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Review

Secreted bone morphogenetic protein antagonists of the Chordin family

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Abstract

Chordin, Chordin-like 1, and Chordin-like 2 are secreted bone morphogenetic protein (BMP) antagonists with highly conserved Chordin-like cysteine-rich domains. Recently, Brorin and Brorin-like have been identified as new Chordin-like BMP antagonists. A *Chordin* ortholog, *Short gastrulation*, has been identified in *Drosophila*, a protostome, but not other orthologs. By contrast, *Chordin*, *Chordin-like 1*, and *Chordin-like 2* have been identified in *Ciona intestinalis*, the closest living relatives of the vertebrates, but *Brorin* and *Brorin-like* have not. However, all these genes have been identified in most vertebrates. These results indicate that *Chordin*, *Chordin-like 1*, and *Chordin-like 2* were generated early in the metazoan lineage. Later on, *Brorin* and *Brorin-like* were potentially generated by a genome duplication event in early vertebrate evolution. All four cysteine-rich domains of Chordin are essential for the regulation of its action. However, Chordin-like 1, Chordin-like 2, Brorin, and Brorin-like contain only two or three cysteine-rich domains. Although their mechanisms of action remain unclear, they might be distinct from that of Chordin. The expression profiles of these genes in mice and zebrafish indicate unique roles at embryonic and postnatal stages. Mutant/knockdown mouse and zebrafish phenotypes indicate roles in morphogenesis during gastrulation, dorsoventral axis formation, ear, pharyngeal, and neural development, and venous and arterial patterning. Aberrant *Chordin* expression might result in hereditary diseases and cancer. In addition, altered serum Chordin and Chordin-like 1 levels are also observed in non-hereditary diseases. Together, these results indicate pathophysiological roles.

Keywords: bone morphogenetic protein; Chordin; development; disease; evolution.

Introduction

Bone morphogenetic proteins (BMPs) are secreted growth factors or cytokines that belong to the transforming growth factor β (TGFβ) superfamily. Originally identified in the protein extracts of deminerized bone, BMPs promote endochondral bone formation. However, they also play diverse roles in developmental and metabolic processes at the embryonic and postnatal stages. BMPs are secreted as dimers and activate specific Ser/Thr kinase receptors at cell surfaces. The activated receptors propagate BMP signals via the phosphorylation of Smad proteins and other non-canonical intracellular effectors (1, 2).

The actions of BMPs are inhibited by several secreted BMP antagonists. Most extracellular BMP antagonists inhibit BMPs by binding to them. The amino acid sequences of secreted BMP antagonists are characterized by cysteine-rich (CR) domains. On the basis of the spacing of cysteine residues in the CR domains, secreted BMP antagonists can be classified into five groups; the Dan family, Twisted gastrulation (Twsg1), Follistatin, Noggin, and Chordin (2–5). However, Twsg1 either inhibits or enhances BMP signaling in different cellular contexts (6). Chordin was originally identified as a novel dorsalizing factor in *Xenopus*. Chordin is a secreted BMP antagonist with highly conserved CR domains (3, 7, 8). Chordin-like 1 (Chrdl1) and Chordin-like 2 (Chrdl2) were also identified as secreted BMP antagonists with Chordin-like CR domains (9, 10). Recently, Brorin and Brorin-like have been identified also (11, 12). These BMP antagonists with Chordin-like CR domains have been identified in most vertebrates including humans, mice, and zebrafish.

Several secreted BMP regulators with Chordin-like CR domains also have been identified. Kielin/Chordin-like protein (Kcp) with 18 Chordin-like CR domains, one thrombospondin domain (Tsp), and one von Willebrand factor D (vWF-D) domain enhances BMP signaling (13). Crossveinless-2 with five Chordin-like CR domains and one vWF-D domain is a BMP agonist/antagonist (14, 15). Connective-tissue growth factor, which has four structural domains including the Chordin-like CR domain, insulin-like growth factor domain, Tsp domain, and C-terminal knot, modulates BMP signaling and TGFβ signaling negatively and positively, respectively (16). However, these regulators are functionally and structurally distinct from secreted BMP antagonists, Chordin, Chrdl1, Chrdl2, Brorin, and Brorin-like, with only Chordin-like CR domains as structural domains.

