

The pituitary TGF β 1 system as a novel target for the treatment of resistant prolactinomas

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Abstract

Prolactinomas are the most frequently observed pituitary adenomas and most of them respond well to conventional treatment with dopamine agonists (DAs). However, a subset of prolactinomas fails to respond to such therapies and is considered as DA-resistant prolactinomas (DARPs). New therapeutic approaches are necessary for these tumors. Transforming growth factor β 1 (TGF β 1) is a known inhibitor of lactotroph cell proliferation and prolactin secretion, and it partly mediates dopamine inhibitory action. TGF β 1 is secreted to the extracellular matrix as an inactive latent complex, and its bioavailability is tightly regulated by different components of the TGF β 1 system including latent binding proteins, local activators (thrombospondin-1, matrix metalloproteases, integrins, among others), and TGF β receptors. Pituitary TGF β 1 activity and the expression of different components of the TGF β 1 system are regulated by dopamine and estradiol. Prolactinomas (animal models and humans) present reduced TGF β 1 activity as well as reduced expression of several components of the TGF β 1 system. Therefore, restoration of TGF β 1 inhibitory activity represents a novel therapeutic approach to bypass dopamine action in DARPs. The aim of this review is to summarize the large literature supporting TGF β 1 important role as a local modulator of pituitary lactotroph function and to provide recent evidence of the restoration of TGF β 1 activity as an effective treatment in experimental prolactinomas.

Key Words

- ▶ resistant prolactinomas
- ▶ dopamine
- ▶ estradiol
- ▶ TGF β 1

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Pituitary tumors

Pituitary tumors are commonly benign, slow growing adenomas, and account for 10–15% of all intracranial neoplasms (Farrell 2006, Melmed 2015). The prevalence of these tumors is relatively high in the general population, with \sim 77 cases per 100 000 (Daly *et al.* 2009, Fernandez *et al.* 2010), and studies of autopsy specimens identified up to a 20% prevalence of clinically occult pituitary adenomas (Ezzat *et al.* 2004).

Despite their benign features, pituitary tumors can cause considerably morbidity due to both hypersecretion of pituitary trophic hormones and excessive tumor growth that can affect surrounding tissue. Common symptoms of a pituitary tumor compressive ‘mass effect’ include visual impairment, headaches, neurological disorders, and hypopituitarism caused by disruption of the hypothalamic–pituitary axis (Arafah & Nasrallah 2001, Melmed 2011). Based on their size, pituitary adenomas are classified as

microadenomas (<10 mm), macroadenomas (>10 mm), or giant adenomas (>40 mm).

Pituitary tumors usually present with monoclonal growth and can also be classified according to their cell-type origin and hormone secretion. Thus, somatotropinomas secrete growth hormone, prolactinomas secrete prolactin (PRL), thyrotropinomas secrete thyroid-stimulating hormone, and corticotropinomas secrete adrenocorticotropin hormone. In contrast, the non-functioning pituitary adenomas (NFPAs) do not produce any hormone and usually derive from gonadotropes (Kovacs *et al.* 2001, Syro *et al.* 2015).

Prolactinomas

Among functioning pituitary tumors, prolactinomas are the most frequently observed in the clinic (40%) (Ciccarelli *et al.* 2005). Excessive PRL secretion by these tumors leads to hyperprolactinemia, which primarily affects gonadal/reproductive function, causing hypogonadism, galactorrhea, decreased libido, and infertility both in men and women. Large macroprolactinomas can also cause neurological symptoms due to compression of adjacent tissues.

Prolactinomas are usually benign, and although some tumors show invasion into the parasellar compartment and/or sphenoid sinuses, malignant transformation and metastatic spread are extremely rare. Macroprolactinomas tend to be more aggressive and resistant to therapies than microprolactinomas (Wong *et al.* 2015a).

Differences in prolactinoma incidence, tumor size, and behavior have been described among genders. The prevalence of prolactinomas is higher in women during the fertile period (20–50 years), while the frequency is similar between sexes after the fifth decade of life (Colao *et al.* 2003, Gillam *et al.* 2006). Also, women usually present with microprolactinomas whereas men more often present with macroprolactinomas (Delgrange *et al.* 1997, Nishioka *et al.* 2003). These differences have been associated to the earlier diagnosis in woman due to the readily detection of symptoms caused by high prolactin (amenorrhea/galactorrea) (Delgrange *et al.* 1997, Colao *et al.* 2003, Nishioka *et al.* 2003, Gillam *et al.* 2006). However, delayed diagnosis in men may not be the only explanation for the differences in tumor size, since young men also present with macroprolactinomas, and prolactinomas in men tend to be more aggressive, with higher proliferative indexes and lower rates of surgical cure, suggesting a sex-specific behavior for these tumors (Delgrange *et al.* 1997, Gillam & Molitch 2015).

Prolactinoma treatment

The major goals of treatment in patients with prolactinomas are to normalize serum PRL levels, to restore gonadal function, to reduce tumor size, and to preserve or improve residual pituitary function. Prolactin secretion in the normal pituitary is tonically inhibited by hypothalamic dopamine through dopamine D2 receptors (Drd2) expressed on lactotroph cell membranes (Ben Jonathan & Hnasko 2001). The majority of prolactinomas retain an intact response to dopamine inhibition; therefore, medical treatment with dopamine agonists (DAs), such as cabergoline and bromocriptine, represents the first-line therapy for this tumors, including microprolactinomas, macroprolactinomas, and giant prolactinomas (Wong *et al.* 2015b). DAs are highly effective in achieving therapeutic aims with a favorable benefit/risk balance compared with surgical treatment.

DA-resistant prolactinomas

Despite the universal use of DAs and their high efficiency in reducing PRL levels and decreasing tumor size, there is a subset of prolactinomas (10–15%) that do not respond appropriately to the treatment, even at high doses of DA (Vroonen *et al.* 2012). These tumors represent a major challenge for clinical management. DA-resistant prolactinomas (DARPs) are more prevalent in men than woman, occur most frequently as macroprolactinomas, and tend to be invasive, exhibiting extension to the cavernous sinuses.

The molecular mechanisms underlying the escape from dopaminergic regulation in DARPs are not fully elucidated. The main candidate thought to be responsible for resistance is the Drd2 itself. However, to date, no point mutation in the Drd2 gene has been identified in DARPs (Friedman *et al.* 1994, Molitch 2003, 2014, Gillam *et al.* 2006, Vroonen *et al.* 2012). Nevertheless, several mechanisms that lead to reduced Drd2 sensitivity were described in resistant prolactinomas, including evidence of decreased Drd2 mRNA expression, and differential expression of short and long Drd2 isoforms (Caccavelli *et al.* 1994, Vasilev *et al.* 2011, Shimazu *et al.* 2012) reduced Drd2 density and dopamine binding sites in plasma membranes of DARP cells (Pellegrini *et al.* 1989). Alterations in dopamine signaling, such as decreased expression of the inhibitory alpha G protein subunit (G $_{\alpha i2}$), have also been described (Caccavelli *et al.* 1996), as well as decreased expression of the nerve growth factor receptor, which indirectly modulates Drd2 expression (Passos *et al.* 2009). Histological studies on DARPs also revealed increased

angiogenesis, cellular atypia (multinucleated cells, irregular nuclei), and increased proliferation index measured by Ki67 staining, indicating an overall increase in invasiveness (reviewed in Gurlek *et al.* (2007)).

