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Focus on Molecules: Gremlin

Robert J. Wordinger^{a,*}, Gulab Zode^a, and Abbot F. Clark^{a,b}

^a Department of Cell Biology and Genetics, University of North Texas Health Science Center at Fort Worth, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107-2699, USA

^b Glaucoma Research, Alcon Laboratories Ltd., Fort Worth, TX 76134-2099, USA

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1. Structure

Gremlin (Accession numbers: Nucleotide NM_013372, Protein NP_037504.1) is a highly conserved 184 amino acid protein (20.7 kDa). The human gremlin gene (*GREM1*) has been mapped to chromosome 15q13–q15. The signaling peptide (1–24) and a predicted glycosylation site (42) have been identified. In addition, the protein contains a cysteine-rich region and a cysteine knot motif (94–184) whose structure is shared by members of the TGF- β superfamily. Post-translational modifications include *N*-glycosylation and phosphorylation. Gremlin exists in both secreted and cell-associated (e.g. membrane associated) forms. A secreted form of gremlin is reported to be 28 kDa and represents the glycosylated form (Fig. 1). Three alternative splicing patterns for gremlin have been reported.

2. Function

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor- β (TGF- β) superfamily of growth factors and were originally identified as osteoinductive cytokines. BMPs are now known to control various cell functions in multiple organs including the eye (Wordinger and Clark, 2007). Signaling by BMP ligands involves interaction with two transmembrane serine/threonine kinase receptors termed type I (BMPRI) and type II (BMPRII). Combinations of intracellular and extracellular BMP inhibitors maintain tight control of BMP action in any given tissue.

Secreted BMP antagonists have been identified. Examples of secreted BMP antagonists include noggin, chordin, follistatin, Dan, cerebus, caronte and gremlin. Gremlin exerts a potent inhibitory action via binding to and forming heterodimers with BMP-2, BMP-4, and BMP-7. The binding of gremlin to selective BMPs prevents ligand–receptor interaction and subsequent downstream signaling. A delicate balance exists in tissues between BMP activity and BMP

*Corresponding author. E-mail addresses: rwording@hsc.unt.edu (R.J. Wordinger), gzode@hsc.unt.edu (G. Zode), abe.clark@alconlabs.com (A.F. Clark).

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inhibition. The balance is maintained through spatial and temporal expression of specific BMPs and specific BMP antagonist proteins. BMP antagonists such as gremlin, play an important role in regulating multiple cell functions both during early development and in adult tissues. Gremlin inhibition of BMPs is important for limb and retina development. Gremlin knockout mice are neonatal lethal because of the lack of kidneys and lung defects. In the adult, gremlin regulates cell proliferation and stem cell differentiation.

In addition to the ability of gremlin to directly bind and inhibit BMP action, gremlin may exert direct effects on cell function via BMP-independent mechanisms. Exogenous gremlin may bind to and act directly on endothelial cells to modulate angiogenesis including endothelial cell migration. Thus a receptor-mediated mechanism of action may exist for gremlin. In support of this concept is a report that gremlin interacts with Slit proteins and acts as a direct negative regulator of monocyte chemotaxis.

3. Disease involvement

The involvement of gremlin in various diseases has primarily centered on fibrotic changes in the kidney, lung, liver, and osteoarthritis. The most widely studied disease is in fibrotic kidney disease including diabetes. Neutralization of BMP-7 via gremlin increased the expression of fibronectin and collagen type III. In addition, both gremlin and connective tissue growth factor are upregulated by TGF- β in kidneys of diabetic animals. With respect to the pathophysiology of ocular diseases there are reports that elevated glucose, mechanical strain, and TGF- β stimulate gremlin expression. Thus the involvement of gremlin in ocular diseases such as diabetic retinopathy (e.g. high glucose levels) and glaucoma (e.g. elevated TGF- β 2 in aqueous humor and the optic nerve head and mechanical strain) is of great interest.

Kane et al. (2005) demonstrated that high glucose increased gremlin mRNA in bovine retinal pericytes. Gremlin expression was modulated by anti-TGF- β 1 antibody and by inhibition of MAPK activation. Using immunohistochemistry, gremlin was localized in the mouse retina to the nerve fiber layer, ganglion cell layer, and inner plexiform layer. In C57Bl/6 mice with streptozotocin induced diabetes, gremlin localization also appeared in the outer retina and in the wall of large retinal vessels. Their results implicate gremlin in the pathogenesis of diabetic retinopathy.