Several excellent reviews on BMPs and secreted BMP regulators have been published (2–4, 16, 17). In this article, we briefly review secreted BMP antagonists with only Chordin-like CR domains as structural domains, focusing on their possible evolutionary history, physiological roles in mice and zebrafish, and pathophysiological roles in humans.

[a] Both authors contributed equally to this work.

Identification of *Chordin*

Chordin was originally identified as a novel dorsalizing factor gene, the Spemann organizer gene, activated by organizer-specific homeobox genes in *Xenopus* (5). Chordin is a secreted BMP antagonist that binds to BMP2, BMP4, and BMP7 but not to TGFβ1 and Activin (18). *Chordin* is the vertebrate ortholog of *Short gastrulation* (*Sog*) in *Drosophila* (19, 20), and has been identified in most vertebrates including humans, mice, and zebrafish (21). Chordin is a secreted protein that contains four highly conserved CR domains of 60 to 80 amino acids (Figure 1A).

Cysteine-rich domains in Chordin

As described above, secreted BMP antagonists can be classified into five groups; the DAN family, Noggin, Twsg1, Follistatin, and Chordin, based on characteristic CR domains (2–5). The DAN family includes Gremlin, Cerberus, Coco, DAN, SOST, and Ectodin/USAG-1/Wise with eight-membered conserved CR domains. Twsg1 has a nine-membered CR domain. Follistatin has a CR domain containing 36 cysteine residues. By contrast, Chordin and Noggin have different types of 10-membered CR domains (3). Chordin is therefore a unique BMP antagonist.

The CR domain in Chordin has a CXXCXC motif in the middle and a CCXXC motif at the carboxyl terminus ('X'; any amino acid) (3). This domain is also known as the von Willebrand factor type C domain (22). Chordin-like CR domains have been identified in approximately 200 extracellular proteins. Procollagen IIA, also has a Chordin-like CR domain, of which the disulfide bond pattern is unambiguously assigned as C_1-C_4, C_2-C_8, C_3-C_5, C_6-C_9, and C_7-C_{10} (7). The CR domain could be divided into two sub-domains, the N-terminal and C-terminal sub-domains. The N-terminal sub-domain is similar to the fibronectin type 1 domain. However, no structural matches could be identified for the C-terminal sub-domain (7).

Identification of *Chrdl1, Chrdl2, Brorin,* and *Brorin-like*

A gene encoding a secreted protein was identified in mouse bone stromal cells by signal sequence trap screening. The protein, Chrdl1, is significantly similar to Chordin, but has only three Chordin-like CR domains not four (Figure 1A). *Chrdl1* transcripts have long and short forms generated by alternative splicing. However, although the two forms differ in the lengths of their C-terminal regions, they have the same three CR domains. Chrdl1 is a Bmp antagonist that binds to BMP4 and BMP6 but not BMP5 and Activin. In contrast to Chordin, Chrdl1 weakly interacts with TGFβ1 and TGFβ2 (9). *Neuralin 1* was identified in the human genome by a homology-based search (23). *Ventroptin* was identified as a gene expressed with a double-gradient pattern in the chick retina (24). *Neurogenesin 1* was identified in rat hippocampal astrocytes by a homology-based polymerase chain reaction (25). *Neuralin 1*, *Ventroptin*, and *Neurogenesin* 1 are synonyms of *Chrdl1*. *Chrdl2* was also identified in mouse placenta by signal sequence trap screening. Chrdl2 is a secreted protein that is structurally homologous to Chrdl1 and Chordin (41% and 33% amino acid identity, respectively). Chrdl2 also has three Chordin-like CR domains (Figure 1A). Chrdl2 is a BMP antagonist that binds to BMP2, BMP4, BMP5, BMP6, and BMP7 but not to TGFβ1, TGFβ, and Activin (10).

