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Perspective Article

Hylonome

The vWFC domain of Type IIA procollagen amino-propeptide functions as an antagonist of bone morphogenetic proteins

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Introduction

Knowledge of how growth factors are regulated in the extracellular matrix is crucial for understanding processes of tissue development and repair. A complex series of processes occurs in the extracellular matrix to control the activity and distribution of bone morphogenetic proteins (BMPs) which are important factors during cartilage and bone development[1-3]. It is known that tissue regeneration is a recapitulation of processes in embryonic development and morphogenesis. Thus, in the case of cartilage repair during osteoarthritis, we can apply our knowledge of BMP function/regulation during embryogenesis to developing therapeutic strategies that will induce and even prolong natural tissue repair.

BMPs are members of the transforming growth factor (TGF-β) superfamily and were originally identified as factors promoting the ectopic formation of cartilage and bone. Major subdivisions within the superfamily include the TGF-βs, BMPs (excluding BMP-1), growth/differentiation factors (GDFs), inhibins, activins, Vg-related genes, nodal related genes, Drosophila genes (e.g., decaplentaplegic, dpp and Drosophila 60A) and glial-derived neurotropic factor. Bone morphogenetic proteins (BMPs) induce skeletal differentiation and participate in the development of other organ systems[4]. Binding proteins located in the extracellular matrix regulate the availability of BMPs to the cellular receptors. These proteins can function to store or present BMPs, and even function to establish a gradient of morphogenetic activity. This mechanism of BMP regulation is conserved throughout evolution and is similar to the dorsal-ventral patterning of flies and frogs to skeletal differentiation in vertebrates. BMP binding proteins potentially involved in skeletal development include noggin, chordin and type IIA procollagen. Their temporal and spatial expression, binding affinity, and mechanism of BMP release distinguish the BMP binding proteins from each other. For example, chordin and type IIA procollagen share homologous BMP binding domains and can be inactivated by enzyme digestion, releasing active BMP. On the other hand, they are synthesized by different cells, are spatially and temporally distinct, and differ in specific enzyme susceptibility. Noggin binds BMPs were tightly and it cannot be cleaved to release active BMP[5]. Type IIA procollagen is deposited in the extracellular matrix of chondroprogenitor mesenchyme and other developmental systems. The cysteine-rich domain of the NH2-propeptide binds to BMPs and TGF-β and can be liberated from the fibrillar collagen by matrix metalloproteinases. Multiple cleavage sites have been found for MMPs 3, 7, 9, 13 and 14 located in the cysteine-rich domain, the interruption of the minor helix, the N-proteinase cleavage site and the telopeptide of the IIA NH2-propeptide. BMP can be released as free protein or bound to the trimeric NH2-propeptide, depending on the cleavage site. The functional activity of trimeric and monomeric type IIA procollagen NH2-propeptide and cleavage products has been easily and reproducibly established both in Xenopus dorsalization assay and in a unique BMP-dependent cell differentiation assay system.

BMP regulation by chordin and matrix metalloproteinases

There have been many reports describing the binding and regulation of BMPs by chordin. Studies of Xenopus development showed that over-expression of chordin *in vivo* dorsalized frog embryos by antagonizing the activity of BMPs. Biochemical analyses by the same investigators showed that

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ColIIA CVQDGQRYNDKDVW – KPE-PCRICVCDTGTVLCDDIICEDVKD-CLSPE – -IPFGECCPICPT
ColI CVQNGLRYHDRDVW – KPE-PCRICVCDNGKVLCDDVICDETKN-CPGAE – -VPEGECCPVCPD
ColIII CSHLGQSYADRDVW – KPE-PCQICVCDSGSVLCDDIICDDQELDCPNPE – -IPFGECCAVCPQ
ColV CTQNGQMYLNRDIW – KPA-PCQICVCDNGAILCDKIECQDVLD-CADP – -VTPPGECCPVC
TSP1 CYHNGVQYRNNEEW – TVD-SCTECHCQNSVTICKKVSCPIMP – CSNAT – -VPDGECCPRCWP
TSP2 CWQDGRFFAENETW – VVD-SCTTCTCKKFKTICHQITCPPAT – CASPS – -FVEGECCPSCLH
SOG CFHSGRFYNESEQW-RSAQDSCQMCACLRGQSSCEVIKCPALK – -CKSTEQLLQRDGECCPSCVP
CHDG(chi) CFFEGQHHAHGTRWAPDYDKKCSICSCQKRTVICDPILCQPLN – CTRQ – -VHPEELCCPIC
CHDDR(dr)CFFEGEQHTHGSQWTPQY-NTCFTCTCQKKTVICDPVMCPTLS – CTHT – -VQPEDQCCPIC
CHDR(rat)CFFEGQQRPHGARWAPNYDPLCSLCTCQRRTVICDPVVCPPPR – CSQPV – -QALDQWCPVC

chordin interacts with BMP via the cysteine-rich, von Willebrand C (vWFC) type domains present in chordin[6]. Another level of complexity exists to release the BMP dimer from the binary complex and permit binding to its cell receptor. In Xenopus, over-expression of the astacin protease, Xolloid, resulted in ventralization of the embryos suggesting a BMP agonistic activity[7]. Xolloid cleaves chordin at two specific sites within the chordin molecule to release and hence activate the BMP dimer. Thus, not only does regulation of BMP activity depend on distribution of the various binding proteins present in the extracellular matrix, but also on the concentration and location of matrix metalloproteinases.

