

A scientific basis for the biologic regeneration of synovial joints

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Temporomandibular joint disorders represent a large group of conditions involving a local or more generalized musculoskeletal disease process. The disorders have many features and many causes and may result in limited or more severe damage of the joint associated tissues including the disks, the articular surface, the underlying bone, and the ligamentous structures. As our understanding of the molecular processes guiding skeletal tissue formation progresses, new opportunities arise in the field of skeletal tissue and joint repair. Reconstruction of the temporomandibular joint by means of scientifically designed approaches will revolutionize surgical treatment modalities. (*Oral Surg Oral Med Oral Pathol Radiol Endod* 1997;83:167-9)

Temporomandibular disorders (TMD) are a heterogeneous group of musculoskeletal disorders. Relatively few are associated with joint tissue damage. Therefore indications for surgical interventions are limited. However, the use of less invasive surgical procedures for internal derangements and osteoarthritic joints has become more frequent, and so it is clear that better defined criteria for surgical interventions have to be established. Regardless of the clinical indications for temporomandibular joint (TMJ) surgery, it is our responsibility to develop scientifically based treatments and to avoid the trial-and-error approaches so characteristic of many medical interventions.

TISSUE REGENERATION RECAPITULATES TISSUE FORMATION

It is widely accepted and all the experimental data suggest that the molecular processes involving tissue regeneration recapitulate to a large extent the molecular cascade of events associated with tissue formation. Therefore there is a continuous exchange of information between developmental biologists and biomedical researchers, who focus on tissue repair and tissue engineering.

This concerted effort has been particularly strong in the field of the formation of skeletal tissues and skeletal repair. One of the major advances in this field has been the discovery of the bone morphogenetic proteins¹⁻³ (BMPs), a group of morphogens structurally belonging to the transforming growth Factor- β superfamily. These potent molecules were originally discovered because of their capacity to induce *in vivo* de novo cartilage and bone formation in an ectopic

site. Subsequently, it has been demonstrated that they are involved in many developmental processes, such as regulation of epithelial-mesenchymal interactions. In addition, recombinantly expressed BMPs in combination with a variety of carriers have been shown to heal craniofacial and periodontal lesions successfully in nonhuman primates.⁴

These data are a good example of the use of developmentally crucial signaling molecules in postnatal tissue repair. Successful tissue repair with minimal scar-tissue formation also involves other critical parameters such as the delivery vehicle, the extracellular environment, and the availability of responding cells. Recent advances in the understanding and use of precursor cell populations (stem cells, mesenchymal stem cells, bone marrow stromal cells, and progenitor cells) and the use of porous degradable synthetic polymers combined with tissue specific cells are additional and powerful approaches in the field of tissue engineering.⁵ It is likely that the ultimate repair protocol will be a combination of any of these components and will depend on the clinical indication, the individual patient, and the underlying disease processes.

JOINT MORPHOGENESIS

The TMJ is a diarthrodial joint, and its formation may involve most of the molecular events associated with the formation of the synovial joints of the appendicular skeleton. Most diarthrodial joints develop from a distinct mesenchymal cell population. The joint-forming process in limbs takes place between 5 and 8 weeks of human embryonic development and starts from a homogeneous interzone between the chondrifying bone primordia. This assembly of mesenchymal cells further evolves into a three-layered zone: two more densely organized layers surrounding the epiphyseal ends of the bones and a more loosely organized middle layer. The dense

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zones further differentiate into the cartilaginous layers at either end of the future joint in contact with the adjacent bone. A cavitation process takes place in the loose zone and leads to the formation of vacuoles that will ultimately coalesce to form the synovial cavity. There is no agreement and few data establishing the developmental origin of all of the joint-associated structures.

