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MOLECULES IN FOCUS

Cartilage-derived Morphogenetic Protein-1

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A new morphogenic secreted protein has been identified with direct evidence for its involvement in skeletal development and joint morphogenesis. Cartilage-derived morphogenetic protein-1 (*Cdmp*1) and its mouse homologue growth/differentiation factor 5 (*Gdf*5) were discovered independently using a degenerate PCR screen for bone morphogenetic protein-like genes. *Cdmp*1/*Gdf*5 belongs to the TGF- β superfamily, a large group of signaling molecules that are secreted as biologically active dimers with a carboxyl-terminal domain containing seven highly conserved cysteines. Its temporal and spatial expression pattern is mostly restricted to the developing appendicular skeleton. Genetic studies revealed that effective null mutations in the gene are associated with short limbs, *brachypodism* (*bp*) in mice and acromesomelic chondrodysplasia in humans. Recombinantly expressed protein initiates and promotes chondrogenesis and to a limited extent osteogenesis *in vitro* and *in vivo*. This makes this polypeptide a potential therapeutic agent in the regeneration of skeletal tissues. © **1997 Elsevier Science Ltd. All rights reserved**

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INTRODUCTION

The discovery of growth/differentiation factor 5 (*Gdf*5) was first reported through its association with *brachypodism* mice (Storm *et al.*, 1994). *Gdf*5 is a member of a novel subfamily of genes structurally related to the bone morphogenetic protein (BMP) family (for a review see Hogan, 1996). This new distinct subfamily of genes consists of three highly related members called *Gdf*5, *Gdf*6 and *Gdf*7. These genes were identified by homology screening using degenerate polymerase chain reaction (PCR). Chromosomal mapping of *Gdf*5 in mice revealed the proximity of the *brachypodism* (*bp*) locus. Brachypodism is typified by shortening of the limbs with the severity of long bone abnormalities in a proximal to distal direction. Sequencing of the *Gdf*5 gene in homozygote *bp* mice demonstrated the presence of effective null mutations (Storm *et al.*, 1994).

Shortly thereafter, two other groups reported independently the cloning of the human homologue (called, respectively, *Cdmp*1 and h*Gdf*5)(Chang *et al.*, 1994; Hötten *et al.*, 1994). Genetic mapping of *Cdmp*1 in mice showed its proximity to the *bp* locus, thereby confirming that *Cdmp*1 is indeed the human homologue of *Gdf*5 (Chang *et al.*, 1994).

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STRUCTURE

*Gdf*5/*Cdmp*1 is most closely related to the BMPs, members of the TGF-β superfamily. Like all members of this family, it is an intermolecular disulfide-bonded homodimeric or heterodimeric molecule. The core of the mature monomer has a conserved cystine knot stabilized by six intramolecular cysteine interactions (Fig. 1) (Venkatamaran *et al.*, 1995). The fourth of the seven highly conserved cysteines forms the intermolecular disulfide bond of the biologically active dimer.

SYNTHESIS AND DEGRADATION

The *Gdf*5/*Cdmp*1 gene product is assembled intracellularly as a large dimeric precursor, as observed for most BMPs. The dimerized precursor is subsequently cleaved at a consensus

RXXR (Arg, X, X, Arg) site, and the biologically active dimer is secreted. Two possible cleavage sites are present in the recognition site RRKRRA[382]. Primary sequencing of the *N*-terminus indicates that the position of the cleavage might be dependent on the cell type, the mature protein starting either with A[382] or with R[381] (Hötten *et al.*, 1996). The role of the pro-region is also speculative but may be involved in the proper assembly of homodimers or heterodimers, and the efficiency of processing and secretion of the mature dimer.

Transcripts of *Gdf5/Cdmp1* and location of the protein appear to be mostly restricted to elements of the appendicular skeleton. Although discrete differences have been observed across species (human, mouse and chick), transcripts are mainly detected during embryonic development in and around skeletal precursors. *Gdf5/Cdmp1* is expressed in precartilaginous mesenchymal condensations of the skeletal elements, in the perichondrium around the cartilaginous cores and, most strikingly, in the joint interzones (Chang *et al.*, 1994; Storm *et al.*, 1994, 1996). *Gdf5/Cdmp1* is therefore one of the only known molecular markers for early joint formation. Postnatally, low level expression has been seen in a variety of tissues including cartilage, brain and placenta, but the functional significance of this is unclear (Storm *et al.*, 1994; Chang *et al.*, 1994; Kriegstein *et al.*, 1995).