Alternative treatments for DARPs

At present, there is no alternative medical treatment for DARPs, and transsphenoidal surgery is indicated if the tumor is still resectable (Primeau *et al.* 2012, Smith *et al.* 2015). However, some aggressive prolactinomas recur post-operatively and show progressive growth, in which case radiotherapy is the next therapeutic option, but with limited efficiency (Molitch 2014).

The chemotherapy agent temozolomide (TMZ) has been recently used as a last resort therapy and showed a moderately successful response in large aggressive DARPs (McCormack *et al.* 2011, Whitelaw *et al.* 2012, Liu *et al.* 2015). However, the efficacy of TMZ therapy in aggressive pituitary adenomas remains controversial (Bruno *et al.* 2015), and clinical trials are now necessary to establish the indications, doses, and duration of TMZ administration to more accurately determine the efficacy of this agent.

New therapeutic approaches are necessary for those prolactinomas that are resistant to conventional treatments. Few reports of experimental treatments can be found in the literature and show variable effectiveness in pre-clinical and *in vitro* models. For instance, treatment with somatostatin receptor (SSTR) analogs failed to inhibit prolactin secretion by cultured cells derived from DARPs (Fusco *et al.* 2008) despite the expression of all subtypes of SSTR in human prolactinomas (Jaquet *et al.* 1999).

Based on the counteracting effects of estradiol on dopamine action in lactotrophs, targeting of the estrogen receptor with tamoxifen was evaluated in the precabergoline era in patients with bromocriptine-resistant prolactinomas, but only a moderated reduction in PRL levels was observed (Volker *et al.* 1982). A novel anti-estrogen agent, fulvestrant, also reduced PRL secretion in pituitary cell lines and decreased tumor growth and serum PRL in estrogen-induced prolactinomas in rats (Cao *et al.* 2014).

In the search for new therapeutic targets for DARPs

Studies in animal models of prolactinomas with altered sensitivity to DA, such as the estrogen-induced prolactinomas in rats and the Drd2 knockout mice (Drd2^{-/-}), have been very helpful to identify molecular pathways altered in these tumors and to test potential future

therapies. Many of these studies suggest that the deregulation of local growth factors and extracellular matrix (ECM) remodeling participate in the pathogenesis of prolactinomas by promoting cell proliferation, angiogenesis, and invasiveness (Paez-Pereda *et al.* 2005, Cristina *et al.* 2005, 2007, Recouvreux *et al.* 2013).

Transforming growth factor β 1 (TGF β 1), a well-known inhibitor in lactotroph physiology, has been recently identified as a novel target for the development of new therapies in resistant prolactinomas.

The complexity of the TGF β system and biology

TGF β s are multifunctional cytokines known to play crucial regulatory roles in cellular proliferation and differentiation, angiogenesis, ECM modification, and immunomodulation (Yoshinaga *et al.* 2008), and have powerful effects on embryogenesis, development, and tissue homeostasis (Heldin *et al.* 2009, Galvin-Burgess *et al.* 2013, Itoh *et al.* 2014). The TGF β family comprises more than 30 highly pleiotropic molecules including activins, inhibins, nodal, bone morphogenetic proteins (BMPs), the anti-Müllerian hormone, and several growth and differentiation factors among others (Derynck & Akhurst 2007). Three isoforms of TGF β have been identified (TGF β 1, 2, and 3).

The importance of TGF β 1 is clearly demonstrated by the fact that TGF β 1 null mutation causes excessive inflammatory response and early death (Kulkarni *et al.* 1993). On the contrary, an excess of TGF β 1 activity is associated to connective tissue diseases, fibrosis and inflammation, cirrhosis, arthritis and sclerosis, cardiovascular diseases, and cancer, making TGF β an interesting target for therapeutic research (Pohlars *et al.* 2009, Akhurst & Hata 2012, Doyle *et al.* 2012).

Nearly all cell types are sensitive to TGF β 1, but TGF β action is highly dependent on cell type, developmental stage, physiological-pathological conditions, interaction with components of the ECM and, once bound to its receptor, interaction with other signaling pathways.

The potent biological activity of TGF β 1 is tightly regulated at different levels, including its synthesis, secretion, storage, and activation. The three TGF β isoforms are synthesized as homodimeric precursor molecules that contain a pro-peptide sequence, so-called latency-associated peptide (LAP), and the functional mature TGF β sequence (Fig. 1, 1). After proteolytic processing by furin within the trans-Golgi, LAP remains associated with the mature TGF β by non-covalent interactions in a small latent

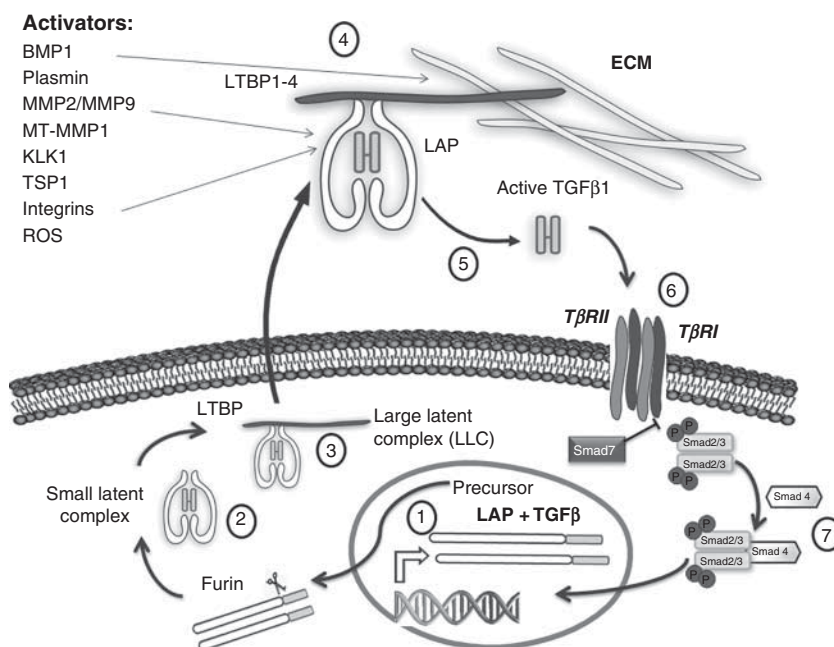


Figure 1

The biology of TGF β system. TGF β is synthesized as homodimeric precursor containing a pro-peptide sequence LAP (1) and then processed by furin. LAP remains associated with the mature TGF β by non-covalent interactions in a small latent complex (2), which in turn is linked by disulfide bonds to one of the latent TGF β -binding proteins (LTBP1–4) (3). TGF β is secreted as part of this large latent TGF β complex (LLC) (3), and it is incorporated as

component of the extracellular matrix (ECM), which acts as a reservoir of the cytokine (4). TGF β must undergo a highly regulated activation process by which mature cytokine is released (5) to enable binding to its receptor complex (T β RI and T β RII) (6) and signal through Smad2/Smad3 pathway (7). Known TGF β activators are listed in the upper left.

complex (Fig. 1, 2). While in the endoplasmic reticulum, LAP is linked, by disulfide bonds, with a latent TGF β -binding protein (LTBP) (Fig. 1, 3). LTBPs belong to a family of large secretory ECM glycoproteins. Although LTBPs are not required for maintenance of TGF β latency, they facilitate the secretion, storage, and activation of the TGF β –LAP complex (Rifkin 2005). TGF β is secreted as part of this large latent TGF β complex (LLC; Fig. 1, 3) and is incorporated as component of the ECM, which acts as a cytokine reservoir (Fig. 1, 4). Trapped in the ECM, TGF β remains latent because of persistent binding of LAP and must undergo a highly regulated activation process by which mature cytokine is released from its latent complex to enable the active form to bind and signal through its receptor (Fig. 1, 5). Latent TGF β activation is a crucial event in governing the cytokine biological function and availability in the ECM (Annes *et al.* 2003, 2004, Rifkin 2005).