Brorin was identified in mouse embryos by searching for cDNAs encoding unknown secreted proteins. Although not significantly homologous to Chordin, Chrdl1, and Chrdl2, Brorin has two only Chordin-like CR domains (Figure 1A). The second CR domain (CR2) has the conserved CXXCXC and CCXXC motifs observed in Chordin and Chrdl1, and Chrdl2. By contrast, the first CR domain (CR1) has a unique CXCXC and CCXXC motif slightly distinct from the conserved motifs. Brorin is a BMP antagonist that inhibits BMP2 and BMP6 in cultured preosteoblastic cells (11). *Brorin-like* was also identified in mouse embryos by searching for cDNAs encoding unknown secreted proteins. Brorin-like is a secreted protein that is significantly similar (48.6% amino acid identity) to Brorin. It also has only two CR domains similar to those of Brorin (Figure 1A). Brorin-like is also a BMP antagonist that inhibits BMP2 and BMP6 in cultured preosteobalstic cells (12). *von Willebrand factor C domain containing 2* (*Vwc2*) and *von Willebrand factor C domain containing protein 2-like* (*Vwc2l*) (GenBank nucleotide sequence database, http://www.ncbi.nlm.nih.gov/nucleotide/) are synonyms of *Brorin* and *Brorin-like*, respectively.

Possible evolutionary history

The evolutionary lineage of metazoan organisms (metazoa) is shown in Figure 2 (26). The fruit fly, *Drosophila melanogaster*, is a protostome. The ascidian, *Ciona intestinalis*, is a urochordate, the closest living relative of the vertebrates (27). The zebrafish, *Danio rerio*, is a teleost. Humans and mice are tetrapods. The genomes of all these metazoa have been sequenced. Most gene expansions occurred in two major phases during metazoan evolution (Figure 2). The first phase (Phase I) occurred in the metazoan lineage, after the divergence of protostomes and deuterostomes. In the first phase, gene duplications occurred. The second phase (Phase II) took place in the chordate lineage, during the early emergence of vertebrates. In the second phase, two genome duplication events occurred (Figure 2) (26, 28, 29).

Chordin, *Chrdl1*, *Chrdl2*, *Brorin*, and *Brorin-like* have been identified in most vertebrates (Figure 2) (Ensemble, http://www.ensemble.org/). These results indicate that they are common genes in vertebrates. *Sog* is the *Drosophila* ortholog of the vertebrate *Chordin* (19, 20). By contrast, no ortholog of *Chrdl1* and *Chrdl2* has been identified in *Drosophila*. However, *Chrdl1* and Chrdl2 might be lost at later evolution stages, because extensive gene loss occurred in metazoan lineages (30). The ortholog of *Chordin* has been