The morphogenesis of the TMJ has some unique characteristics. During human development the TMJ has two distinct stages: the primary and secondary TMJ.⁶ The primary early embryonic TMJ consists of the Meckel's cartilage and the first branchial arch ear ossicles. This primary TMJ is a mostly uniaxial hinge joint and is progressively replaced (after 16 weeks) by the secondary TMJ, the articulation between the mandibular condyle, and mandibular fossa of the temporal bone. The secondary TMJ serves as the diarthrodial joint throughout life. This secondary TMJ forms mostly between 7 and 11 weeks of gestation and develops from a developmental field of mesenchymal cells surrounding the upper posterior end of the developing bony mandible. This block of mesenchymal cells gives rise to most of the joint structures, including the articular surface, the joint capsule, ligaments, articular disk, the superior and inferior cavities, and the synovial linings. In contrast to most other synovial joints, the articular surfaces of the condyle and fossa remain covered by a thin fibrous layer of connective tissue cells. This tissue layer may be important in the protection and maintenance of the integrity of the articulating surfaces.

This information on joint development is still very descriptive. Few data have emerged with respect to the molecular basis of these developmental events. Our understanding of the signals and cells involved in these formation processes are critical. Recent work has identified at least some molecular participants apparently involved in the early stages of joint development and also important in the synovial joint postnatally.

CARTILAGE-DERIVED MORPHOGENETIC PROTEINS: ROLE IN SKELETAL DEVELOPMENT

We have identified a group of morphogens, designated cartilage-derived morphogenetic proteins^{7,8} (CDMPs) closely related to the BMPs. They were discovered by means of a reverse-transcription polymerase chain reaction with articular-cartilage messenger RNA as a template. This work evolved from earlier findings in our laboratory that cartilaginous tissues contain *in vivo* chondrogenic activity as assayed by a subcutaneous implantation assay, and this activity appeared to be distinct from the previ-

ously identified BMPs in bone matrix. CDMP-1, the human homologue of mouse growth differentiation factor-5,⁹ is expressed predominantly in the precartilaginous condensations of the developing limbs and subsequently in the epiphyseal cartilage of the cartilaginous cores. Most strikingly, CDMP-1 is also expressed in the interzone or future site of the synovial joints. The expression levels were the highest in the more distal parts of the limbs. This localization pattern suggests an important role for this gene in the development of the appendicular skeleton and joint morphogenesis.

The role of CDMP-1 as a regulator of skeletal growth has been confirmed by the presence of mutations in the gene in acromesomelic chondrodysplastic diseases. The Hunter-Thompson chondrodysplasia is an autosomal dominant disorder characterized by short limbs. The distal parts of the limbs are especially poorly developed. We have identified a 22-base-pair frameshift mutation in the mature region of *cdmp 1* that results in a complete loss of function of the growth factor.¹⁰ The corresponding clinical characteristics of affected persons show not only short limbs but also a joint phenotype with hypoplastic condyles and dislocations that may be associated with ligament laxity, although it is difficult to establish whether this feature is a primary or a secondary feature. In addition, several joints are missing, as is the case for the fifth medial phalanx in the hands and feet. These data have been expanded and new mutations found in the *cdmp 1* gene in other acromesomelic chondrodysplasias (J. T. Thomas et al., manuscript in preparation).

In these human phenotypes we could not see any major abnormalities in the sternoclavicular joint, acromioclavicular joint, or TMJ. This result suggests that the molecular signals guiding the formation of the TMJ may be distinct from those involved in appendicular joint morphogenesis. This possibility is not surprising because jaws and jaw joints preceded all other joints in evolution. Their developmental sequence was established before the appendicular joints and is therefore expected to be distinct. This area presents opportunities for further investigation because very little is known about TMJ morphogenesis at the molecular level.