Several latent TGF β 1 activators have been described, including proteases such as plasmin, matrix metalloproteinase 2 (MMP2), matrix metalloproteinase 9 (MMP9), BMP-1, thrombospondin-1 (TSP1), kallikrein 1 (KLK1), integrins α v β 6 and α v β 8, and reactive oxygen species or

pH changes in the local environment, among others. However, their individual biological importance in releasing TGF β 1 from its latent complex and their local regulation in different tissues are not fully understood (Annes *et al.* 2003, 2004, Yoshinaga *et al.* 2008). Since all these factors are related to ECM perturbations, the latent TGF β complex has been postulated as a ‘sensor’ of environment disturbances (Annes *et al.* 2003).

Once TGF β 1 is released from the ECM, the active cytokine binds to its transmembrane receptor, the type II TGF β receptor (T β RII), a constitutively active kinase that recruits and phosphorylates type I TGF β receptor (T β RI) forming a heterotetrameric complex of serine/threonine kinase receptors containing two type I and two type II subunits (Fig. 1, 6). Next, T β RI phosphorylates the downstream receptor-associated Smads (R-Smads: Smad2/Smad3), which form a heteromeric complex with Smad4, and translocate to the nucleus to regulate the transcription of target genes (Fig. 1, 7). Additionally, an inhibitory Smad, Smad7, competes with the Smad2/3 for binding to the activated T β RI, thereby exerting a negative effect on TGF β /Smad signaling (Shi & Massague 2003, Han *et al.* 2015).

This pathway is known as 'the canonical' TGFβ signaling pathway. However, TGFβ can also signal through Smad-independent pathways including the mitogen-activated protein kinases (ERK1/2, JNK, p38), small GTP-binding proteins (Ras, RhoA, Rac1, CDC42, mTOR), the NF-κB pathway and Wnt/β-catenin pathway, the AKT/PKB pathway, and phosphatidylinositol-4, 5-bisphosphate 3-kinase (PI3K) (Attisano & Wrana 2002, Derynck & Zhang 2003, Moustakas & Heldin 2005).

As a multifunctional cytokine with powerful effects on cell proliferation, cellular migration, and inflammation, TGFβ signaling has been targeted for drug development, and numerous strategies have proceeded through preclinical to clinical trials (reviewed in Akhurst & Hata (2012)).

TGFβ1 in the pituitary: a brief history

The earliest publications on TGFβ1 action in the pituitary date from the late 1980s and early 1990s (Ying *et al.* 1986, Mueller & Kudlow 1991). Sarkar *et al.* (1992) were the first to demonstrate local TGFβ1 mRNA and protein expression in the pituitary gland, and the inhibitory action of TGFβ1 on prolactin secretion and lactotrophic growth in 1992. Although these first evidences were found in animal models (rat), TGFβ1 and TβRII expression were promptly found to be expressed in human pituitaries (Halper *et al.* 1992, Fujiwara *et al.* 1995), as well as in human pituitary adenomas (Fujiwara *et al.* 1995, Jin *et al.* 1997).

The main physiological modulators of lactotroph function are dopamine and estradiol, which exert inhibitory and stimulatory actions respectively (Ben Jonathan & Hnasko 2001). The pro-mitotic effect of estradiol (pharmacological doses) and its role in prolactinoma induction is very well described in the literature (Heaney *et al.* 2002). However, estrogens also participate in the lactotroph cell turnover in normal pituitary glands, sensitizing lactotroph cells to apoptotic stimuli. Therefore, the effect of estradiol on lactotroph function depends on the dose and normal/tumoral condition of the cells (Pisera *et al.* 2004, Zaldivar *et al.* 2009, Jaita *et al.* 2015). Interestingly, dopamine and estradiol also regulate the expression of both TGFβ1 and its receptor, but in opposite ways. Thus, while estrogen stimulation increases serum PRL levels and lactotroph proliferation, it decreases the expression of TGFβ1 in the anterior pituitary. On the contrary, dopamine, acting through the Drd2, up-regulates TGFβ1 expression and secretion *in vivo* and *in vitro*, with a concomitant reduction in the proliferation rate of lactotrophs. Moreover, it has been proposed that TGFβ1 partially mediates the

inhibitory effect of dopamine on lactotroph proliferation (Sarkar *et al.* 2005). Our group has recently described that the amount of pituitary active TGFβ1 is also locally regulated by dopamine and estradiol treatment in mice; moreover, we found an inverse correlation between active TGFβ1 levels and serum PRL (Recouvreux *et al.* 2011). It is worth noting that <8% of total pituitary TGFβ1 was found in the active form, similar to what has been described in other tissues (Yoshinaga *et al.* 2008). This underscores the tightly regulation of the latent TGFβ activation process.

Other important factor regulating lactotroph homeostasis is PRL itself, acting through the PRL receptor (PRLR). It has been shown that endogenous PRL exerts paracrine/autocrine anti-proliferative and proapoptotic effects on lactotrophs; moreover, knockout mice lacking PRLR develop prolactinomas, further demonstrating the important role of PRL in the negative feedback on lactotroph function (Schuff *et al.* 2002, Ferraris *et al.* 2012, 2014).

Whether PRL can as well regulate TGFβ1 expression or function in the pituitary gland is an open question that has not yet been addressed.

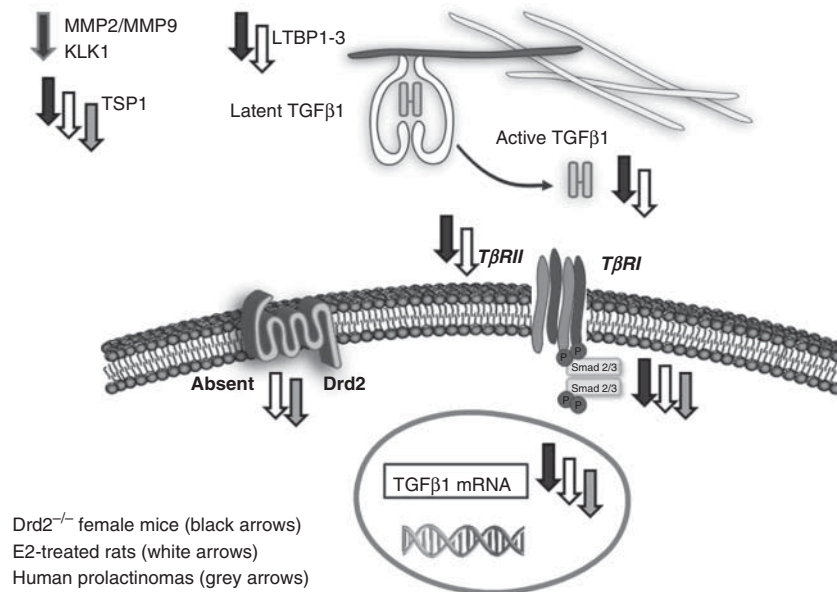
Alterations in the components of the TGFβ1 system during prolactinoma development

Evidences of TGFβ1 alterations in estradiol-induced prolactinomas in rats

The estrogen-treated rat is a well-known model for prolactinoma development with increased pituitary weight, hyperprolactinemia, lactotroph hyperplasia, and reduced dopaminergic action at the pituitary level (Heaney *et al.* 1999, 2002). Furthermore, estradiol treatment decreases pituitary TGFβ1 and TβRII mRNA and protein, together with an increase in PRL levels (Sarkar *et al.* 1992, Pastorcic *et al.* 1995, De *et al.* 1996) (Fig. 2). Therefore, the inhibition of TGFβ1 and TβRII might cooperate in the development of prolactinomas induced by estradiol (Hentges & Sarkar 2001). In agreement with this idea, pituitary tumorigenesis induced by estrogen treatment is greatly accelerated in TβRII heterozygous knockout mice (TβRII^{+/-}) where the expression of TβRII is markedly reduced (Shida *et al.* 1998).

