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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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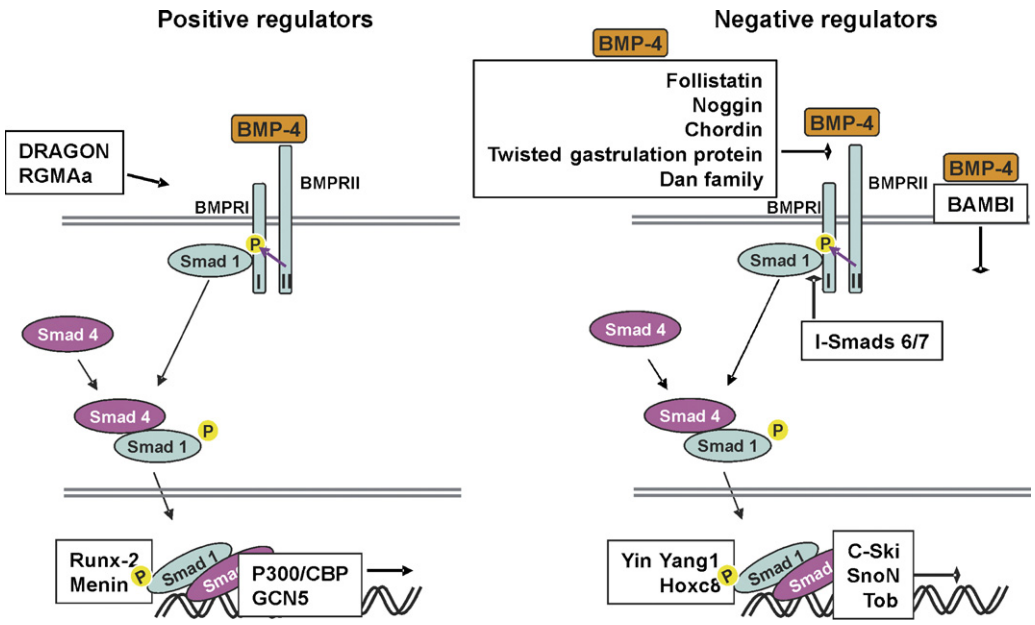

Fig. 1. Control of BMP-4 signaling. BMP-4 activity is modulated by negative and positive regulators operating at different levels. Dot ended arrow: repression of BMP-4 signaling. Triangle ended arrow: activation of BMP-4 signaling.

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

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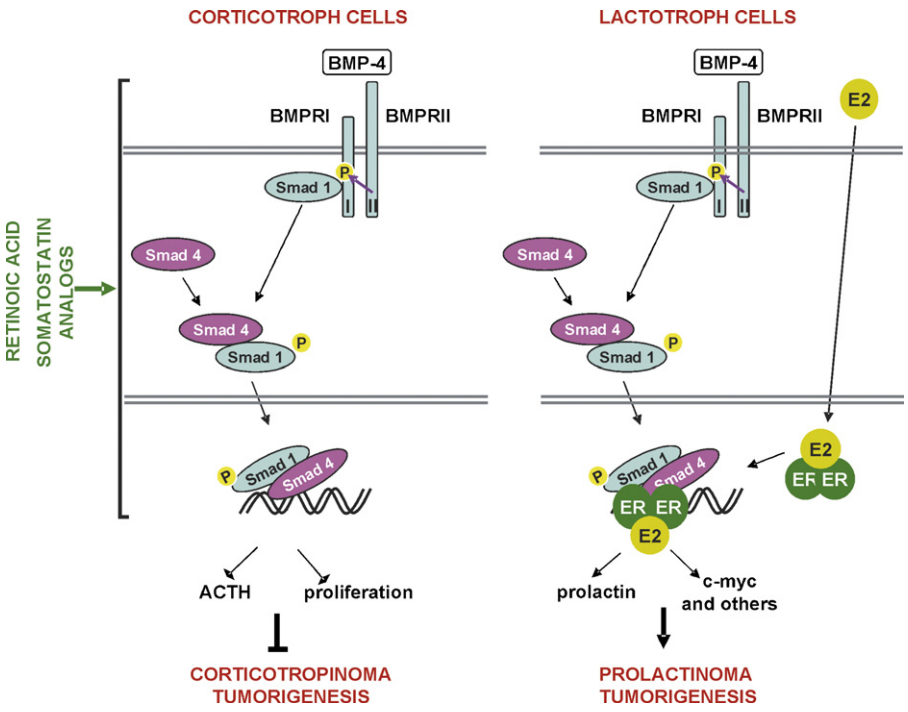

Fig. 2. Differential action of BMP-4 on lactotroph and corticotroph cells. BMP-4 induces prolactin secretion and cell proliferation in lactotroph cells meanwhile inhibits corticotroph cell proliferation and ACTH secretion. Crosstalk between BMP-4 and estrogen signaling due to physical interaction between the Smad1/Smad4 complex with the estrogen receptor was found in GH3 cells. Involvement of BMP-4 in the response to retinoic acid and somatostatin analogs in corticotroph cells.