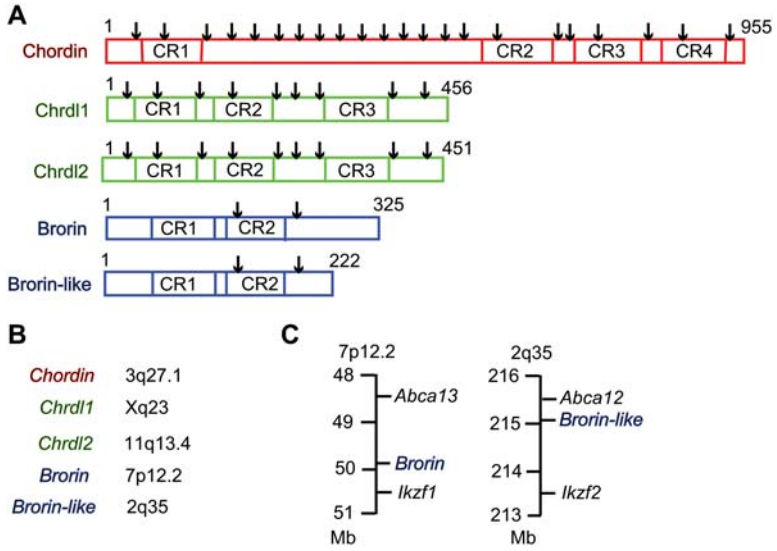

Figure 1 Schematic representation of BMP antagonists of the human Chordin family and their gene loci.
(A) Schematic representation of the human Chordin family. CR1, CR2, CR3, and CR4 indicate the first, second, third, and fourth cysteine-rich domains, respectively. Arrows indicate the positions of introns. Numbers refer to amino acid positions. (B) Chromosomal locations of the human *Chordin* family. (C) Syntenic relation between 7p12.2 and 2q35. *Brorin* is closely linked to *Abca13* and *Ikzf1*. *Brorin-like* is closely linked to *Abca12* and *Ikzf2*. *Abca13*; *ATP-binding cassette, sub-family A (ABC1), member 13*, *Ikzf1*; *IKAROS family zinc finger 1*, *Abca12*; *ATP-binding cassette, sub-family A (ABC1), member 12*, *Ikzf2*; *IKAROS family zinc finger 2*, Mb; megabase.

found in the *C. intestinalis* genome. Potential orthologs of *Chrdl1* and *Chrdl2* have also been found in the *C. intestinalis* genome (unpublished observation). These results indicate that ancestral genes of the vertebrate *Chordin*, *Chrdl1*, and *Chrdl2* were generated in the early metazoan lineage (Figure 2).

Human *Chordin, Chrdl1, Chrdl2, Brorin,* and *Brorin-like* are widespread in the genome (Figure 1B). The organization and location of the genes potentially suggest the evolutionary history of genes (31). Although the conserved gene location (synteny) between *Chrdl1* and *Chrdl2* genes is not observed (data not shown), *Chrdl1* and *Chrdl2* genes have nearly the same exon-intron organization (Figure 1A) (10), indicating they were generated from a common ancestor by gene duplication at Phase I in the early metazoan lineage (Figure 2). By contrast, no ortholog of *Brorin* and *Brorin-like* has been identified in *C. intestinalis* and *Drosophila* genomes (Figure 2) (unpublished observation). In addition, the conserved exon-intron organization and synteny between *Brorin* and *Brorin-like* are observed (Figure 1A and C) (12). These results indicate that *Brorin* and *Brorin-like* were potentially generated from an ancestral gene by a genome duplication event at Phase II in early vertebrate evolution (Figure 2). However, to make the origin of the ancestral gene clear, other protostome and deuterostome genomes should be further examined.

Chordin, Chrdl2, Brorin, and *Brorin-like* have been identified in zebrafish (12, 32), but *Chrdl1* has not (Figure 2) (28). *Chrdl1* has not been identified in other teleosts including fugu and stickleback either (unpublished observation). These results indicate that *Chrdl1* might have been lost early during teleost evolution. Comparisons of mammalian and