TGFβ1 alterations in the prolactinoma development in Drd2^{-/-} mice

Another well-characterized model to study prolactinoma development are the transgenic knockout mice lacking functional Drd2 (Drd2^{-/-}). This model represents an

**Figure 2**

Alterations of TGF β 1 system in prolactinomas. Decreased expression of different components of pituitary TGF β 1 system in prolactinoma models are represented with down-pointed arrows. Grey arrows indicate findings

in human prolactinoma specimens, black arrows indicate findings in Drd2^{-/-} mice, and white arrows indicate findings in estradiol-induced prolactinomas in rats.

excellent model to mimic DA resistance. Because of the absence of inhibitory dopaminergic control, these mice display chronic hyperprolactinemia and lactotroph hyperplasia (Kelly *et al.* 1997, Diaz-Torga *et al.* 2002, Cristina *et al.* 2006), but the loss of dopamine inhibition has deeper effect on pituitary function in female than in male mice (Saiardi *et al.* 1997, Diaz-Torga *et al.* 2002). In females, the increase in serum prolactin levels is much more pronounced than in males, and females develop lactotroph hyperplasia from 6 months onwards, while age-matched Drd2-deficient males develop pituitary lactotroph adenomas at 17–20 months of age (Asa *et al.* 1999).

Interestingly, active and total TGF β 1 levels, as well as T β RII and LTBP1 expression, are reduced in Drd2^{-/-} pituitaries compared to controls (WT), highlighting the stimulatory role of dopamine on pituitary TGF β 1 system (Recouvreux *et al.* 2011) (Fig. 2). On the other hand, the impact of the chronic loss of dopaminergic tone on the TGF β 1 system was also stronger in females, evidenced by the down-regulation of several putative TGF β 1 activators (MMP2, MMP9, MT1–MMP, TSP1, and kallikrein) as well as the decreased expression of TGF β 1 target genes observed only in females (Drd2^{-/-} vs their WT counterpart). In this model, we found sex differences in the regulation of the TGF β 1 system: males express higher levels of several components of the TGF β 1 system, and it could be due to

the lower serum estradiol levels present in males, as estradiol negatively controls most of the components of the system (Recouvreux *et al.* 2013). We suggest that stronger pituitary TGF β 1 system could protect males from excessive lactotroph proliferation and prolactinoma development. Then, sex differences found in the regulation of the TGF β 1 system could explain sex differences found in the incidence of prolactinoma development in this model.

TGF β 1 alterations in human pituitary tumors

In humans, the expression of several components of the TGF β signaling pathway was recently compared in five normal human anterior pituitaries, 29 invasive NFPAs and 21 non-invasive NFPAs (Zhenye *et al.* 2014). This report demonstrated that TGF β 1 mRNA expression and p-Smad3 protein levels gradually decreased, while Smad7 mRNA levels gradually increased from normal anterior pituitaries to non-invasive NFPAs and invasive NFPAs. The authors concluded that the activity of TGF β signaling would be limited during tumor development.

Recent work also described a significant down-regulation of the TGF β 1/Smad signaling cascade in 12 cases of DARPs compared to normal human anterior pituitaries. The authors showed that TGF β 1 mRNA levels,

Smad2 and Smad3 mRNA, and protein expression were significantly decreased in human prolactinomas (Li *et al.* 2015, Fig. 2).

Overall, decreased TGF β 1 activity and decreased expression of different components of the TGF β 1 system have been described in animal models of prolactinomas as well as in human prolactinomas. Taking into account that TGF β 1 inhibits lactotroph proliferation and PRL synthesis and secretion, we speculate that recovering local TGF β 1 activity could contribute to revert the adenoma development and to normalize hyperprolactinemia.

Recovery of local TGF β 1 activity: successful treatment in an experimental model of prolactinoma

TSP1 is one of the main physiologic latent TGF β 1 activators *in vitro* and *in vivo* (Schultz-Cherry *et al.* 1994). TSP1 is a large multifunctional matrix glycoprotein involved in cell growth, adhesion, and migration (Lawler 2002). TSP1 also functions as an endogenous anti-angiogenic factor, inhibiting the proliferation and migration of endothelial cells by interaction with its cell surface receptor CD36 and by antagonizing VEGF activity (Lawler & Lawler 2012).

Based on the CD36-binding peptide sequence from TSP1, small molecules were developed to mimic TSP1 anti-angiogenic properties (Haviv *et al.* 2005). Several of these new drugs were able to slow tumor growth in preclinical models (Anderson *et al.* 2007, Yang *et al.* 2007, Garside

et al. 2010). Among them, ABT-510 and ABT-898 (Abbott Laboratories), two of such TSP1 analogs, were assayed in several solid tumors (Haviv *et al.* 2005). ABT-510 was evaluated in phase II clinical trials for the treatment of head and neck cancer, non-small cell lung cancer, lymphoma, and renal cell carcinoma (Haviv *et al.* 2005, Ebbinghaus *et al.* 2007, Markovic *et al.* 2007, Yang *et al.* 2007, Gordon *et al.* 2008, Nabors *et al.* 2010). The second-generation TSP1 synthetic analogue, ABT-898, was found to have greater potency than ABT-510 and is expected to have greater efficacy than the other available TSP1 mimetic peptides (Garside *et al.* 2010, Campbell *et al.* 2011) due to its lower clearance rate.

Immunoreactive TSP1 is present in the anterior pituitary, particularly in endothelial cells (Burns & Sarkar 1993), and TSP1 levels and its anti-angiogenic effect are reduced in prolactinomas induced by estradiol in rats (Sarkar *et al.* 2007) and in the hyperplastic pituitaries of *Drd2*^{-/-} mice (Recouvreux *et al.* 2013). TSP1 expression was also found down-regulated in invasive vs non-invasive prolactinomas in humans (Jiang *et al.* 2012).

Given that: i) TSP1 is an anti-angiogenic factor, ii) TSP1 expression is reduced during prolactinoma development, iii) TSP1 is a known TGF β 1 activator, iv) TGF β 1 activity is also reduced during the development of prolactinomas, and v) TGF β 1 is an inhibitory factor of lactotroph proliferation and synthesis, we speculated that treatments that improve pituitary TSP1 and/or TGF β 1 activities could reduce the progression of prolactinomas.