We have previously shown that cultured human trabecular meshwork (TM) cells express BMPs, BMP receptors, and mRNA for selective BMP antagonists including gremlin and are capable of secreting BMPs. We recently reported that BMP-4 selectively counteracts the action of TGF- β 2 in TM cells with respect to ECM-related proteins (Wordinger et al., 2007). Thus it appears that BMP-4 may play a significant role in maintaining the normal function of the TM by modifying the action of TGF- β 2. With respect to gremlin, we reported that it inhibits BMP-4 activity in cultured TM cells and increased outflow resistance in a perfusion cultured human eye anterior segment model. Significantly, we noted that both gremlin mRNA and protein are increased in glaucomatous human TM cell lines. We have suggested that, in POAG, elevated gremlin expression by TM cells inhibits BMP-4 antagonism of TGF- β 2 leading to increased ECM deposition and elevated IOP (Wordinger et al., 2007).

4. Future studies

The BMPs are emerging as a significant sub-family of the TGF- β superfamily of growth factors within the eye. In most normal tissues, equilibrium exists between BMP levels and BMP antagonists in order to maintain normal tissue functions. An emerging paradigm posits that specific developmental genes such as gremlin become activated during pathological changes in adult tissues. The recapitulation of gremlin expression has been demonstrated in fibrotic diseases especially of the kidney, lung, and liver. In the future, it will be important to examine

gremlin expression during ocular pathology. For example, Lee et al. (2007) reported that epithelial-to-mesenchymal transition in proliferative vitreoretinopathy is induced by gremlin. Since it is known that TGF- β , elevated glucose, and mechanical strain can upregulate gremlin expression, it will be particularly important to expand our studies in the glaucomatous TM and ONH as well as the diabetic retina. Specifically, future ocular studies of BMP antagonists including gremlin should determine (a) the pathophysiology of BMP antagonist expression in ocular diseases, (b) the regulators of ocular BMP antagonist expression, (c) if BMP antagonists can act in a BMP-independent manner to directly bind ocular cells and elicit a direct response, and (d) the expression pattern and functional significance of BMP antagonist splice variants in ocular tissues.

References

- Kane R, Stevenson L, Godson C, Stitt AW, O'Brien CO. Gremlin gene expression in bovine retinal pericytes exposed to elevated glucose. *Br J Ophthalmol* 2005;89:1638–1642. [PubMed: 16299147]
- Lee H, O'Meara SJ, O'Brien C, Kane R. The role of gremlin, a BMP antagonist and epithelial-to-mesenchymal transition in proliferative vitreoretinopathy. *Invest Ophthalmol Vis Sci* 2007;48:4291–4299. [PubMed: 17724219]
- Wordinger RJ, Clark AF. Bone morphogenetic proteins and their receptors in the eye. *Exp Biol Med* 2007;232:979–992.
- Wordinger RJ, Fleenor D, Hellberg P, Pang IH, Tovar T, Zode G, Fuller J, Clark AF. Effects of TGF- β 2, BMP-4, and gremlin in the trabecular meshwork: implications for glaucoma. *Invest Ophthalmol Vis Sci* 2007;48:1191–1200. [PubMed: 17325163]

Fig. 1. (A): Structural features of gremlin protein. The predicted positions of structural features of gremlin protein are shown. Signal Seq., signal sequence (positions 1–24); DAN, cysteine-rich motif (positions 69–184); , glycosylation site (position 42); *, phosphorylation sites (positions 6, 29, 44, 47, 55, 66, 76, 77, 88, 102 and 151); , PKC specific eukaryotic protein phosphorylation site (position 165); NLS, nuclear localization signal sequences (positions 145, 166, 163, 164); (B and C): gremlin immunolocalization in cultured ONH astrocytes and LC cells, respectively. ONH astrocytes and LC cells were fixed and stained for gremlin protein. Gremlin is localized in nucleus and cytoplasm of ONH astrocytes and LC cells; (D): western blot analysis of gremlin protein in ONH, retina, and brain tissues. Human ONH, retina, and

brain tissue lysate were analyzed for gremlin protein by western blot. Gremlin protein was found in ONH, retina, and brain tissues.