available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/modo

Unexpected activities of Smad7 in *Xenopus* mesodermal and neural induction

Irene de Almeida[a], *Ana Rolo*[a], *Julie Batut*[b,1], *Caroline Hill*[b], *Claudio D. Stern*[a,*], *Claudia Linker*[a,*,2]

[a]*Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK*
[b]*Laboratory of Developmental Signalling, Cancer Research UK, London Research Institute 44 Lincoln's Inn Fields, London WC2A 3PX, UK*

ARTICLE INFO

Article history:
Received 12 June 2007
Received in revised form
1 February 2008
Accepted 4 February 2008
Available online 12 February 2008

Keywords:
Xenopus
Chick
Chicken
Neural induction
FGF
BMP
Wnt
Hedgehog
Retinoic acid
Nodal
Smad6
Smad7
TGFβ

ABSTRACT

Neural induction is widely believed to be a direct consequence of inhibition of BMP pathways. Because of conflicting results and interpretations, we have re-examined this issue in *Xenopus* and chick embryos using the powerful and general TGFβ inhibitor, Smad7, which inhibits both Smad1- (BMP) and Smad2- (Nodal/Activin) mediated pathways. We confirm that Smad7 efficiently inhibits phosphorylation of Smad1 and Smad2. Surprisingly, however, over-expression of Smad7 in *Xenopus* ventral epidermis induces expression of the dorsal mesodermal markers Chordin and Brachyury. Neural markers are induced, but in a non-cell-autonomous manner and only when Chordin and Brachyury are also induced. Simultaneous inhibition of Smad1 and Smad2 by different approaches does not account for all Smad7 effects, indicating that Smad7 has activities other than inhibition of the TGFβ pathway. We provide evidence that these effects are independent of Wnt, FGF, Hedgehog and retinoid signalling. We also show that these effects are due to elements outside of the MH2 domain of Smad7. Together, these results indicate that BMP inhibition is not sufficient for neural induction even when Nodal/Activin is also blocked, and that Smad7 activity is considerably more complex than had previously been assumed. We suggest that experiments relying on Smad7 as an inhibitor of TGFβ-pathways should be interpreted with considerable caution.

1. Introduction

BMPs are members of the transforming growth factor β (TGFβ) family of secreted proteins. The main mechanism of signal transduction for these proteins involves two serine/threonine kinase receptors: type-I and type-II. Upon ligand binding the receptor complex phosphorylates particular members of the Smad family of proteins, the receptor regulated Smads (R-Smads) (Hill, 2001; Massague et al., 2005; Park, 2005; Shi and Massague, 2003; ten Dijke and Hill, 2004;

* Corresponding authors.
E-mail addresses: c.stern@ucl.ac.uk (C.D. Stern), claudia.linker@cancer.org.uk (C. Linker).
[1] Present address: Université de Toulouse, Centre de Biologie du Développement, CBD, UMR 5547, IFR 109, Bat 4R3, 118 route de Narbonne, 31062 Toulouse Cedex 9, France.
[2] Present address: Vertebrate Development Laboratory, Cancer Research UK, London Research Institute, 44 Lincoln's Inn Fields, London WC2A 3PX, UK.
0925-4773/$ - see front matter
doi:10.1016/j.mod.2008.02.002

von Bubnoff and Cho, 2001). Phosphorylated R-Smads are released from the receptor complex and bind to Smad4, allowing the translocation of this complex to the nucleus, where it regulates transcription of target genes (von Bubnoff and Cho, 2001). Other than the R-Smads and Smad4, inhibitory Smads (I-Smads) have been shown to be important regulators of this pathway. Two major I-Smads have been characterized: Smad-6, which preferentially inhibits the BMP pathway and Smad-7, which blocks all TGFβ signalling. I-Smads bind to the intracellular domain of receptor type-I, recruit Smurf ubiquitin ligases and induce degradation of the receptor (Hill, 2001; ten Dijke and Hill, 2004). In addition, Smad6 inhibits BMP signalling by competing with Smad4 for binding to phosphorylated Smad1, yielding inactive Smad1–Smad6 complexes (Hata et al., 1998). Through these mechanisms I-Smads inhibit the TGFβ pathway in a cell-autonomous way.

BMP signalling plays numerous roles in development. One of the best studied processes involving regulation of BMP signalling is neural induction – an early embryonic event first demonstrated clearly almost a century ago when it was shown that signals emanating from the organizer (the dorsal lip of the blastopore in amphibians) can instruct ectoderm to acquire a neural fate (Spemann and Mangold, 1924). The first molecular explanation for this process (the "default model") is that BMPs, which are broadly expressed in the early embryo, act as epidermal inducers and need to be inhibited in the prospective neural plate for this structure to form (Harland, 2000; Hemmati-Brivanlou and Melton, 1997a; Hemmati-Brivanlou and Melton, 1997b; Muñoz-Sanjuán and Brivanlou, 2002). Although there is some controversy concerning whether or not the default model provides a sufficient explanation for this process (see for example Bachiller et al., 2000; Belo et al., 2000; Bertrand et al., 2003; Delaune et al., 2005; Linker and Stern, 2004; McMahon et al., 1998; Mukhopadhyay et al., 2001; Stern, 2004; Streit et al., 2000, 1998; Streit and Stern, 1999a) it is clear that inhibition of BMP signalling is part of the neural induction process in vertebrates.