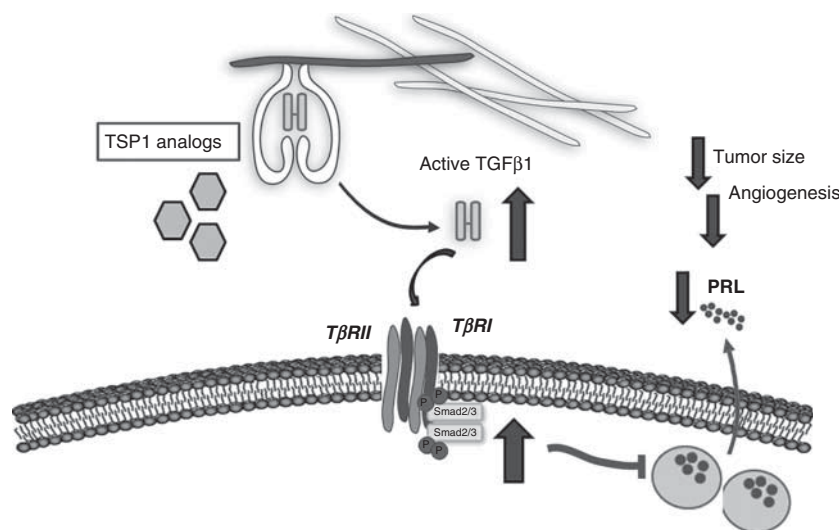


Figure 3

Recovery of TGF β 1 activity emerges as a novel therapeutic target for treatment of dopamine agonist resistant prolactinomas. Treatment with thrombospondin-1 analogs (ABT-510, ABT-898) recover pituitary TGF β 1

activity, reduce tumor size, tumor angiogenesis, and proliferation markers, as well as serum prolactin in estradiol-induced prolactinomas in female rats.

We first evaluated whether the TSP1 analogs were able to activate TGFβ1 in the pituitary. In fact, an *in vivo* short-term treatment (100 mg/kg ABT-510 i.p.; 30 min) enhanced the biological activity of pituitary TGFβ1, with a concomitant reduction in serum prolactin levels (Recouvreux *et al.* 2012). Notably, same effect was observed after short-term treatment with ABT-510 in female rats carrying prolactinoma induced by chronic estradiol treatment. We next evaluated whether an *in vivo* treatment for 2 weeks with the TSP1 analogs could counteract the development of estradiol-induced prolactinomas in rats. ABT-510 and ABT-898 treatment (100 mg/kg i.p., thrice a week for 2 weeks) significantly decreased pituitary tumor size and reduced tissue angiogenesis and pituitary proliferation markers, as well as serum prolactin levels, in female rats with prolactinomas induced by chronic treatment with estradiol (Recouvreux *et al.* 2012). Furthermore, ABT-510 and ABT-898 treatment markedly increased active TGFβ1 content, measured by ELISA within the tumors. The increase in cytokine activation was also reflected in the recovery of intrapituitary p-Smad2/3 expression (Fig. 3). Besides from the well-known anti-angiogenic effect of these TSP1 mimetic peptides, the improvement of the local TGFβ1 biological activity most likely contributed to the reduction in serum prolactin and in the inhibition of prolactinoma growth.

Overall conclusions and perspectives

Prolactinomas are the most frequent pituitary tumors in adults accounting for 60% of all functioning pituitary tumors (Ciccarelli *et al.* 2005). Even though prolactinomas are usually benign and in most cases respond well to treatment with dopaminergic agents, 15% of these tumors are resistant to classical therapy, become invasive and aggressive, and require extirpation. The mechanisms underlying the escape from dopaminergic regulation in DARPs are not fully understood, and the main candidate to be responsible for resistance is the Drd2 itself. Since TGFβ1 mediates, at least partially, the inhibitory action exerted by dopamine on lactotrophs, and reduced TGFβ1 activity is a common feature of prolactinoma development, treatments that improve pituitary TGFβ1 activity represent a rational approach to develop alternative therapies for DARPs. Supporting this, we provide evidence of the effectiveness of a treatment with the small TSP1 analog peptides ABT-510 and ABT-898 to restore TGFβ1 activity and to counteract prolactinoma development in rats.

Taken together the data summarized here, the recovery of TGFβ1 activity emerges as a novel therapeutic target for treatment of DARPs.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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References

- Akhurst RJ & Hata A 2012 Targeting the TGFβ signalling pathway in disease. *Nature Reviews. Drug Discovery* **11** 790–811. (doi:10.1038/nrd3810)
- Anderson JC, Grammer JR, Wang W, Nabors LB, Henkin J, Stewart JE Jr & Gladson CL 2007 ABT-510, a modified type 1 repeat peptide of thrombospondin, inhibits malignant glioma growth *in vivo* by inhibiting angiogenesis. *Cancer Biology & Therapy* **6** 454–462. (doi:10.4161/cbt.6.3.3630)
- Annes JP, Munger JS & Rifkin DB 2003 Making sense of latent TGFβ activation. *Journal of Cell Science* **116** 217–224. (doi:10.1242/jcs.00229)
- Annes JP, Chen Y, Munger JS & Rifkin DB 2004 Integrin αVβ6-mediated activation of latent TGF-β requires the latent TGF-β binding protein-1. *Journal of Cell Biology* **165** 723–734. (doi:10.1083/jcb.200312172)
- Arafah B & Nasrallah M 2001 Pituitary tumors: pathophysiology, clinical manifestations and management. *Endocrine-Related Cancer* **8** 287–305. (doi:10.1677/erc.0.0080287)
- Asa SL, Kelly MA, Grandy DK & Low MJ 1999 Pituitary lactotroph adenomas develop after prolonged lactotroph hyperplasia in dopamine D2 receptor-deficient mice. *Endocrinology* **140** 5348–5355. (doi:10.1210/endo.140.11.7118)
- Attisano L & Wrana JL 2002 Signal transduction by the TGF-β superfamily. *Science* **296** 1646–1647. (doi:10.1126/science.1071809)
- Ben Jonathan N & Hnasko R 2001 Dopamine as a prolactin (PRL) inhibitor. *Endocrine Reviews* **22** 724–763. (doi:10.1210/edrv.22.6.0451)
- Bruno OD, Juarez-Allen L, Christiansen SB, Manavela M, Danilowicz K, Vigovich C & Gomez RM 2015 Temozolomide therapy for aggressive pituitary tumors: results in a small series of patients from Argentina. *International Journal of Endocrinology* **2015** 587893. (doi:10.1155/2015/587893)
- Burns G & Sarkar DK 1993 Transforming growth factor-beta1-like immunoreactivity in the pituitary gland of the rat: effect of estrogen. *Endocrinology* **133** 1444–1449. (doi:10.1210/endo.133.3.8365375)
- Caccavelli L, Feron F, Morange I, Rouer E, Benarous R, Dewailly D, Jaquet P, Kordon C & Enjalbert A 1994 Decreased expression of the two D2 dopamine receptor isoforms in bromocriptine-resistant prolactinomas. *Neuroendocrinology* **60** 314–322. (doi:10.1159/000126764)
- Caccavelli L, Morange-Ramos I, Kordon C, Jaquet P & Enjalbert A 1996 Alteration of G alpha subunits mRNA levels in bromocriptine resistant