It is apparent that proteins containing these cysteine rich vWFC type domains play an important role in binding to and hence regulating other components of the matrix whether during developmental or other processes. These domains are also found in other matrix proteins including thrombospondin-1, connective tissue growth factor and the amino propeptide of the fibrillar procollagens (types I, II, III and V). Type II collagen is the main structural component of the extracellular matrix of articular cartilage and the following section will discuss the role of the developmentally-regulated type IIA procollagen molecule as a regulator of growth factor activity.

The cysteine-rich domain of type IIA procollagen is homologous to the BMP binding domains of chordin

The type IIA NH2-propeptide is encoded by eight exons[8]. The translated protein consists of a short globular domain followed by the cysteine-rich, exon 2 encoded domain, a Gly-X-Y triple-helical domain encoded by exons[3-7] and a short telopeptide domain encoded by exon 8. Exon 2 is alternatively spliced, i.e. included in pre-chondrogenic cells and excluded in chondrocytes[9-11]. The spatial organization of the 10 cysteines within the exon 2 encoded domain is representative of a von Willebrand factor type C sequence and is homologous to the BMP binding domains of chordin, in particular the CR2 domain shown above.

Monomeric recombinant proteins containing the exon 2-encoded cysteine rich domain binds to BMP-2 and TGF-β *in vitro*

Immunoprecipitations and solid-phase binding assays carried out by Zhu et al. showed that the type IIA procollagen cysteine-rich domain fused to GST bound to BMP-2 and TGF-β with a kD similar to that for an individual chordin CR domain binding to BMP[12]. From these results, we hypothesized that a correctly-folded, trimeric IIA NH2-propeptide containing three cysteine-rich domains would be more efficient in binding BMP than the monomer. This led us to produce a recombinant, trimeric IIA NH2-propeptide to study its growth factor binding potential compared to the monomers. We also showed that the trimeric N-propeptide could be cleaved by a number of matrix metalloproteinases including MMP-3, MMP-9, MMP-13 and MMP-713.

Over-expression of full-length Xenopus procollagen dorsalized frog embryos

Recent evidence suggests that full-length, trimeric Xenopus IIA procollagen has a similar role to that of chordin during embryonic development. Interestingly, Larrain and colleagues showed that over-expression of Xenopus type IIB procollagen or a monomer fragment of type IIA procollagen in the same system had no dorsalizing effects. This concluded that the dorsalizing (i.e. BMP binding) function resides in the exon 2-encoded domain and that function also depends on structural organization of the trimeric procollagen NH2-propeptide containing three cysteine-rich domains. Xenopus procollagen IIA, like chordin, was localized in the dorsal mesoderm (notochord and somites) of frog embryos. The spatial distribution and timing of IIA procollagen expression is consistent with a role in dorsal development of the embryos, perhaps replacing the activity of chordin at later stages of Xenopus embryogenesis.

Structure/function studies

At present, research in our laboratory is specifically

directed towards detailed analysis of growth factor interactions with the cysteine-rich vWFC domain of type IIA procollagen and determining its function in a biological system. For these studies, our IIA-SPD fusion protein containing the human, trimeric IIA NH2-propeptide will be an invaluable tool. In conjunction with biological studies, we are currently exploring the use of computational biology to model the vWFC domains that bind to BMPs. These studies will provide insight into the function of this critical domain. We intend to compare binding efficiencies of monomer versus trimer IIA NH2-propeptide as well as the effect of various MMP cleavages on the growth factor binding potential. Mutational analysis will also be applied to examine which amino acids within the IIA procollagen cysteine-rich domain are important for growth factor binding.

IIA procollagen in disease and repair

We are also interested in the role of IIA procollagen during tissue repair. It is known that IIA procollagen is re-expressed in osteoarthritis and thus may prove to be a useful candidate as a marker of degenerative disease. To date, little is known about the distribution and function of BMPs and BMP binding proteins in osteoarthritic tissue. If indeed there is a recapitulation of developmental processes to induce cartilage repair, then it is likely that IIA procollagen also plays a significant role. In terms of therapy, researchers are exploring ways to deliver BMPs to the repair site with the hope that the growth factor will remain at that site for long periods to induce an effect. It is possible that delivery of such BMPs in conjunction with a binding protein, such as IIA procollagen, will retain the growth factor in the matrix for longer. In addition, the presence of specific MMPs, which are known to be elevated in diseased cartilage, may function to cleave the IIA binding protein resulting in release of the active BMP. Thus from development to disease it is becoming apparent that type IIA procollagen is more to cartilage than just a pretty fiber!

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