Foreword: A Short History of Bone and Embryonic Induction

The repair of bone fractures provides one of the best examples of tissue regeneration in adult humans. It is of great medical interest to repair bone in those cases of fractures that fail to heal or in wounds involving large bone defects. Orthopedic surgeons have been interested in the biology of bone regeneration for many decades, as explained in this timely book that relates the unexpected discovery by Ugo Ripamonti of a central role of transforming growth factor-βs (TGF-βs) in bone regeneration. Remarkably, these novel insights in the treatment of bone fractures were achieved by a research team in faraway South Africa, under the blue skies of the Witwatersrand plateau near Johannesburg. They used similarities between human biology and the baboon to make discoveries that would not have been possible in other mammalian models.

The TGF-β superfamily constitutes the largest family of growth factors in humans, with a total of 33 different secreted ligands. They are commonly divided into the bone morphogenetic protein (BMP) and the TGF-β branches. Of these, the TGF-β themselves (TGF-β1 to -3) are the most recent evolutionary acquisitions. This family of ligands is not solely of interest in orthopedic medicine, but has proven of enormous importance during the signaling that takes place in the early embryo,
in which a single cell, the fertilized egg, is converted into a body plan containing many tissue types. Curiously, it was the search for agents that repair fractures that led to the isolation of many of the diffusible substances that control embryonic development.

The story told in this book starts in 1938 with the publication by Gustave Levander, from Uppsala, Sweden, of a study of bone regeneration. He showed that the regenerating callus of long bones could be cut into tiny fragments, fixed in ethanol, stored for several days, diluted to 40% with water, and injected into the thigh muscles of rabbits. He made the astonishing finding that these dead tissues were able to induce bone and cartilage from muscle mesenchyme in 22% of the cases.

In his paper, Levander made the intellectual connection between the induction of bone in mesenchyme and the studies on embryonic development by Hans Spemann of Freiburg University, who had recently received the 1935 Nobel Prize for Medicine. Spemann and his student Hilde Mangold had shown that a region of the gastrula embryo, called the organizer, was able to change the differentiation of neighboring cells into different tissue types. He called this process embryonic induction. During the 1930s, many attempts were made to isolate the mysterious inducing substances from embryos, but all met with failure. It was the work on bone that was to lead the way in the isolation of embryonic inducers. Levander concluded his paper (1938) in this way: “On the basis of these experiments the author holds that bone regeneration takes place as the result of some specific bone forming substance activating the non-specific mesenchymal tissue. The theory also agrees with the views advanced by Spemann with regard to embryonic development.”

One can only stand in awe of the prescience of Levander’s insight, for it was only in the 1990s that Noggin and Chordin, two BMP antagonists secreted by organizer tissue, were found to mediate Spemann’s embryonic induction. Noggin (isolated by Richard Harland at the University of California–Berkeley) and Chordin (isolated by us at the University of California–Los Angeles) generate a gradient of BMP signaling that is maximal in the ventral side of the embryo. In fact, dorsal–ventral patterning has been found to be the ancestral function of BMPs in all bilateral animals. For example, in *Drosophila* the function of the morphogen Decapentaplegic (Dpp) can be replaced by human BMP-2 or BMP-4. Teleost fish co-opted this ancient dorsal–ventral signaling system to generate an evolutionarily novel tissue, bone.
The next key step in this saga was the discovery in 1965 by orthopedic surgeon Marshall Urist, of the University of California–Los Angeles, that the matrix of long bones decalcified by incubation in 0.6 N HCl over many days had very powerful bone-inducing activity. While dead bone fragments only generated a low proportion of bone induction cases after transplantation into abdominal muscle of rabbits, implanted bone matrix was effective in up to 90% of the cases. Furthermore, decalcified matrix could be used to bridge regeneration in long bone defects. He proposed the name BMP for this inducing substance, which much later was found to be a key morphogenetic protein in the embryo as well as in bone.

BMPs proved difficult to separate from the matrix until 1981, when Hari Reddi, then at the National Institutes of Health (Bethesda, Maryland), discovered that BMPs could be solubilized with 4 M guanidine–HCl and 8 M urea. The protein could now be purified biochemically, but in order to restore its bone-inducing activity, it was necessary to implant it together with BMP-depleted matrix. This assay eventually allowed Dr. Sampath at Creative Biomolecules, Hopkinton, Massachusetts, to purify osteogenic protein-1 (OP-1), also known as BMP-7. In 1988, a team from Genetics Institute headed by John Wozney was able to obtain highly purified BMP fractions that yielded protein sequences for BMP-2 to -7. This work revealed that BMPs were members of the TGF-β superfamily, which could now be produced in large amounts by recombinant DNA technology.

The availability of BMP proteins triggered herculean efforts by the biotech industry to apply BMPs in bone and dental regeneration. Surprisingly, so far the bone-inducing effects of BMPs in humans have yielded many disappointments as practical therapeutic agents. However, BMPs continue to have great promise in the treatment of many diseases, in particular now that it is known that BMP-6 circulates in blood. One of the main problems in the application of BMPs to bone fracture repair seems to be the induction of BMP antagonists such as Noggin by high concentrations of the growth factor in the target tissues.

It was therefore a great surprise when the Ripamonti team found that TGF-βs greatly cooperated with BMP, and were even able to induce bone on their own in the baboon system. While the expectation was that BMPs would be the main bone inducers, it may well be TGF-β that provides the therapeutic breakthrough. The BMP branch of the pathway signals by activating receptors that phosphorylate and activate transcription
factors known as Smad-1/5/8. The TGF-β branch of the pathway phosphorylates Smad-2/3 was considered completely independent of BMP. In fact, in many contexts, BMP and TGF-β oppose each other. One exception was the discovery by Peter ten Dijke in Holland that a BMP receptor can phosphorylate Smad-2 in addition to Smad-1, although with slower kinetics. There are seven type-1 receptors that must match with five type-2 receptors in order to transduce the entire spectrum of TGF-β superfamily ligands, so there is room for unexpected mixing and matching of signals. In addition, there are many opportunities for sequential induction of genes in the pathway, as indicated by the recent finding that recombinant human TGF-β3 upregulates endogenous BMP-2.

In sum, this book marks a contribution to the fascinating history of BMP and TGF-β research at the intersection of molecular biology, tissue induction, bone regeneration, and orthopedic surgery.

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