- prolactinomas. *Journal of Neuroendocrinology* **8** 737–746. (doi:10.1046/j.1365-2826.1996.04902.x)
- Campbell N, Greenaway J, Henkin J & Petrik J 2011 ABT-898 induces tumor regression and prolongs survival in a mouse model of epithelial ovarian cancer. *Molecular Cancer Therapeutics* **10** 1876–1885. (doi:10.1158/1535-7163.MCT-11-0402)
- Cao L, Gao H, Gui S, Bai G, Lu R, Wang F & Zhang Y 2014 Effects of the estrogen receptor antagonist fulvestrant on F344 rat prolactinoma models. *Journal of Neuro-oncology* **116** 523–531. (doi:10.1007/s11060-013-1351-8)
- Ciccarelli A, Daly AF & Beckers A 2005 The epidemiology of prolactinomas. *Pituitary* **8** 3–6. (doi:10.1007/s11102-005-5079-0)
- Colao A, Sarno AD, Cappabianca P, Briganti F, Pivonello R, Somma CD, Faggiano A, Biondi B & Lombardi G 2003 Gender differences in the prevalence, clinical features and response to cabergoline in hyperprolactinemia. *European Journal of Endocrinology* **148** 325–331. (doi:10.1530/eje.0.1480325)
- Cristina C, Diaz-Torga G, Baldi A, Gongora A, Rubinstein M, Low MJ & Becu-Villalobos D 2005 Increased pituitary vascular endothelial growth factor-A in dopaminergic D2 receptor knockout female mice. *Endocrinology* **146** 2952–2962. (doi:10.1210/en.2004-1445)
- Cristina C, García-Tornadú I, Diaz-Torga G, Rubinstein M, Low MJ & Becu-Villalobos D 2006 The dopaminergic D2 receptor knockout mouse: an animal model of prolactinoma. *Frontiers of Hormone Research* **35** 50–63. (doi:10.1159/000094308)
- Cristina C, Diaz-Torga G, Gongora A, Guida MC, Perez-Millan MI, Baldi A & Becu-Villalobos D 2007 Fibroblast growth factor-2 in hyperplastic pituitaries of D2R knockout female mice. *American Journal of Physiology, Endocrinology and Metabolism* **293** E1341–E1351. (doi:10.1152/ajpendo.00260.2007)
- Daly AF, Tichomirowa MA & Beckers A 2009 The epidemiology and genetics of pituitary adenomas. *Best Practice & Research. Clinical Endocrinology & Metabolism* **23** 543–554. (doi:10.1016/j.beem.2009.05.008)
- De A, Morgan TE, Speth RC, Boyadjieva N & Sarkar DK 1996 Pituitary lactotrope expresses transforming growth factor beta (TGF β) type II receptor mRNA and protein and contains 125I-TGF β 1 binding sites. *Journal of Endocrinology* **149** 19–27. (doi:10.1677/joe.0.1490019)
- Delgrange E, Trouillas J, Maiter D, Donckier J & Tournaire J 1997 Sex-related difference in the growth of prolactinomas: a clinical and proliferation marker study. *Journal of Clinical Endocrinology and Metabolism* **82** 2102–2107. (doi:10.1210/jcem.82.7.4088)
- Derynck R & Akhurst RJ 2007 Differentiation plasticity regulated by TGF- β family proteins in development and disease. *Nature Cell Biology* **9** 1000–1004. (doi:10.1038/ncb434)
- Derynck R & Zhang YE 2003 Smad-dependent and Smad-independent pathways in TGF- β family signalling. *Nature* **425** 577–584. (doi:10.1038/nature02006)
- Diaz-Torga G, Feierstein C, Libertun C, Gelman D, Kelly MA, Low MJ, Rubinstein M & Becu-Villalobos D 2002 Disruption of the D2 dopamine receptor alters GH and IGF-I secretion and causes dwarfism in male mice. *Endocrinology* **143** 1270–1279. (doi:10.1210/endo.143.4.8750)
- Doyle JJ, Gerber EE & Dietz HC 2012 Matrix-dependent perturbation of TGF β signaling and disease. *FEBS Letters* **586** 2003–2015. (doi:10.1016/j.febslet.2012.05.027)
- Ebbinghaus S, Hussain M, Tannir N, Gordon M, Desai AA, Knight RA, Humerickhouse RA, Qian J, Gordon GB & Figlin R 2007 Phase 2 study of ABT-510 in patients with previously untreated advanced renal cell carcinoma. *Clinical Cancer Research* **13** 6689–6695. (doi:10.1158/1078-0432.CCR-07-1477)
- Ezzat S, Asa SL, Couldwell WT, Barr CE, Dodge WE, Vance ML & McCutcheon IE 2004 The prevalence of pituitary adenomas: a systematic review. *Cancer* **101** 613–619. (doi:10.1002/cncr.20412)
- Farrell WE 2006 Pituitary tumours: findings from whole genome analyses. *Endocrine-Related Cancer* **13** 707–716. (doi:10.1677/erc.1.01131)
- Fernandez A, Karavitaki N & Wass JA 2010 Prevalence of pituitary adenomas: a community-based, cross-sectional study in Banbury (Oxfordshire, UK). *Clinical Endocrinology* **72** 377–382. (doi:10.1111/j.1365-2265.2009.03667.x)
- Ferraris J, Boutillon F, Bernadet M, Seilicovich A, Goffin V & Pisera D 2012 Prolactin receptor antagonism in mouse anterior pituitary: effects on cell turnover and prolactin receptor expression. *American Journal of Physiology, Endocrinology and Metabolism* **302** E356–E364. (doi:10.1152/ajpendo.00333.2011)
- Ferraris J, Zarate S, Jaita G, Boutillon F, Bernadet M, Auffret J, Seilicovich A, Binart N, Goffin V & Pisera D 2014 Prolactin induces apoptosis of lactotropes in female rodents. *PLoS ONE* **9** e97383. (doi:10.1371/journal.pone.0097383)
- Friedman E, Adams HF, Hoog A, Gejman PV, Carson E, Larsson C, De Marco L, Werner S, Fahlbusch R & Nordenskjöld M 1994 Normal structural dopamine type 2 receptor gene in prolactin-secreting and other pituitary tumors. *Journal of Clinical Endocrinology and Metabolism* **78** 568–574. (doi:10.1210/jcem.78.3.7907340)
- Fujiwara K, Ikeda H & Yoshimoto T 1995 Immunohistochemical demonstration of TGF- β -receptor type II in human pituitary adenomas. *Acta Histochemica* **97** 445–454. (doi:10.1016/S0065-1281(11)80071-1)
- Fusco A, Gunz G, Jaquet P, Dufour H, Germanetti AL, Culler MD, Barlier A & Saveanu A 2008 Somatostatinergic ligands in dopamine-sensitive and -resistant prolactinomas. *European Journal of Endocrinology* **158** 595–603. (doi:10.1530/EJE-07-0806)
- Galvin-Burgess KE, Travis ED, Pierson KE & Vivian JL 2013 TGF- β -superfamily signaling regulates embryonic stem cell heterogeneity: self-renewal as a dynamic and regulated equilibrium. *Stem Cells* **31** 48–58. (doi:10.1002/stem.1252)
- Garside SA, Henkin J, Morris KD, Norvell SM, Thomas FH & Fraser HM 2010 A thrombospondin-mimetic peptide, ABT-898, suppresses angiogenesis and promotes follicular atresia in pre- and early-antral follicles *in vivo*. *Endocrinology* **151** 5905–5915. (doi:10.1210/en.2010-0283)
- Gillam MP & Molitch ME 2015 Prolactinoma. In *The Pituitary*, 3rd edn. Ed S Melmed. Philadelphia, PA, USA: Elsevier, Inc.
- Gillam MP, Molitch ME, Lombardi G & Colao A 2006 Advances in the treatment of prolactinomas. *Endocrine Reviews* **27** 485–534. (doi:10.1210/er.2005-9998)
- Gordon MS, Mendelson D, Carr R, Knight RA, Humerickhouse RA, Iannone M & Stopeck AT 2008 A phase 1 trial of 2 dose schedules of ABT-510, an antiangiogenic, thrombospondin-1-mimetic peptide, in patients with advanced cancer. *Cancer* **113** 3420–3429. (doi:10.1002/cncr.23953)
- Gurlek A, Karavitaki N, Ansorge O & Wass JA 2007 What are the markers of aggressiveness in prolactinomas? Changes in cell biology, extracellular matrix components, angiogenesis and genetics *European Journal of Endocrinology* **156** 143–153. (doi:10.1530/eje.1.02339)
- Halper J, Parnell PG, Carter BJ, Ren P & Scheithauer BW 1992 Presence of growth factors in human pituitary. *Laboratory Investigation* **66** 639–645.
- Han J, Alvarez-Breckenridge CA, Wang QE & Yu J 2015 TGF- β signaling and its targeting for glioma treatment. *American Journal of Cancer Research* **5** 945–955.
- Haviv F, Bradley MF, Kalvin DM, Schneider AJ, Davidson DJ, Majest SM, McKay LM, Haskell CJ, Bell RL, Nguyen B *et al.* 2005 Thrombospondin-1 mimetic peptide inhibitors of angiogenesis and tumor growth: design, synthesis, and optimization of pharmacokinetics and biological activities. *Journal of Medicinal Chemistry* **48** 2838–2846. (doi:10.1021/jm0401560)
- Heaney AP, Horwitz GA, Wang Z, Singson R & Melmed S 1999 Early involvement of estrogen-induced pituitary tumor transforming gene and fibroblast growth factor expression in prolactinoma pathogenesis. *Nature Medicine* **5** 1317–1321. (doi:10.1038/15275)
- Heaney AP, Fernando M & Melmed S 2002 Functional role of estrogen in pituitary tumor pathogenesis. *Journal of Clinical Investigation* **109** 277–283. (doi:10.1172/JCI0214264)