BMP-4 increases GH secretion and cAMP production induced by forskolin through BMP receptors and the Smad 1,5,8 pathway in GH3 cells (Miyoshi et al., 2008). We also observed that BMP-4 induces GH secretion in a dose-dependent manner and this effect occurs without interaction with estrogen/ER (Giacomini et al., 2009) (Fig. 2). BMP-4 endogenous expression in GH3 cells has been shown to be involved in the response to therapeutic drugs commonly used to treat somatotropinomas, such as bromocriptine and somatostatin analogs (Miyoshi et al., 2008). BMP-4 increases GH secretion and cAMP production induced by forskolin in GH3 cells. In the presence of BMP-4, high octreotide concentrations inhibits the suppressive effect of bromocriptine on GH secretion and cAMP production, which may provide an explanation to the resistance to the combined treatment in somatotropinomas (Miyoshi et al., 2008).

In gonadotroph cells, gonadotropin releasing hormone and gonadal steroids regulate the secretion of the LH and FSH. It has been shown that BMP-4 suppresses FSH production without affecting LH release in the sheep pituitary. In comparison with other physiological factors, BMP antagonized the activin effect known for stimulating FSH release (Faure et al., 2005). On the other hand, other studies in the murine gonadotroph cell line LβT2 show that BMP-4 and other members of the BMP sub-family regulate FSH production in a manner analogous to the activins, which stimulate FSHβ subunit gene transcription through the activation of Smads (Lee et al., 2007). These different effects may be due to the use of a homogenous transformed cell line in contrast to the mixed primary pituitary cell cultures used in Faure's work.

4. Role of BMP-4 in corticotroph cells

The ACTH excess due to a pituitary corticotroph adenoma, known as Cushing's disease, constitutes one of the main causes of Cushing's syndrome that results in glucocorticoid excess. A recent study assessed the action of BMP signaling on the expression of the ACTH precursor, POMC (Nudi et al., 2005). BMP-4 signaling negatively regulates endogenous POMC expression as well as POMC promoter activity in AtT-20 cells. This negative regulation is mediated by the classical BMP signaling pathway involving ALK3/6 receptors and the Smad1/4 transcription factors. The transcription factors Pitx1 and Tpit are critical for teminal differentiation and identity of corticotroph cells, participating in synergistic interactions that are the basis of cell-specific POMC transcription. Upon BMP4 stimulation of corticotroph AtT-20 cells, activated phospho-Smad1 is recruited to the POMC promoter, where it acts through interactions with the Pitx and Tpit transcription factors and subsequently disrupt transcriptional activity (Nudi et al., 2005). BMP-4 in AtT-20 cells inhibits ACTH secretion and cell proliferation *in vitro* (Giacomini et al., 2006) (Fig. 2). In addition, AtT-20 cells stably transfected with a dominant-negative form of the BMP-4 signal cotransducer Smad-4 or the BMP-4 inhibitor noggin have increased tumorigenicity in nude mice, showing that an increase in BMP-4 action blocks corticotroph tumor growth *in vivo* (Giacomini et al., 2006). In the same study we also showed that BMP-4 expression is reduced in corticotroph tumors associated with Cushing's disease compared with normal corticotroph cells. Retinoic acid, a pharmacological agent for different types of cancer, inhibited AtT-20 cell proliferation and *in vivo* tumor cell growth (Paez-Pereda et al., 2001) Moreover, in these cells, retinoic acid induced both BMP-4 transcription and expression. The overexpression of noggin or Smad-4-dominant negative abolishes the inhibitory effect of retinoic acid, showing that BMP-4 induction mediates the therapeutic effect of retinoic acid (Giacomini et al., 2006) (Fig. 2). Recently it was shown that somatostatin analogs suppress CRH-induced ACTH production in AtT-20 cells and that this inhibition is attenuated by noggin. Moreover, SOM230 and octreotide upregulates the BMP receptor signaling -Smad 1/5/8 signaling, ALK3 and BMP type II receptor- and down-regulates inhibitory Smad 6/7. Thus, somatostatin analogs act to facilitate the BMP signaling, which in turn suppresses ACTH production by inhibiting CRH-induced ERK and p38 pathways in corticotroph cells (Tsukamoto et al., 2010). Thus, the BMP system, which mediates the action

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of various substances acting on corticotroph cells, appears to be a promising target for the development of new treatment modalities for corticotroph adenomas.

5. Conclusions

BMP-4 as well as its receptors and their signal transduction pathways play a crucial role in the control of the differentiation and proliferation of the different cell types that constitute the anterior pituitary. In the adult, similar mechanisms involving BMP-4 participate in the disregulation of hormone secretion and cell proliferation that result in the development and progression of pituitary adenomas of different types. The action of BMP-4 is in all these cases cell type-specific, having clearly contrasting effects on lactotroph and corticotroph tumors. The detailed knowledge of the control of the BMP-4 action during development or in adult tissues provides new opportunities to better characterize the mechanisms of pituitary tumor development and the effects of therapeutic drugs.

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