after administration of an adenovirus gene therapy vector, similar to the one used for the studies of this thesis. The death of Jesse Gelsinger was linked to using very high doses of adenovirus delivered directly into the major blood vessel feeding the liver. This resulted in strong and uncontrollable inflammation known as a “cytokine storm” that led to the patient’s demise. This is not a risk in the bone healing strategies described here because we use a million-fold less virus which is placed locally in the bone defect rather than being targeted to a vital organ such as the liver. The results presented in this thesis contribute not only to the field of bone healing research, but to field of gene therapy research in general as well. The findings that donor, genetically modified cells persist 8 weeks after treatment in various forms, coupled with the role played by the immune suppressor FK506, are of relevance to the broader scientific field of gene therapy. The need for reducing the adenovirus dose, as proposed by our studies into the BRD4 molecule, and using alternative vectors such as chemically modified mRNA, are also of great relevance.

These results offer insights and potential avenues to research groups for future experimental designs. Knowing that genetically modified cells function as both the delivery vehicle and effector in bone healing applications, should prompt new studies to consider the use of cells that are less likely to elicit an immune

response after inflammation, such as cells obtained from the same patient (autologous). Our results show that short periods of immune suppression can be beneficial to achieve full healing of the bone defects.

An important breakthrough from our research, is the demonstration that mRNA has the ability to heal bone defects. This is of great importance because it allows for the use of a non-viral gene therapy vector, which has the potential to be safer and have a more streamlined pathway to the clinic. The use of mRNA in our studies enjoyed the benefits of BMP-2 gene therapy over the commercially available BMP-2 protein, mainly a drastically lower concentration of BMP-2 needed to heal the defect, and a bone regenerate of better quality. The prospect of mRNA for bone healing allows for an off-the-shelf therapy for orthopedic surgeons, with an improved safety profile over both viral gene therapy vectors and the commercially available BMP-2 protein, and at lower cost.

Bone healing complications represent a challenging scenario for both medical staff and society. Non-healed fractures represent a significant form of disability for patients. Interventions needed to treat these complications are costly, and so is the use of the commercial version of BMP-2 protein. While gene therapy using viral vectors represents a promising solution, the regulatory paths to approve these are complicated. Additionally, viral vector production is costly, requiring highly specialized facilities for production and product testing, making these therapies very expensive and potentially unaffordable to most health systems around the world. Thus, strategies such as BRD4 inactivation to lower the adenovirus dose or the use of mRNA, represent important discoveries.

Since we began our studies, mRNA became widely available and approved for use all around the world in the form of vaccines to the SARS-CoV-2 disease of the COVID-19 pandemic. The use of mRNA has been shown to be safe after billions of inoculations, and of almost equal importance, cost-effective. It is therefore that a potential therapy for bone healing complications in the form of mRNA, represents not only a viable regulatory pathway, but also has the potential to be widely adopted.

The results of our research presented in this thesis have been submitted and published by well-known, peer-reviewed scientific journals. These include *Translational Research*, *Molecular Therapy*, *JBMR Plus*, *Science Advances* and *European Cells and Materials*. Moreover, all of these publications are available to anyone interested through Pubmed Central, the free, full-text archive of the United States National Institutes of Health's library of medicine. This ensures that the information presented in this research is readily available with widespread dissemination, for anyone interested in the fields of gene therapy and/or bone healing. The results have also been presented at several distinguished scientific conferences, such as the *Orthopedic Research Society Annual Meeting* and the *Tissue Engineering and Regenerative Medicine International Society*. This dissemination of scientific knowledge is geared towards a vast audience of

orthopedic and gene therapy researchers, orthopedic surgeons, and members of the private industry, all of which have a vested interest in the advancement of bone healing therapies, and/or gene therapy.