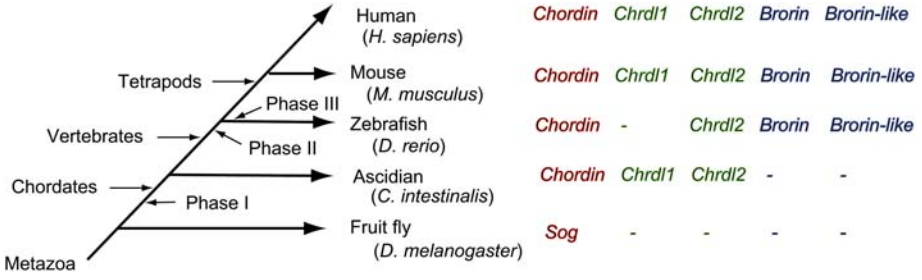

Figure 2 The evolutionary lineage of metazoan organisms and their genes encoding BMP antagonists of the Chordin family.
The sequencing of the fruit fly, ascidian, zebrafish, mouse, and human genomes has been completed. Most gene expansions occurred in two major phases during metazoan evolution. The first phase (Phase 1) occurred in the metazoan lineage, after the divergence of protostomes and deuterostomes. In the first phase, gene duplications occurred. The second phase (Phase II) took place in the chordate lineage, during the early emergence of vertebrates. In the second phase, two genome duplication events occurred. An additional genome duplication event (Phase III) occurred shortly before the teleost radiation.

teleostean genes have shown that there are often two paralogs of the mammalian equivalent in teleosts. This suggests that there was an additional genome duplication event (Phase III) shortly before the teleost radiation (Figure 2). This partial or whole genome duplication event was followed by rapid gene loss because gene duplications account for only approximately 20% of zebrafish genes (33). However, no teleost-specific duplicate of these genes has been identified in teleosts including zebrafish, fugu, and stickleback (unpublished observation).

Mechanism of action

BMPs dimerize to form an active moiety. The active BMPs bind to specific type I and type II Ser/Thr kinase receptors at the cell surface, propagating their signals via the phosphorylation of Smads1/5/8 and other intracellular effectors. The type II receptor then forms a heterodimer with the type I receptor, and the constitutive kinase of the type II receptor activates the type I receptor (4). Chordin inhibits BMP signaling by binding to BMPs. The binding prevents BMPs from binding to their receptors. Twsg1 binds to both Chordin and BMP, facilitating the formation of a Chordin-BMP-Twsg1 complex. The trimolecular complex prevents BMP from binding to its receptors (34). The binding is reversible. Active BMPs are released from the complex by a metalloproteinase, Tolloid, which cleaves Chordin at two specific sites, after the first CR domain and between the third and fourth CR domains. Sizzled, a secreted protein, inhibits Tolloid. Sizzled acts as a negative feedback inhibitor of BMP signaling (34). Ont1 (Olfml3, Olfactomedin-like3), a secreted protein of the Chordin pathway, contains two distinct domains, a coiled-coil domain and an olfactomedin domain. The coiled-coil domain binds to Tolloid/BMP and the olfactomedin domain binds to Chordin. Ont1 functions as an extracellular scaffold protein that brings Tolloid and Chordin together (34, 35).

Chrdl1 and Chrdl2 inhibit BMP signaling by binding directly to BMPs (9, 10). Although Brorin and Brorin-like also inhibit BMP signaling, it is unclear whether they bind to BMPs (11, 12). As described above, four CR domains in Chordin are essential for the regulation of the actions of Chordin. However, Chrdl1, Chrdl2, Brorin, and Brorin-like contain only two or three CR domains. Although their action mechanisms mostly remain unclear, they might be distinct from that of Chordin.

Physiological roles in mouse and zebrafish development

Chordin plays roles in dorsal-ventral patterning and neural induction in *Xenopus* embryos (36). The mouse is widely used as a model for studying gene functions *in vivo*. *Chordin*, *Chrdl1*, *Chrdl2*, *Brorin*, and *Brorin-like* have been identified in mice. *Chordin* is first expressed in the anterior primitive streak and then in the node and the axial mesendoderm in mouse embryos, indicating that Chordin potentially plays a role in patterning in the early embryo (37). During limb development in mouse embryos, *Chordin* is expressed only at the distal ends of the skeletal elements, indicating that Chordin might also play a role in endochondral ossification (38). To examine the physiological roles of Chordin in mammals, *Chordin* knockout mice were generated (Table 1). The mice display a normal early development and neural induction but later defects in inner and outer ear development (37). They also display an extensive array of malformations that encompass most features of DiGeorge syndrome in humans, indicating a role for Chordin in pharyngeal development (39). In addition, their phenotype indicates a role in venous and arterial patterning (40). Noggin has no structural similarity to Chordin (41). However, double *Chordin*/*Noggin* knockout mice display defects in the development of the forebrain, eye, and facial structures, and exhibit a disrupted mesoderm development and left to right patterning. These results indicate that both Chordin and Noggin are required for the proper establishment of the three body axes in mouse embryos (37).