BIOLOGICAL FUNCTIONS

The role of *Gdf5/Cdmp1* in the developing appendicular skeleton has been confirmed by the presence of null mutations in the gene in *bp* mice (Storm *et al.*, 1994) and in the human acromesomelic chondrodysplastic diseases, Hunter–Thompson type (Thomas *et al.*, 1996) and Grebe type (Thomas *et al.*, 1997). The skeletal disorders are characterized by short limbs, altered number and shape of bones with no abnormalities of the axial or craniofacial skeleton. In particular, the distal parts of the limbs are poorly developed and several peripheral phalangeal joints are missing. Larger joints, such as knee and hip, are also affected with hypoplastic condyles and dislocations. These findings support a critical role for *Gdf5/Cdmp1* in the formation of diarthrodal joints. Extensive investigation of affected individuals has not revealed other abnormalities in any other organ systems. Human chromosomal mapping assigned *Cdmp1* to chromosome 20q11.2.

As it is a BMP family member, it is anticipated that GDF-5/CDMP-1 will share a

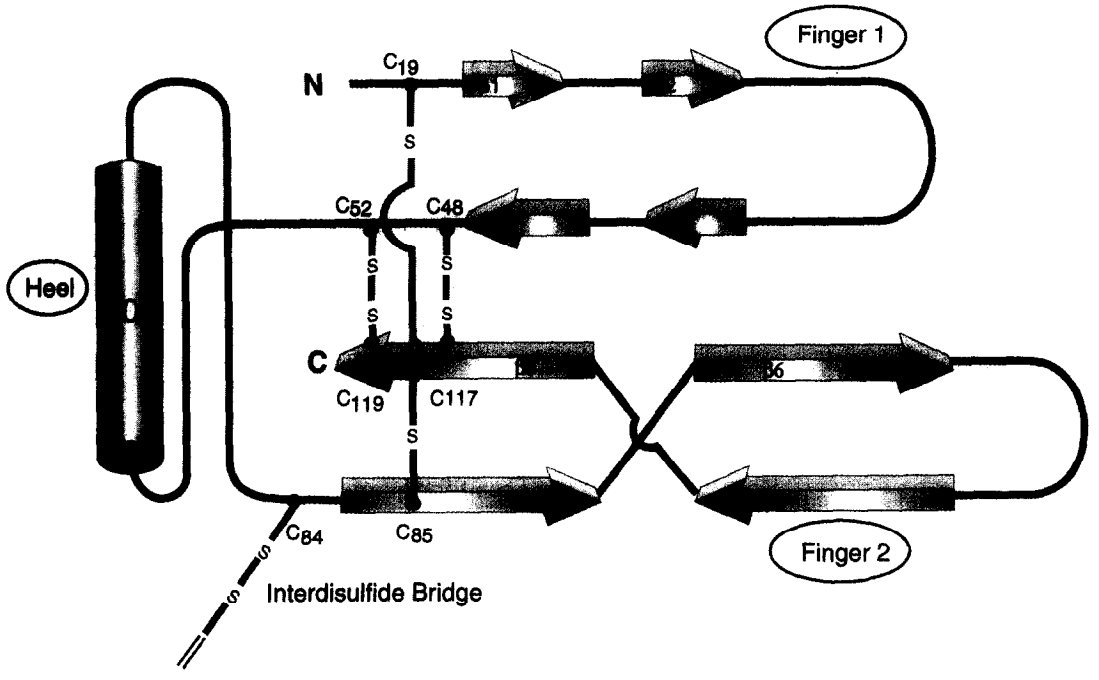

Fig. 1. Schematic diagram of the structure of the GDF-5/CDMP-1 monomer, based on its analogy to BMP-like molecules. N and C correspond to *N*- and *C*-terminal. The conserved cysteine residues forming intramolecular disulfide linkages are indicated. The fourth cysteine (at position 84) forms the intermolecular disulfide bridge. α, alpha helix; β1–8, beta sheets.

