Commentary   **Open Access**

A Better Understanding of the Role of TGF-β Family Members in Tissue Fibrosis

Jose M Muñoz-Felix[1,2] **and Carlos Martinez-Salgado**[1,2,3*]

[1]*Centre for Tumour Biology, Barts Cancer Institute, Queen Mary University of London, UK*

[2]*Translational Research on Renal and Cardiovascular Diseases (TRECARD), Department of Physiology and Pharmacology, University of Salamanca, Salamanca, Spain*

[3]*Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain.*

[*]**Corresponding author:** Carlos Martinez-Salgado, Department of Physiology and Pharmacology, University of Salamanca, Spain, E-mail: carlosms@usal.es

Commentary

Traditionally two different subfamilies of the transforming growth factor-β (TGF-β) superfamily have been associated with different effects on tissue fibrosis: the profibrotic TGF-β subfamily (TGF-β1, TGF-β2 and TGF-β3) and the antifibrotic bone morphogenetic proteins (BMPs) subfamily. TGF-β1 is the most widely studied profibrotic cytokine, as it regulates numerous processes involved in tissue fibrosis: synthesis of extracellular matrix proteins (ECM), apoptosis of the parenchymal cells, regulation of epithelial-to-mesenchymal transition (EMT) and endothelial-to-mesenchymal transition [1,2]. On the other hand, BMPs (especially BMP7) have been considered as antifibrotic proteins [2-4]. TGF-β subfamily members activate ALK5 type I receptors –thus activating Smad2 and Smad3 proteins- whereas BMPs activate ALK1, ALK2, ALK3 and ALK6 receptors and thus activating Smad1, Smad5 and Smad8 proteins.

BMP9 and BMP10 were described as high affinity ligands for ALK1 receptors in endothelial cells [5,6]. These proteins show redundancy in several biological processes such as vascular development, and share numerous similarities in their functions, although BMP10 is necessary for embryonic development and BMP9 is not [7]. Our group has demonstrated that the ALK1 receptor has an antifibrotic role in an experimental model of obstructive nephropathy [8], as ALK1 (previously considered as an endothelial specific receptors) is expressed in fibroblasts and has an important regulatory role in ECM proteins synthesis [8]. In order to explore the antifibrotic role of the ALK1 receptor, we studied the effects of its canonical and high affinity ligand, the BMP9 protein, on renal fibrosis and ECM proteins regulation. We have recently observed that BMP9 stimulates cellular responses in fibroblasts through ALK1 and ALK5 receptors. Although previous studies demonstrated that BMP9 stimulates cellular responses in other non-endothelial cell types such as liver tumour cells, those studies did not show whether the effects are due to ALK1 activation [9].

The findings that we show in our recent studies address a profibrotic role of BMP9 *in vitro*, but further analysis is needed to unveil the potential interest of this molecule in fibrotic diseases. It will be necessary to evaluate the levels of BMP9 in fibrotic tissues as well as in plasma, either in patients or in *in vivo* experimental models of fibrosis. It is well known that BMP9 is produced by hepatocytes in the liver and circulates in an active form at concentrations ranging between 2 and 12 ng/ml [16].

We demonstrate that BMP9 behaves as a profibrotic factor *in vitro*, promoting the synthesis of ECM proteins such as collagen I, fibronectin and connective tissue growth factor (CTGF) [10].
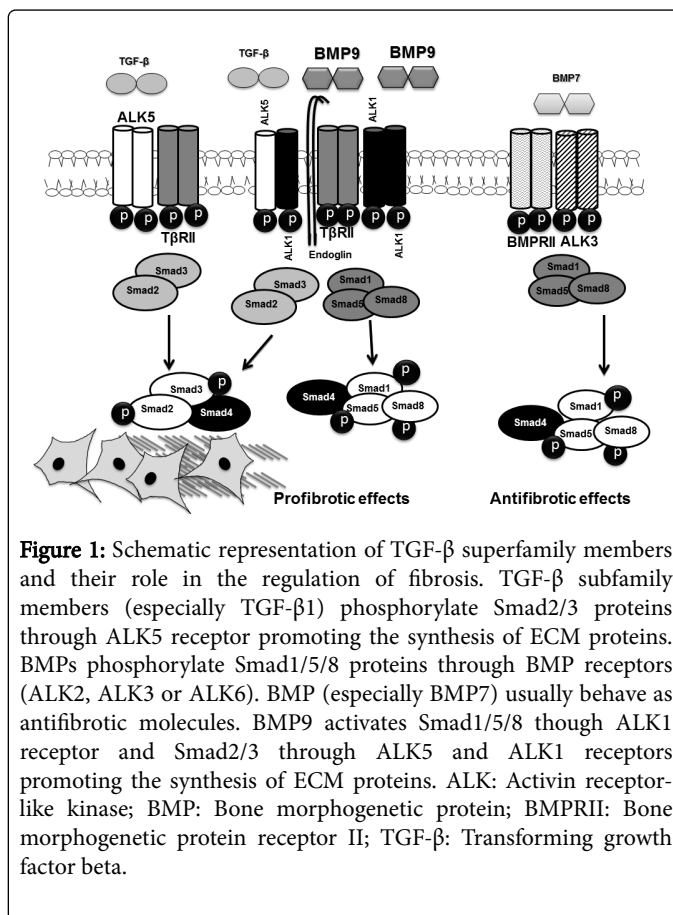