The expression profile of *Chrdl1* in mice is distinct from that of *Chordin*, although the two products have similar activities. *Chrdl1* is first expressed in the neural plate at midgastrulation. Later on, *Chrdl1* is expressed in discrete regions of the central nervous system and derivatives of the neural crest cells. During organogenesis, *Chrdl1* is broadly expressed in many tissues including the dorsal root ganglia, the gut, condensing cartilages of the skeleton, and developing hair follicles (9, 23). It is also expressed in the hippocampal astrocytes and dentate granule cells in the brain (25). The expression profile of *Chrdl1* indicates potential roles in various developmental processes. However, because *Chrdl1* knockout mice have not been reported, its physiological roles remain unclear.

Although Chrdl2 is structurally most similar to Chrdl1, the expression profile of *Chrdl2* differs from that of *Chrdl1*. *Chrdl1* is restrictedly expressed in chondrocytes of developing joint cartilages and connective tissues in reproductive organs in mice. *Chrdl2* is expressed in adult mesenchymal progenitor cells, and its expression decreases during chondrogenic differentiation (10). The expression profile of *Chrdl2* indicates potential roles in developmental processes including chondrogenesis. However, because *Chrdl2* knockout mice have not been reported, the physiological roles of Chrdl2 also remain unclear.

Brorin is predominantly expressed in the developing diencephalon at early stages and in the developing neural tissues at later stages in mouse embryos. Although widely expressed in the brain, *Brorin* is mainly expressed in the diencephalon and medulla oblongata. The cellular distribution of *Brorin* expression indicates that *Brorin* is expressed in neurons but not in glial cells (11). These results indicate potential roles in neural development and functions. Recently, *Brorin* knockout mice have been generated. However, the mice were viable and apparently normal (Table 1) (unpublished observation). The expression profile of *Brorin-like* is also similar to that of *Brorin* in mouse embryos and the adult

Table 1 Roles of BMP antagonists of the Chordin family in mice and zebrafish.

Gene	Phenotype		References
Knockout mice			
Chordin	Lethal	Inner and outer ear development	(37)
		Pharyngeal development	(39)
		Venous and arterial patterning	(40)
Chrdl1	–	–	–
Chrdl2	–	–	–
Brorin	Viable	Apparently normal	Unpublished
Brorin-like	Viable	Apparently normal	Unpublished
Knockout/knockdown zebrafish			
Chordin	Lethal	Morphogenesis during gastrulation	(43–45)
Chrdl2	Lethal	Dorsoventral axis formation	(32)
Brorin	Lethal	Brain and eye formation	Unpublished
Brorin-like	Lethal	Brain and eye formation	(12)

brain (12). *Brorin-like* knockout mice have also been generated. Again, the mice were viable and apparently normal (Table 1) (unpublished observation). Physiological roles of Brorin and Brorin-like in mice remain unclear.

The zebrafish is also widely used for studying gene functions *in vivo*. *Chordin* is initially expressed shortly after the midblastula transition and comes to be strongly expressed in the organizer region at early gastrulation stages in zebrafish. In addition, *Chordin* is also transiently expressed in the developing brain and in the paraxial mesoderm and ectoderm (42). By screening for randomly induced zygotic mutations, *dino* mutant embryos were identified (Table 1). In the mutant embryos, the tail is enlarged at the expense of the head and the anterior region of the trunk. The phenotype suggests that *dino* is required for the establishment of dorsal fates in both the marginal and the animal zone of early gastrula embryos (43). The *dino* phenotype is caused by a mutation in *Chrodin* (44). The function of Chordin during gastrulation is important for the correct morphogenesis of the adult skeleton in zebrafish (45).