One of the ways in which the involvement of BMPs as epidermal inducers and neural inhibitors has been tested in *Xenopus* is by misexpression of the powerful inhibitory Smad, Smad7, a double-inhibitor of both the Smad1-,5-,8- (BMPs) and 2-,3-dependent (Nodal/Activin) TGFβ-pathways (Casellas and Brivanlou, 1998; Chang and Harland, 2007; Nakao et al., 1997; Nakayama et al., 2001). Here, we use this reagent to re-examine the role of BMP signalling in neural induction in *Xenopus* and chick. We confirm that Smad7 efficiently inhibits the phosphorylation of both Smad1 and Smad2. Over-expression of Smad7 does not induce neural markers in chick competent ectoderm. Surprisingly, in *Xenopus*, over-expression of Smad7 in ventral epidermis induces *Chordin* and *Brachyury*, markers of dorsal mesoderm, and as a secondary effect, neural markers are induced in a non-cell-autonomous manner. These effects cannot be explained entirely by inhibition of all (Nodal/Activin and BMP-related) TGFβ signalling, because inhibition of Nodal/Activin-related signalling by co-injection of either Cerberus-Short (CerS) or a truncated form of a type I receptor (tAlk4), together with inhibition of BMP-related signals by either Smad6 or a truncated version of Smad7 (lacking the MH1 domain) does not produce the same effects. These results suggest that Smad7 has activities other than inhibition of TGFβ-pathways and that these activities are due to functional elements outside of the MH2 domain. We also provide evidence that the effects of Smad7 unrelated to TGFβ signalling are independent of Wnt, FGF, hedgehog and retinoid signalling. Although these activities of Smad7 remain only partially understood, this study suggests that great caution should be exercised in interpreting the results of experiments using Smad7 as a BMP/TGFβ antagonist.

2. Results

2.1. *Smad7 does not induce neural markers in the chick*

Previous work has shown that BMP inhibition, through over-expression of soluble or cell-autonomous antagonists, does not induce neural markers in chick epiblast (Linker

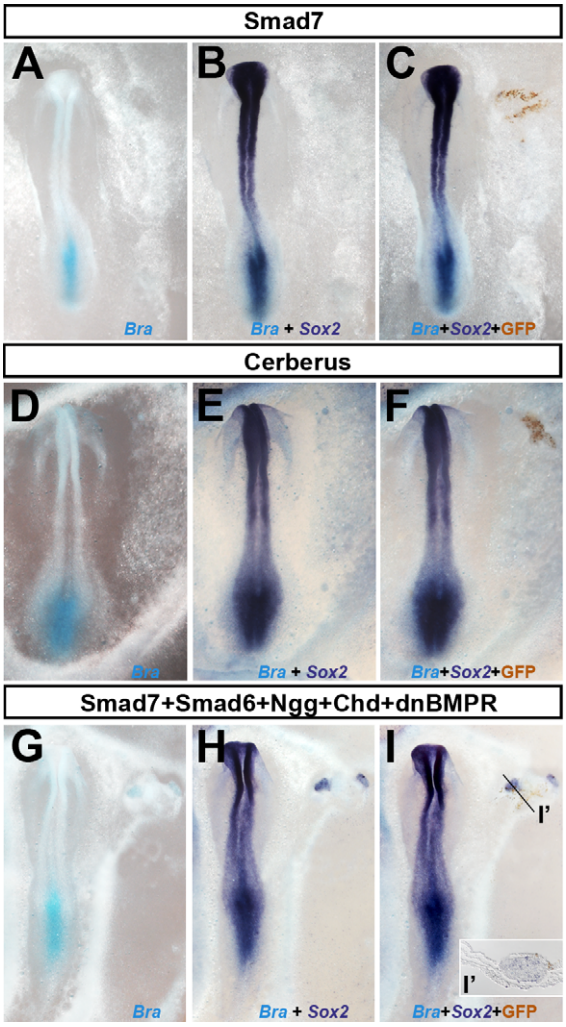

Fig. 1 – Smad7 is not sufficient for neural induction in chick. Electroporation of Smad7 (A–C; 0/12), Cerberus (D–F; 0/4) or a combination of Smad7 + Smad6 + Noggin + Chordin + dnBMPR (0/9) does not induce either *Brachyury* (light blue in A, D and G) or *Sox2* (dark blue in B, E and H; the same embryo to the left). Electroporated cells were recognised by GFP expression (C, F, I and I' in the same embryo to the left). The plane of section is indicated by a black line.