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Pituitary tumors: Cell type-specific roles for BMP-4

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ABSTRACT

BMP-4 plays a crucial role not only in the formation of the anterior pituitary during embryo development but also in the pathogenesis of pituitary tumors in adults. In tumor cells, BMP-4 promotes prolactin secretion and lactotroph cell proliferation through a Smad-estrogen receptor crosstalk but it inhibits ACTH production and cell proliferation of corticotrophs. In addition, BMP-4 increases GH secretion in rat pituitary tumor somatolactotroph GH3 cells and FSHβ subunit gene transcription in the murine gonadotroph cell line, LβT2. Therefore, BMP-4 has a differential role on different types of pituitary tumors: it promotes pituitary prolactinoma while it inhibits corticotroph pathogenesis in Cushing's disease. The modulation of BMP-4 also plays an important role in the therapeutic mechanism of action of bromocriptine, somatostatin analogs and retinoic acid.

1. Introduction

The anterior pituitary gland contains six hormone producing cell types, corticotrophs, gonadotrophs, somatotrophs, lactotrophs and thyrotrophs within the anterior lobe and, in rodents, melanotrophs within the intermediate lobe (Voss and Rosenfeld, 1992). These cell types emerge from a common primordium, exhibiting precise spatial and temporal patterns of expression (Simmons et al., 1990; Japon et al., 1994), which are coordinated by sets of transcription factors during pituitary organogenesis (Rosenfeld et al., 2000). Extrinsic signaling events mediated through the actions of multiple members of a relatively small family of molecules, such as Bone Morphogenic Protein (BMP), specifies the expression and activities of these transcription factors. BMPs belong to the transforming growth factor-β (TGF-β) family of multifunctional secretory peptides that regulate diverse cellular responses, such as cell differentiation, migration, adhesion, proliferation and cell death (Massague, 1998, 2000). More than 20 BMP-related proteins have been indentified, and can be subdivided into several groups based on their structures and functions (Kawabata et al., 1998). BMP-2 and BMP-4, which have 83% aminoacid sequence identity with each other, are the best studied members in the BMP family.

The basic signaling engine consists of a complex of two receptor serine/threonine protein kinases (types I and II) and a family of receptor substrates, the receptor-regulated Smads (R-Smads proteins), that move into the nucleus. BMPs bind to three distinct type II receptors, BMP type II receptor, activin type II receptor and activin type IIB receptors. BMPs bind to three distinct type I receptors, called activin receptor-like kinase (ALK)-2 and ALK-3 and ALK-6. The serine/threonine kinase domains of type II receptors are constitutively active and phosphorylate Gly-Ser domains in the type I receptors upon ligand binding, leading to the activation of type I receptor kinases. Upon receptor activation, BMPs transmit signals through Smad-dependent and independent pathways, including ERK, JNK and p38 MAP kinase pathways (Derynck et al., 2001). Smads are the major signal transducers for the serine/threonine kinase receptors. Upon ligand stimulation and receptor activation, type I receptors phosphorylate R-Smad-1/5/8. Phosphorylated R-Smads subsequently associate with the common-mediator Smad4 and translocate to the nucleus where they can assemble cell type-specific transcription factors and/or transcriptional coactivators/repressors, that regulate target genes (Massague, 1998; Massague and Chen, 2000). Moreover, BMP activity is fundamentally controlled not only by intracellular factors but also by extracellular proteins that modulate BMP action. Thus, BMP effects can be regulated at different levels (Fig. 1). Negative BMP regulators include: 1) inhibition by extracellular binding proteins which bind BMP and prevent its interaction with its specific receptors, such as Follistatin and Noggin, the chordin family, twisted gastrulation protein and the Dan family (vsian-Kretchmer and Hsueh, 2004; Vitt et al., 2001); 2) dominant-negative nonsignaling membrane pseudoreceptors, like Bambi (Onichtchouk et al., 1999); 3) intracellular BMP antagonists, like the inhibitory Smads (I-Smad 6/7) (Wrana, 2000); 4) transcription factors, such as, Yin Yang1 or

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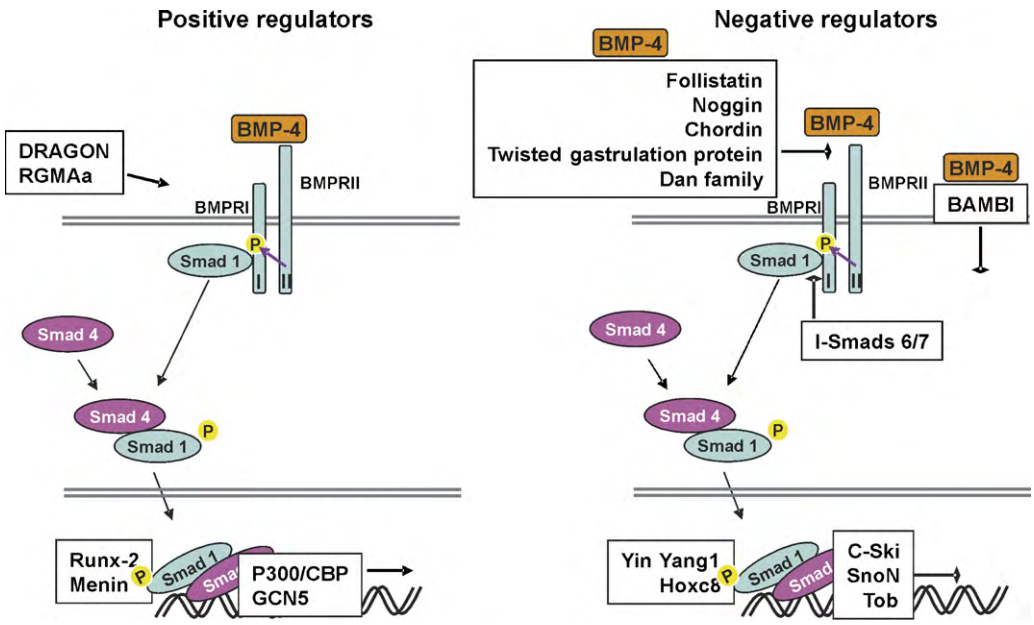