Chrdl2 has been identified in zebrafish, but *Chrdl1* has not. The absence of *Chrdl1* suggests that Chrdl2 could serve the roles of both Chrdl1 and Chrdl2 (32). Although *Chrdl2* is maternally expressed, it is also expressed later in development with spatiotemporal patterns that differ from but overlap those of *Chordin* (32). Loss-of-function experiments involving the injection of Morpholino antisense oligonucleotides have demonstrated that *Chrdl2* serves as a BMP antagonist with functions that overlap those of Chordin in forming the dorsoventral axis (Table 1) (32).

Brorin-like is strongly expressed in the zebrafish embryonic brain including several discrete regions in the forebrain, midbrain, and hindbrain. Its profile of neural tissue-specific expression in zebrafish embryos is essentially consistent with that in mouse embryos (12). Loss-of-function experiments with *Brorin-like* Morpholino antisense oligonucleotides revealed morphological abnormalities in the zebrafish embryonic brain and eye. The formation of the telencephalic and tectal ventricles was apparently impaired. In addition, the formation of the constriction between the midbrain and hindbrain was also impaired (Table 1) (12).

Brorin is also markedly expressed in the zebrafish embryonic brain. Its profile of neural tissue-specific expression is slightly different from that of *Brorin-like* (12). *Brorin* knockdown embryos also show morphological abnormalities in the brain and eye (unpublished) (Table 1). However, the phenotype of these embryos is apparently severer than that of *Brorin-like* knockdown embryos. The formation of the telencephalic and tectal ventricles and constriction between the midbrain and hindbrain is also apparently impaired in *Brorin-like* knockdown embryos (unpublished observation). Because the phenotype of *Brorin* knockdown embryos is similar to, but distinct from, that

Table 2 Human diseases potentially caused or affected by Chordin or Chrdl1 signaling disorders.

Gene	Diseases	Gene mutation/signaling	References
	Hereditary disease		
Chordin	Holoprosencephaly	Loss-of-function mutation	(46)
	DiGeorge syndrome	Loss-of-function mutation	(39)
	Cancer		
Chordin	Ovarian cancers	Decrease signaling	(47)
	Non-hereditary disease		
Chordin	Osteoarthritis	Decrease signaling	(48)
Chrdl1	Hypoxia	Increase signaling	(49)
Chrdl1	Kidney injury	Decrease signaling	(51)

of *Brorin-like* knockdown embryos, Brorin and Brorin-like potentially play roles, which are similar to but distinct from each other, in neural development.

Pathophysiological roles in humans

Holoprosencephaly (HPE) is the common malformation of the forebrain resulting from a failure of the left and right hemispheres to completely septate at the rostral end of the neural tube. Mouse mutants with reduced levels of *Chordin* and *Noggin* display mutant phenotypes remarkably analogous to the range of malformations seen in HPE. The study of *Chordin* and *Noggin* mutant mice will help us to understand the molecular, cellular, and genetic pathogenesis of HPE and associated malformations (Table 2) (46). Several loci for HPE have been mapped to specific chromosomal sites [Online Mendelian Inheritance in Man (OMIM); http://www.ncbi.nlm.nih.gov/omim/]. However, *Chordin* and *Noggin* are located at 3q27.1 and 17q22, different from any of these sites, indicating that they are not directly causative of HPE.

DiGeorge syndrome covers a spectrum of head and neck malformations. *Chordin* mutant mice display an extensive array of malformations that encompass most features of the syndrome. The *Chordin* mutation provides a mouse model for head and neck congenital malformations and suggests that Chordin/BMP signaling participates in the pathogenesis (Table 2) (39). However, the gene locus for DiGeorge syndrome has been mapped to 22q11.2 (OMIM), different from that of *Chordin*, 3q27.1, indicating that *Chordin* is not a direct cause of the syndrome.