Figure 1: Schematic representation of TGF-β superfamily members and their role in the regulation of fibrosis. TGF-β subfamily members (especially TGF-β1) phosphorylate Smad2/3 proteins through ALK5 receptor promoting the synthesis of ECM proteins. BMPs phosphorylate Smad1/5/8 proteins through BMP receptors (ALK2, ALK3 or ALK6). BMP (especially BMP7) usually behave as antifibrotic molecules. BMP9 activates Smad1/5/8 though ALK1 receptor and Smad2/3 through ALK5 and ALK1 receptors promoting the synthesis of ECM proteins. ALK: Activin receptor-like kinase; BMP: Bone morphogenetic protein; BMPRII: Bone morphogenetic protein receptor II; TGF-β: Transforming growth factor beta.

The identification of BMP9 as a profibrotic factor complicates the scheme of the BMP/TGF-β network. BMP9 does not induce the same effects than BMP7 –the most powerful antifibrotic cytokine, as their mechanisms of action are different. While BMP7 activates Smad1/5/8 through ALK3 we observed that BMP9 activates Smad1/5/8 through the ALK1 receptor and Smad2/3 proteins through the ALK5 receptor, being ALK1 essential in both processes. In this experimental scenario, both Smad1/5/8 and Smad2/3 are necessary for the induction of ECM proteins synthesis (Figure 1). In several cellular types, BMP9 can induce non-Smad signalling (MAPK, PI3K/AKT, NF-κB, Wnt etc.) [11]. In our experimental model with mouse embryonic fibroblasts,

BMP9 induces Erk1/2 phosphorylation, which is necessary for BMP9-induced fibrogenic response [10]. However, further studies should be performed to determine if Erk1/2 activation induced by BMP9 is an indirect effect of Smad phosphorylation. This is not the first time that BMP9 is related to ECM protein synthesis, as it was previously shown that this cytokine stimulates the synthesis of the ECM components Col2A1 and aggrecan in human multipotent mesenchymal cells [12]. Moreover, BMP9 regulates Col9a1, Col9a3 and Col1a synthesis in forebrain cholinergic neurons [13]. In endothelial cells, BMP9 regulates CTGF expression through a Yes-associated protein (YAP)-dependent mechanism [14]. BMP9 and BMP10 stimulate the expression of fibronectin and collagen I in cultured endothelial cells [15]. Recently, Bailly et al. have demonstrated that BMP9 and BMP10 are both necessary for the proper closure of the *ductus arteriosus* in newborns; one of the mechanisms involved in this process is BMP9-promoted ECM deposition [15].

Zhu et al. have discovered 4-fold increased levels of BMP9 in the serum of dialysis-patients with chronic kidney disease with respect to pre-dialysis patients [17]. It will be interesting to elucidate whether there is any correlation between BMP9 levels and kidney fibrosis. Previous work from our laboratory showed that the predominant form of endoglin (L-endoglin), which is now considered as a receptor required for BMP9-induced signalling [7,18] promotes renal tubule-interstitial fibrosis after the unilateral ureteral obstruction experimental model through Smad1/5 and Smad2/3 activation. The possible involvement of BMP9 in these mechanisms should be addressed.

Moreover, the study of the role of BMP9 in liver fibrosis is of undoubted relevance for different reasons: First, BMP9 shows its major expression in the liver in physiological conditions. Second, during the development of fibrosis, ALK1 activates Id1 through the Smad1 pathway promoting the transdifferentiation of hepatic stellate cells into myofibroblasts [19]. Moreover, BMP9 induces EMT in hepatocellular carcinoma cells [20]. Finally, another important target of BMP9 in liver cells is hepdicin, a polypeptide involved in the inhibition of iron absorption and recycling, being these processes associated with liver fibrosis and cirrhosis [21].

Further studies will be needed for the complete characterization of BMP9 as a regulator of tissue fibrosis and to its validation as a good therapeutic target. The deeper knowledge of the complex and large TGF-β superfamily is widening as new members and their roles are being identified. New studies will be necessary to integrate the interaction of all these molecules that share receptor complexes and intracellular Smads.