Fig. 1. Control of BMP-4 signaling. BMP-4 activity is modulated by negative and positive regulators operating at different levels. Dot ended arrow: repression of BMP-4 signaling. Triangle ended arrow: activation of BMP-4 signaling.

Hoxc8 (Miyazono et al., 2005); 5) transcriptional corepressors, like, c-Ski, SnoN and Tob (Gazzerro and Canalis, 2006; Massague and Chen, 2000) and 6) ubiquitination and proteasomal degradation of BMP signaling effectors (Gazzerro and Canalis, 2006). Positive regulators are: 1) BMP activating co-receptors, DRAGON and RGMAa, which form a complex with BMP type I receptors and enhance receptor binding to BMP2/4, potentiating their biological effects (Gazzerro and Canalis, 2006); 2) transcriptional coactivators, such as, p300/CBP and GCN5 (Kahata et al., 2004; Derynck et al., 1998) and 3) transcription factors such as Runx-2 and Menin (Miyazono et al., 2005). In this review article, among the various BMP members, we will focus on BMP-4 in different pituitary cell types, emphasizing BMP-4 differential action on them.

2. BMP-4 effects on lactotroph cells

Tumors arising from prolactin-secreting adenohypophysial cells, prolactinomas, are the most common pituitary adenomas. The mechanisms of the pathogenesis of these common adenomas has only partially been described. In rats, estrogen causes lactotroph hyperplasia and enhanced expression of vascular endothelial growth factor (Lohrer et al., 2001), pituitary tumor transforming gene (Heaney et al., 2002) and galanin (Shen et al., 1999). Moreover, estrogen is implicated in lactotroph proliferation during pregnancy (Asa et al., 1982). Conversely, dopamine maintains tonic inhibition of lactotrophs. Dopamine signaling is mediated by a family of receptors including dopamine 1 receptors (D1Rs), which stimulate adenylyl cyclase, and dopamine 2 receptors (D2Rs), the predominant in the anterior pituitary, which inhibit this enzyme. D2R-deficient female mice spontaneously develop massive lactotroph hyperplasia (Kelly et al., 1997) and subsequently invasive lactotroph adenomas (Asa et al., 1999). Using mRNA differential display to compare these tumors versus normal pituitary, we found that the BMP antagonist noggin is down-regulated in prolactinomas from D2R-deficient female mice, whereas BMP-4 itself is overexpressed. Moreover, the analysis of different prolactinoma models, such as estradiol-induced rat prolactinomas and human prolactinomas, confirmed the BMP-4 overexpression compared to normal tissue and other pituitary adenoma types. BMP-4 stimulates, and noggin blocks human prolactinoma development measured as cell proliferation and expression of the cell cycle regulator and target for Smad pathway, c-Myc. GH3 cells stably transfected with a dominant negative of Smad4 or noggin expression vector that inhibit BMP-4 action showed reduced tumorigenicity when injected in nude mice as compared to cells transfected with the corresponding empty vectors. These results indicate a stimulatory role for BMP-4 in lactotroph tumor development *in vivo*. We also demonstrated a crosstalk between BMP-4 and estrogens, which at low concentrations interact through an overlapping additive intracellular signaling mechanism on GH3 cell proliferation and c-myc expression. Co-immunoprecipitation studies demonstrate that under BMP-4 stimulation, Smad4 and Smad1 physically interact with the estrogen receptor (Paez-Pereda et al., 2003) (Fig. 2).

Recently, we described the regulatory mechanism of BMP-4 on hormone secretion and gene transcription in prolacting-producing rat GH3 cells (Giacomini et al., 2009). The crosstalk between BMP-4 and estradiol occurs at both levels, prolactin secretion and promoter transcriptional activity. Whereas BMP-4 inhibited the transcriptional activity of ER at low doses of estradiol, estrogens stimulated transcriptional activity of BMP-4 specific Smads. This reciprocal regulation promotes the specific control of prolactin synthesis in lactotroph cells. The BMP-4 and estrogen crosstalk depends on a BMP-4 response element within the promoter, since mutations of the estrogen response element in the prolactin promoter do not inhibit the cross talk while a Smad1 dominant negative abolished it. Moreover, by serial deletions of the prolactin promoter and CHIP analysis, we defined the region responsive to BMP-4/Smad1 located upstream to the transcriptional start site. Thus, BMP-4/Smad/ER molecular regulatory mechanism plays a central role on prolactin promoter transcriptional regulation (Giacomini et al., 2009) (Fig. 2).

3. BMP-4 effects on somatotroph and gonadotroph cells

Somatotroph adenomas arise from GH-producing cells. GH excess in adults manifests as acromegaly and gigantism results from excessive GH production. Growth hormone-releasing hormone stimulates somatotroph proliferation and GH secretion, whereas somatostatin inhibits it. Recently, it has been shown that