- Heldin CH, Landstrom M & Moustakas A 2009 Mechanism of TGF- β signaling to growth arrest, apoptosis, and epithelial-mesenchymal transition. *Current Opinion in Cell Biology* **21** 166–176. (doi:10.1016/j.ccb.2009.01.021)
- Hentges S & Sarkar DK 2001 Transforming growth factor- β regulation of estradiol-induced prolactinomas. *Frontiers in Neuroendocrinology* **22** 340–363. (doi:10.1006/frme.2001.0220)
- Itoh F, Watabe T & Miyazono K 2014 Roles of TGF- β family signals in the fate determination of pluripotent stem cells. *Seminars in Cell & Developmental Biology* **32** 98–106. (doi:10.1016/j.semcdb.2014.05.017)
- Jaita G, Zarate S, Ferraris J, Gottardo MF, Eijo G, Magri ML, Pisera D & Seilicovich A 2015 Estradiol upregulates c-FLIPLong expression in anterior pituitary cells. *Hormone and Metabolic Research* [in press]. (doi:10.1055/s-0035-1565068)
- Jaquet P, Ouafik L, Saveanu A, Gunz G, Fina F, Dufour H, Culler MD, Moreau JP & Enjalbert A 1999 Quantitative and functional expression of somatostatin receptor subtypes in human prolactinomas. *Journal of Clinical Endocrinology and Metabolism* **84** 3268–3276. (doi:10.1210/jcem.84.9.5962)
- Jiang M, Mou CZ, Han T, Wang M & Yang W 2012 Thrombospondin-1 and transforming growth factor- β 1 levels in prolactinoma and their clinical significance. *Journal of International Medical Research* **40** 1284–1294. (doi:10.1177/147323001204000407)
- Jin L, Qian X, Kulig E, Sanno N, Scheithauer BW, Kovacs K, Young WF Jr & Lloyd RV 1997 Transforming growth factor- β , transforming growth factor- β receptor II, and p27Kip1 expression in nontumorous and neoplastic human pituitaries. *American Journal of Pathology* **151** 509–519.
- Kelly MA, Rubinstein M, Asa SL, Zhang G, Saez C, Bunzow JR, Allen RG, Hnasko R, Ben-Jonathan N, Grandy DK *et al.* 1997 Pituitary lactotroph hyperplasia and chronic hyperprolactinemia in dopamine D2 receptor-deficient mice. *Neuron* **19** 103–113. (doi:10.1016/S0896-6273(00)80351-7)
- Kovacs K, Horvath E & Vidal S 2001 Classification of pituitary adenomas. *Journal of Neuro-oncology* **54** 121–127. (doi:10.1023/A:1012945129981)
- Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM & Karlsson S 1993 Transforming growth factor β 1 null mutation in mice causes excessive inflammatory response and early death. *PNAS* **90** 770–774. (doi:10.1073/pnas.90.2.770)
- Lawler J 2002 Thrombospondin-1 as an endogenous inhibitor of angiogenesis and tumor growth. *Journal of Cellular and Molecular Medicine* **6** 1–12. (doi:10.1111/j.1582-4934.2002.tb00307.x)
- Lawler PR & Lawler J 2012 Molecular basis for the regulation of angiogenesis by thrombospondin-1 and -2. *Cold Spring Harbor Perspectives in Medicine* **2** a006627. (doi:10.1101/cshperspect.a006627)
- Li Z, Liu Q, Li C, Zong X, Bai J, Wu Y, Lan X, Yu G & Zhang Y 2015 The role of TGF- β /Smad signaling in dopamine agonist-resistant prolactinomas. *Molecular and Cellular Endocrinology* **402** 64–71. (doi:10.1016/j.mce.2014.12.024)
- Liu JK, Patel J & Eloy JA 2015 The role of temozolomide in the treatment of aggressive pituitary tumors. *Journal of Clinical Neuroscience* **22** 923–929. (doi:10.1016/j.jocn.2014.12.007)
- Markovic SN, Suman VJ, Rao RA, Ingle JN, Kaur JS, Erickson LA, Pitot HC, Croghan GA, McWilliams RR, Merchan J *et al.* 2007 A phase II study of ABT-510 (thrombospondin-1 analog) for the treatment of metastatic melanoma. *American Journal of Clinical Oncology* **30** 303–309. (doi:10.1097/O1.coc.0000256104.80089.35)
- McCormack AI, Wass JA & Grossman AB 2011 Aggressive pituitary tumours: the role of temozolomide and the assessment of MGMT status. *European Journal of Clinical Investigation* **41** 1133–1148. (doi:10.1111/j.1365-2362.2011.02520.x)
- Melmed S 2011 Pathogenesis of pituitary tumors. *Endocrine Reviews* **7** 257–266. (doi:10.1038/nrendo.2011.40)
- Melmed S 2015 Pituitary tumors. *Endocrinology and Metabolism Clinics of North America* **44** 1–9. (doi:10.1016/j.ecl.2014.11.004)
- Molitch ME 2003 Dopamine resistance of prolactinomas. *Pituitary* **6** 19–27. (doi:10.1023/A:1026225625897)
- Molitch ME 2014 Management of medically refractory prolactinoma. *Journal of Neuro-oncology* **117** 421–428. (doi:10.1007/s11060-013-1270-8)
- Moustakas A & Heldin CH 2005 Non-Smad TGF- β signals. *Journal of Cell Science* **118** 3573–3584. (doi:10.1242/jcs.02554)
- Mueller SG & Kudlow JE 1991 Transforming growth factor-beta (TGF β) inhibits TGF α expression in bovine anterior pituitary-derived cells. *Molecular Endocrinology* **5** 1439–1446. (doi:10.1210/mend-5-10-1439)
- Nabors LB, Fiveash JB, Markert JM, Kekan MS, Gillespie GY, Huang Z, Johnson MJ, Meleth S, Kuo H, Gladson CL *et al.* 2010 A phase 1 trial of ABT-510 concurrent with standard chemoradiation for patients with newly diagnosed glioblastoma. *Archives of Neurology* **67** 313–319. (doi:10.1001/archneurol.2010.16)
- Nishioka H, Haraoka J & Akada K 2003 Growth potential of prolactinomas in men: is it really different from women? *Surgical Neurology* **59** 386–390. (doi:10.1016/S0090-3019(03)00012-0)
- Paez-Pereda M, Kuchenbauer F, Arzt E & Stalla GK 2005 Regulation of pituitary hormones and cell proliferation by components of the extracellular matrix. *Brazilian Journal of Medical and Biological Research* **38** 1487–1494. (doi:10.1590/S0100-879X2005001000005)
- Passos VQ, Fortes MA, Giannella-Neto D & Bronstein MD 2009 Genes differentially expressed in prolactinomas responsive and resistant to dopamine agonists. *Neuroendocrinology* **89** 163–170. (doi:10.1159/000156116)
- Pastorcic M, De A, Boyadjieva N, Vale W & Sarkar DK 1995 Reduction in the expression and action of transforming growth factor- β 1 on lactotropes during estrogen-induced tumorigenesis. *Cancer Research* **55** 4892–4898.
- Pellegrini I, Rasolonjanahary R, Gunz G, Bertrand P, Delivet S, Jedynak CP, Kordon C, Peillon F, Jaquet P & Enjalbert A 1989 Resistance to bromocriptine in prolactinomas. *Journal of Clinical Endocrinology and Metabolism* **69** 500–509. (doi:10.1210/jcem-69-3-500)
- Pisera D, Candolfi M, Navarra S, Ferraris J, Zaldivar V, Jaita G, Castro MG & Seilicovich A 2004 Estrogens sensitize anterior pituitary gland to apoptosis. *American Journal of Physiology. Endocrinology and Metabolism* **287** E767–E771. (doi:10.1152/ajpendo.00052.2004)
- Pohlars D, Brenmoehl J, Löffler I, Müller CK, Leipner C, Schultze-Mosgau S, Stallmach A, Kinne RW & Wolf G 2009 TGF- β and fibrosis in different organs – molecular pathway imprints. *Biochimica et Biophysica Acta* **1792** 746–756. (doi:10.1016/j.bbdis.2009.06.004)
- Primeau V, Raftopoulos C & Maiter D 2012 Outcomes of transsphenoidal surgery in prolactinomas: improvement of hormonal control in dopamine agonist-resistant patients. *European Journal of Endocrinology* **166** 779–786. (doi:10.1530/EJE-11-1000)
- Recouvreux MV, Guida MC, Rifkin DB, Becu-Villalobos D & Diaz-Torga G 2011 Active and total transforming growth factor- β 1 are differentially regulated by dopamine and estradiol in the pituitary. *Endocrinology* **152** 2722–2730. (doi:10.1210/en.2010-1464)
- Recouvreux MV, Camilletti MA, Rifkin DB, Becu-Villalobos D & Diaz-Torga G 2012 Thrombospondin-1 (TSP-1) analogs ABT-510 and ABT-898 inhibit prolactinoma growth and recover active pituitary transforming growth factor-beta1 (TGF- β 1). *Endocrinology* **153** 3861–3871. (doi:10.1210/en.2012-1007)
- Recouvreux MV, Lapyckyj L, Camilletti MA, Guida MC, Ornstein A, Rifkin DB, Becu-Villalobos D & Diaz-Torga G 2013 Sex differences in the pituitary transforming growth factor- β 1 system: studies in a model of resistant prolactinomas. *Endocrinology* **154** 4192–4205. (doi:10.1210/en.2013-1433)
- Rifkin DB 2005 Latent transforming growth factor-beta (TGF- β) binding proteins: orchestrators of TGF- β availability. *Journal of Biological Chemistry* **280** 7409–7412. (doi:10.1074/jbc.R400029200)
- Saiardi A, Bozzi Y, Baik J-H & Borrelli E 1997 Antiproliferative role of dopamine: loss of D2 receptors causes hormonal dysfunction and pituitary hyperplasia. *Neuron* **19** 115–126. (doi:10.1016/S0896-6273(00)80352-9)