Acknowledgements

References

1. Munoz-Felix JM, Gonzalez-Nunez M, Martinez-Salgado C, Lopez-Novoa JM (2015) TGF-beta/BMP proteins as therapeutic targets in renal fibrosis. Where have we arrived after 25 years of trials and tribulations? Pharmacol Ther 156: 44-58.

2. Meng XM, Nikolic-Paterson DJ, Lan HY (2016) TGF-β: the master regulator of fibrosis. Nat Rev Nephrol 12: 325-338.

3. Meng XM, Chung AC, Lan HY (2013) Role of the TGF-β/BMP-7/Smad pathways in renal diseases. Clin Sci (Lond) 124: 243-254.

4. Zeisberg M, Hanai J, Sugimoto H, Mammoto T, Charytan D, et al. (2003) BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. Nat Med 9: 964-968.

5. David L, Mallet C, Mazerbourg S, Feige JJ, Bailly S (2007) Identification of BMP9 and BMP10 as functional activators of the orphan activin receptor-like kinase 1 (ALK1) in endothelial cells. Blood 109: 1953-1961.

6. David L, Mallet C, Keramidas M, Lamandé N, Gasc JM, et al. (2008) Bone morphogenetic protein-9 is a circulating vascular quiescence factor. Circ Res 102: 914-922.

7. Tillet E, Bailly S (2015) Emerging roles of BMP9 and BMP10 in hereditary hemorrhagic telangiectasia. Front Genet 5: 456.

8. Munoz-Felix JM, Lopez-Novoa JM, Martinez-Salgado C (2014) Heterozygous disruption of activin receptor-like kinase 1 is associated with increased renal fibrosis in a mouse model of obstructive nephropathy. Kidney Int 85: 319-332.

9. Herrera B, García-Álvaro M, Cruz S, Walsh P, Fernández M, et al. (2013) BMP9 is a proliferative and survival factor for human hepatocellular carcinoma cells. PLoS One 8: e69535

10. Muñoz-Félix JM, Cuesta C, Perretta-Tejedor N, Subileau M, López-Hernández FJ, et al. (2016) Identification of bone morphogenetic protein 9 (BMP9) as a novel profibrotic factor in vitro. Cell Signal 28: 1252-1261.

11. Herrera B, Dooley S, Breitkopf-Heinlein K (2014) Potential roles of bone morphogenetic protein (BMP)-9 in human liver diseases. Int J Mol Sci 15: 5199-5220.

12. Majumdar MK, Wang E, Morris EA (2001) BMP-2 and BMP-9 promotes chondrogenic differentiation of human multipotential mesenchymal cells and overcomes the inhibitory effect of IL-1. J Cell Physiol 189: 275-284.

13. Lopez-Coviella I, Follettie MT, Mellott TJ, Kovacheva VP, Slack BE, et al. (2005) Bone morphogenetic protein 9 induces the transcriptome of basal forebrain cholinergic neurons. Proc Natl Acad Sci U S A 102: 6984-6989.

14. Young K, Tweedie E, Conley B, Ames J, FitzSimons M, et al. (2015) BMP9 Crosstalk with the Hippo Pathway Regulates Endothelial Cell Matricellular and Chemokine Responses. PLoS One 10: e0122892.

15. Levet S, Ouarné M, Ciais D, Coutton C, Subileau M, et al. (2015) BMP9 and BMP10 are necessary for proper closure of the ductus arteriosus. Proc Natl Acad Sci U S A 112: 3207-3215.

16. Bidart M, Ricard N, Levet S, Samson M, Mallet C, et al. (2012) BMP9 is produced by hepatocytes and circulates mainly in an active mature form complexed to its prodomain. Cell Mol Life Sci 69: 313-324.

17. Zhu D, Mackenzie NC, Shanahan CM, Shroff RC, Farquharson C, et al. (2015) BMP-9 regulates the osteoblastic differentiation and calcification of vascular smooth muscle cells through an ALK1 mediated pathway. J Cell Mol Med 19: 165-174.

18. Nolan-Stevaux O, Zhong W, Culp S, Shaffer K, Hoover J, et al. (2012) Endoglin requirement for BMP9 signaling in endothelial cells reveals new mechanism of action for selective anti-endoglin antibodies. PLoS One 7: e50920.

19. Wiercinska E, Wickert L, Denecke B, Said HM, Hamzavi J, et al. (2006) Id1 is a critical mediator in TGF-beta-induced transdifferentiation of rat hepatic stellate cells. Hepatology 43: 1032-1041.

20. Li Q, Gu X, Weng H, Ghafoory S, Liu Y, et al. (2013) Bone morphogenetic protein-9 induces epithelial to mesenchymal transition in hepatocellular carcinoma cells. Cancer Sci 104: 398-408.

21. Bi J, Ge S (2014) Potential roles of BMP9 in liver fibrosis. Int J Mol Sci 15: 20656-20667.