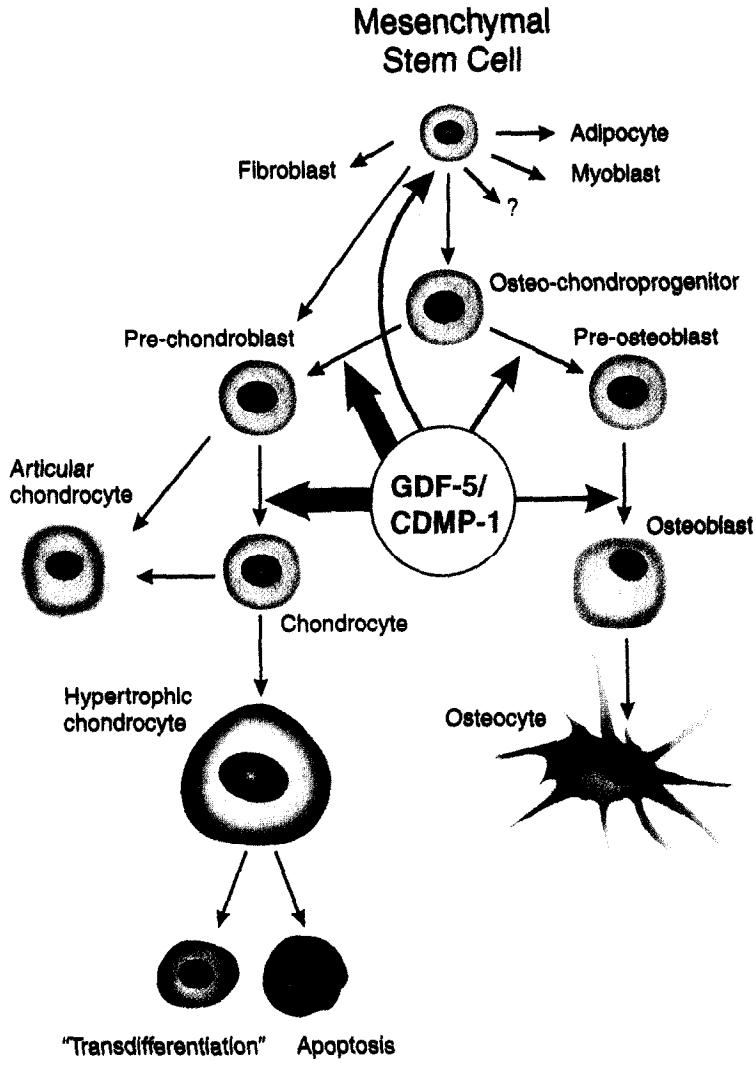

Fig. 2. Effects of GDF-5/CDMP-1 on the progression of the chondrogenic and osteogenic lineage.

number of biological activities described previously for other BMPs. However, its restricted expression pattern suggests a more limited range of biological functions. *In vitro* studies using recombinant protein indicate that GDF-5/CDMP-1 promotes aggregation of mesenchymal cells and chondrogenesis (Hötten *et al.*, 1996; Erlacher *et al.*, in press), thereby providing possible mechanistic insights into its mode of action in early skeletal development. The *in vitro* data are suggestive of its preferential stimulation and promotion of chondrogenesis, although using a variety of cell lines and in serum free cultures we also found some stimulatory effect on osteogenic differentiation (Erlacher *et al.*, in press Fig. 2). This promotion of the progression in the osteogenic lineage appears to be more discrete when compared with OP-1 (osteogenic protein-1) or BMP-2. Interestingly, in an ectopic site GDF-5/CDMP-1 does initiate cartilage and some bone formation *in vivo*, an activity typically shared by a number of BMPs. It should be noted that this bioassay measures mainly endochondral bone formation activity. In this assay, the induction of chondrogenesis initiates a cascade of events ultimately and inevitably leading to bone formation probably because of the micro-environment, the matrix and blood vessel invasion, and precursor cell population recruited in the implant.

Ectopic overexpression of GDF-5/CDMP-1, using a replication competent retroviral vector, in the developing chick limb bud increases the length and width of the affected cartilage elements with a relatively normal morphology (Francis-West *et al.*, 1996). Although the mechanism by which this enlargement of the chick limb takes place is still unclear, it seems likely that GDF-5/CDMP-1 promotes the

recruitment of cells into the chondrocytic lineage and expands the chondrocyte precursor populations. Subsequently, this morphogen may contribute to the differentiation and the maintenance of the chondrocytic phenotype. A schematic diagram of its presumed effects on skeletal cells is shown in Fig. 2. Additional *in vivo* and *in vitro* experiments are required to evaluate further the influence of GDF-5/CDMP-1 on the progression of the chondrocytic and osteogenic lineage.

Limited data are available with regard to the possible role of GDF-5/CDMP-1 in other models or organ systems. One report describes its trophic and protective effects on midbrain dopaminergic neurons (Krieglstein *et al.*, 1995). This activity has also been shown for other TGF-β superfamily members and TGF-β3 (Krieglstein *et al.*, 1995). However, its mechanism of action in this model might be distinct from other family members.

In conclusion, it appears that GDF-5/CDMP-1 is more restricted in its effects on primary cells or cell lines, and its osteogenic activity is more discrete when compared with other BMPs. This suggests that GDF-5/CDMP-1 acts through distinct receptors/receptor complexes. It was demonstrated recently that GDF-5/CDMP-1 signals preferentially through a heteromeric complex of BMPR-IB (Alk 6, Type I receptor) with BMPR-II or ActR-II (type II receptor) (Nishitoh *et al.*, 1996; Erlacher *et al.*, in press).

POSSIBLE APPLICATIONS

The use of GDF-5/CDMP-1 as a therapeutic agent is still speculative. Owing to its relationship with other BMPs and its chondrogenic/osteogenic activities, one can envision GDF-5/CDMP-1 as a useful therapeutic agent in protocols designed to enhance cartilage and maybe endochondral bone formation. The use of "devices" enriched with recombinant protein to promote skeletal repair is a logical extension of its biological activities. GDF-5/CDMP-1 might have some advantage in the repair of the cartilaginous skeleton, including the joint surface, because of its apparent preferential promotion of chondrogenesis and maintenance of the cartilage phenotype *in vitro*. Appropriate *in vivo* experiments will allow the evaluation of this potential.

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