CARTILAGE-DERIVED MORPHOGENETIC PROTEINS: ROLE IN SKELETAL AND JOINT REPAIR

The above-mentioned genetic studies reveal some information about the physiologic role of CDMP-1. However, they do not provide information about its mechanism of action, about the biologic activities of the CDMPs, or about the actions of these morphogens

in postnatal tissues. Preliminary studies using recombinantly expressed protein indicate that CDMP-1 protein is chondrogenic and osteogenic in vitro and in vivo. These biologic traits confirm the BMP-like nature of the protein and confirm the potential of CDMP-1 as an agent promoting cartilage and bone differentiation. Our data indicate that CDMP-2, the second member of this family, is expressed at high levels in postnatal cartilage tissues and that the third member of this family is also present in the ligamentous structures of the developing limbs. This finding suggests the possibility that cell- and tissue-specific differentiation can be directed. From this knowledge tissue-engineering applications, including new joint resurfacing protocols, may become possible and will include the development of appropriate delivery systems and enrichment with the proper precursor cells.

Recent studies with autologous articular chondrocytes, expanded in vitro, for the repair of local cartilage defects in the human knee joint surface support the concept of this tissue-engineering approach.¹¹ Although additional studies are necessary to evaluate the data, this protocol may provide the first evidence of the importance of the use of the proper cell population to repair tissues. The relative success of this study may be attributed at least in part to the use of the articular chondrocyte to repair the articular cartilage defects. Therefore it is important to identify further the molecular markers associated with specific cell types that would allow scientists and clinicians to repair tissue damage with the appropriate cells and to avoid scar formation to the greatest extent possible.

CONCLUSIONS

The identification and study of the molecular signals guiding tissue morphogenesis and tissue differentiation contribute to the development of new tissue regeneration protocols. The scientific information providing insight into the molecular basis of the development and biologic characteristics of the joint structures, including the TMJ, are still preliminary. The further development of tissue-specific markers (articular surface, bone, tendon, disk, and synovium) will greatly enhance our capabilities to use the proper precursor cells, morphogens, and delivery

systems in future repair protocols. It is possible that the biologic repair of tissues based on this new research will dramatically change surgical approaches in TMD.

REFERENCES

1. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, et al. Novel regulators of bone formation: molecular clones and activities. *Science* 1988;242:1528-34.
2. Luyten FP, Cunningham NS, Ma S, Muthukumaran N, Hammonds RG, Nevins WB, et al. Purification and partial amino acid sequence of osteogenin, a protein initiating bone cell differentiation. *J Biol Chem* 1989;264:13377-80.
3. Sampath TK, Coughlin JE, Whestone RM, Banach D, Corbett C, Opperman H, et al. Bovine osteogenic protein is composed of dimers of OP-1 and BMP-2A, two members of the transforming growth factor B superfamily. *J Biol Chem* 1990;265:13198-205.
4. Ripamonti U, Reddi AH. Bone morphogenetic proteins: applications in plastic and reconstructive surgery. *Plast Reconstr Surg* 1995;11:47-74.
5. Langer R, Vacanti JP. Tissue engineering. *Science* 1993;260:920-6.
6. Burdi AR. Morphogenesis. In: Sarnat BG, Laskin DM, editors. *The temporomandibular joint: a biological basis for clinical practice*. Philadelphia: WB Saunders, 1992:36-47.
7. Chang S, Hoang B, Thomas JT, Vukiceric S, Luyten FP, Rybo NJP, et al. Cartilage-derived morphogenetic proteins: new members of the TGF- β superfamily, predominantly expressed in long bones during human embryonic development. *J Biol Chem* 1994;269:28227-34.
8. Luyten FP. Cartilage-derived morphogenetic proteins: key regulators in chondrocyte differentiation? *Acta Ortho Scand* 1995;66:51-4.
9. Storm EE, Huynh TV, Copeland NG, Jenkins NA, Kingsley DM, Lee SJ. Limb alterations in brachypodism mice due to mutations in a new member of the TGF beta-superfamily. *Nature* 1994;368:639-43.
10. Thomas JT, Lin K, Nandedkar M, Camargo M, Cervenka J, Luyten FP. A human chondrodysplasia due to a mutation in a TGF- β superfamily member. *Nature Genetics* 1996;12:315-7.
11. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 1994;331:889-95.

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