NEWS AND VIEWS / NUUS EN MENINGS

Molecules initiating bone differentiation: osteogenin and related bone morphogenetic proteins

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Research into the molecular initiators of bone differentiation aims to provide new therapeutic approaches for the correction of craniofacial and orthopaedic conditions and to bone diseases such as osteoporosis.

There is a direct relationship between growth and differentiation in early development and regeneration: postfoetal osteogenesis, such as fracture repair, may be considered to recapitulate events that occur in the normal course of embryonic bone development.[1] The remarkable potential for repair and regeneration of bone is well known. This has stimulated an increasing interest in the isolation of osteogenic factors, originally postulated by Lacroix,[2] present within the bone matrix. Additional impetus for the identification of molecules that are potential regulators of bone differentiation originated from the phenomenon of bone formation by induction:[3] demineralized bone matrix, when implanted extraskeletally in laboratory animals, induces a developmental cascade of biochemical and morphogenetic events culminating in the local differentiation of endochondral bone.[4,5]

Which are the molecular signals that initiate the cascade of bone differentiation? Identification of osteogenic proteins in mammalian bone has been a difficult task owing to the relative inaccessibility of small quantities of proteins tightly bound to the organic and inorganic components of the extracellular matrix of bone. The observation that demineralized bone matrix could be dissociatively extracted and inactivated with denaturants, and the osteogenic activity restored by reconstituting the inactive residue (mainly insoluble collagenous matrix) with solubilized protein fractions confirmed the existence of osteogenic proteins, and provided the starting point for their purification.[6] This has led to the identification of an entirely new family of protein initiators that induce cartilage and bone differentiation *in vivo*.[7-9] Osteogenin, a bone morphogenetic protein, has been isolated by heparin-affinity chromatography,[10] and recently purified to homogeneity from bovine bone matrix.[8] The amino acid sequence of bovine osteogenin is identical to the amino acid sequence deduced from the cDNA clones of one of the recently characterized human bone morphogenetic proteins (BMPs), BMP-3.[7,8] Bovine osteogenin, in conjunction with insoluble collagenous bone matrix, initiates the local differentiation of endochondral bone *in vivo*.[8,11] Osteogenin and related BMPs are members of the transforming growth factor-β (TGF-β) gene superfamily.[7,12] The TGF-β family includes five distinct forms of TGF-β, activins and inhibins, implicated in follicle-stimulating hormone release, the Mullerian inhibiting substance, the decapentaplegic (dpp) gene product of *Drosophila melanogaster*, the Vg-1 gene product of *Xenopus laevis*, and the Vg-1-related murine protein Vgr-1. Of the seven BMPs that have been characterized, six show sequence homologies with TGF-β molecules.[12] As gene products of the TGF-β superfamily, BMP-2 to 7 are synthesized as precursor polypeptide chains containing a hydrophobic leader sequence, a relatively poorly conserved N-terminal proregion of several hundred amino acids, and a shorter and highly conserved carboxy terminal region of 114 to 139 amino acids containing a characteristic cysteine motif.[12,13] In contrast to TGF-β molecules, however, none of the BMPs has been shown to form latent complexes.[13] The biologically active native osteogenin (BMP-3) and the human recombinant BMP-2A and BMP-2B (now known as BMP-2 and BMP-4) share limited homology with TGF-β molecules.[7,8,12,14] The carboxy termini of BMP-2 and BMP-4 are more closely related to the corresponding region of *Drosophila* ddp gene product than to any known member of the TGF-β family. BMP-1, the only BMP that is not a TGF-β family member, has multiple structural motifs, including an EGF-like domain,[7] and a region of similarity to a known protease.[15] Interestingly, the *Drosophila* tolloid (tld) gene product, involved in dorso-ventral patterning, contains at least three distinct sequence motifs also found in human BMP-1.[16] It is possible that the tld gene product and BMP-1 are proteases that cleave the precursor forms of ddp and the bone-inductive BMPs, respectively, liberating the mature (and active) carboxy termini.[7,16] This suggests that a phylogenetically ancient signalling process, used in dorso-ventral patterning in the fly, may also operate to produce a unique vertebrate trait such as bone differentiation.[16] Indeed, recent experiments indicate that, in addition to postfoetal osteogenesis, osteogenin (BMP-3) may play a role in embryonic skeletogenesis.[17] Furthermore, the temporally and spatially distinct patterns of BMP-2, BMP-4 and TGF-β type-2 expression in different populations of mesenchymal cells in the developing embryo suggest that BMPs may be involved in inductive events unrelated to bone induction that control pattern formation during embryonic development.[18-20]