Ovarian cancers mostly derive from the monolayer epithelium that covers the ovary. *Chordin* is underexpressed in epithelium ovary cancers and epithelial cancer cell lines. Chordin could participate in regulating BMP activity in the normal ovarian epithelium and its misexpression could lead to cancer incidence and/or progression (Table 2) (47).

In normal human cartilage, Chordin is detected at low levels, mainly in the very superficial layers. In osteoarthritic cartilage, Chordin is detected at significantly higher levels in the very superficial layers. By contrast, *Chordin* expression is significantly downregulated in osteoarthritic chondrocytes by growth factors. The differential distribution and regulation of Chordin in normal and osteoarthritic cartilage and chondrocytes suggest an involvement of Chordin in the osteoarthritic process (Table 2) (48).

Pericytes play a specialized role in regulating angiogenesis and vascular function by providing vascular stability and controlling endothelial cell proliferation. Disorders in pericyte function and pericyte-endothelial interaction have been observed in several disease states including tumor angiogenesis and diabetic microangiopathy. In ischemic retinal disease, hypoxia is a potent driver of retinal angiogenesis. Hypoxia induces the expression of *Chrdl1* in human retinal pericytes (49). BMP4 has been implicated in angiogenesis through a VEGF-dependent mechanism (50). *Chrdl1* expression by retinal pericytes in response to hypoxia could play an important role in regulating retinal angiogenesis through modulation of BMP4 actions on endothelial cells (49).

Stimulation of the BMP pathway protects the kidney from acute and chronic injury. *Chrdl1* is expressed in the ischemia-sensitive S3 segment in humans. Chrdl1 specifically antagonizes BMP7, which is expressed in the neighboring distal nephron. Upon ischemia-induced degeneration of the S3 segment, *Chrdl1* expression is decreased. *Chrdl1* expression coincides with intense BMP signaling in tubules of the recovering kidney. These results indicate that Chrdl1 reduces BMP7 signaling in healthy proximal tubules, and the loss of this activity upon sloughing of injured epithelia promotes BMP7 signaling in repopulating, dedifferentiated epithelia (51).

Expert opinion and outlook

Disulfide bonds formed by pairs of cysteine residues in proteins are essential for the formation of unique functional motifs and protein folds. Two intrachain disulfide bonds formed by four cysteine residues represent a unique framework for the formation of a cystine ring. Chordin contains four highly conserved cysteine domains but not other functional domains. Chordin is a secreted BMP antagonist. Most secreted BMP antagonists including the DNA family, Follistatin, Noggin, and Twsg1 also contain CR domains. However, the CR domains in Chordin are unique.

Several secreted BMP antagonists, Chrdl1, Chrdl2, Brorin, and Brorin-like, have been identified. They also contain the Chordin-like CR domains but not other functional domains. *Chordin*, *Chrdl1*, *Chrdl2*, *Brorin*, and *Brorin-like* have been identified in most vertebrates. *Chordin*, *Chordin-like 1*, and *Chordin-like 2* were generated early in the metazoan lineage. However, *Brorin* and *Brorin-like* were generated by a genome duplication event early during vertebrate evolution.

The action mechanism of Chordin has been extensively elucidated. Four CR domains appear to be essential for the regulation of its actions. However, Chrdl1, Chrdl2, Brorin, and Brorin-like contain only two or three cysteine-rich domains. Although their action mechanisms mostly remain unclear, they might be distinct from that of Chordin.

The mouse and zebrafish are widely used vertebrate models for studying gene functions *in vivo*. These BMP antagonist gene knockout/knockdown in mice and zebrafish indicate physiological roles. In addition, their aberrant expression and serum levels might result in disease, indicating pathophysiological roles in humans. Further analysis will promote our understanding of their roles in developmental and metabolic processes.

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