The interaction of osteogenin with other extracellular matrix components, including type-IV collagen, is another fertile area for research.[21,22] Recent experiments are now providing a conceptual framework for the supramolecular assembly of the extracellular matrix of bone, as well as novel insights into the regulatory role of growth factors in the solid state. Therefore, type-IV collagen and other basement membrane components may function as a delivery system by sequestering both angiogenic and bone morphogenetic proteins.[21,24] This may initiate and promote angiogenesis and vascular invasion, a prerequisite for osteogenesis. The affinity of osteogenin for type-IV collagen helps to relate the bone matrix-induced bone differentiation to uroepithelial osteogenesis,[25] a phenomenon which we have also shown to occur in primates.[26]

A point of fundamental clinical importance is the potential therapeutic use of osteogenin as an initiator of osteogenesis in man. The bone-inductive potential of osteogenin and related BMPs is mainly based on work in rodents.[8,27] Information concerning bone induction in adult primates is a prerequisite for therapeutic applications in man.[28,29] Previous work in our laboratory has shown bone formation by induction in extraskeletal sites of adult baboons (*Papio ursinus*).[30,31] Ongoing collaboration with Dr Hari Reddi, a world authority on bone induction, formerly at the National Institute of Dental Research in Bethesda and now Director

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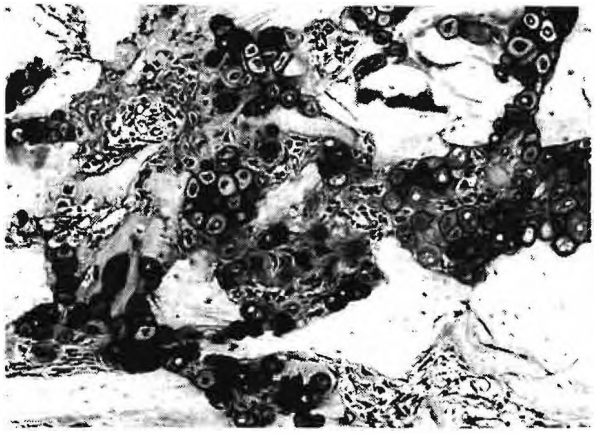

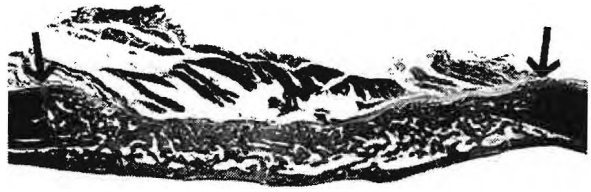

Fig. 1. (*left*) Cartilage and bone differentiation in the subcutaneous space of a rat 11 days after implantation of osteogenin purified from baboon bone matrix.
Fig. 2. (*top*) Complete osseous regeneration of a skull defect of an adult baboon after implantation of baboon osteogenin. Arrows indicate the original margins of the defect.

of the Laboratory of Musculoskeletal Cell Biology at the Johns Hopkins School of Medicine in Baltimore, has led to the isolation and purification to homogeneity of osteogenin from baboon bone matrix (Fig. 1).[32] Testing the efficacy of osteogenin in primates has stimulated our laboratory to create animal models using baboons[33–35] which share similar, if not identical, mechanisms of bone remodelling with man.[36] We have recently demonstrated that osteogenin can be used for the architectural reconstruction of the bone–bone marrow organ in skull defects of adult baboons (Fig. 2).[37] In our experiments, reconstitution with the insoluble collagenous matrix is a requirement for optimal delivery of osteogenin. However, the collagenous carrier, with its potential problems of antigenicity and viral contamination, may limit the widespread utilization of osteogenin and related BMPs as therapeutic agents. A major goal for reconstructive surgeons and molecular biologists alike is the formulation of inorganic non-immunogenic carriers with defined geometries capable of delivering osteogenin in the absence of the collagenous matrix.[38] This will result in the construction of osteogenic delivery systems with potential therapeutic use.[39,40]

Research into the molecular initiators of bone differentiation is at a very exciting stage because it will answer basic research questions that will help to formulate new therapeutic approaches to bone diseases, including osteoporosis. The correction of congenital and acquired craniofacial and orthopaedic conditions will require the combined use of several of the human recombinant BMPs delivered by appropriate carriers.[7] This prediction, however, needs to be evaluated experimentally in nonhuman primates, to test the efficacy of recombinant BMPs. In the end, it will be necessary to identify the mechanistic processes that regulate sequential gene expression of the different BMPs, their specificity on target cells and their receptor function. This will help to design therapeutic strategies based on the cell biology of matrix–cell interactions.

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