- Sarkar DK, Kim KK & Minami S 1992 Transforming growth factor- β 1 messenger RNA and protein expression in the pituitary gland: its action on prolactin secretion and lactotropic growth. *Molecular Endocrinology* **6** 1825–1833. (doi:10.1210/mend.6.11.1480172)
- Sarkar DK, Chaturvedi K, Oomizu S, Boyadjieva NI & Chen CP 2005 Dopamine, dopamine D2 receptor short isoform, transforming growth factor (TGF)- β 1, and TGF- β type II receptor interact to inhibit the growth of pituitary lactotropes. *Endocrinology* **146** 4179–4188. (doi:10.1210/en.2005-0430)
- Sarkar AJ, Chaturvedi K, Chen CP & Sarkar DK 2007 Changes in thrombospondin-1 levels in the endothelial cells of the anterior pituitary during estrogen-induced prolactin-secreting pituitary tumors. *Journal of Endocrinology* **192** 395–403. (doi:10.1677/joe.1.06925)
- Schuff KG, Hentges ST, Kelly MA, Binart N, Kelly PA, Iuvone PM, Asa SL & Low MJ 2002 Lack of prolactin receptor signaling in mice results in lactotroph proliferation and prolactinomas by dopamine-dependent and -independent mechanisms. *Journal of Clinical Investigation* **110** 973–981. (doi:10.1172/JCI0215912)
- Schultz-Cherry S, Ribeiro S, Gentry L & Murphy-Ullrich JE 1994 Thrombospondin binds and activates the small and large forms of latent transforming growth factor- β in a chemically defined system. *Journal of Biological Chemistry* **269** 26775–26782.
- Shi Y & Massague J 2003 Mechanisms of TGF- β signaling from cell membrane to the nucleus. *Cell* **113** 685–700. (doi:10.1016/S0092-8674(03)00432-X)
- Shida N, Ikeda H, Yoshimoto T, Oshima M, Taketo MM & Miyoshi I 1998 Estrogen-induced tumorigenesis in the pituitary gland of TGF- β (+/-) knockout mice. *Biochimica et Biophysica Acta* **1407** 79–83. (doi:10.1016/S0925-4439(98)00024-6)
- Shimazu S, Shimatsu A, Yamada S, Inoshita N, Nagamura Y, Usui T & Tsukada T 2012 Resistance to dopamine agonists in prolactinoma is correlated with reduction of dopamine D2 receptor long isoform mRNA levels. *European Journal of Endocrinology* **166** 383–390. (doi:10.1530/EJE-11-0656)
- Smith TR, Hulou MM, Huang KT, Gokoglu A, Cote DJ, Woodmansee WW & Laws ER Jr 2015 Current indications for the surgical treatment of prolactinomas. *Journal of Clinical Neuroscience* **22** 1785–1791. (doi:10.1016/j.jocn.2015.06.001)
- Syro LV, Rotondo F, Ramirez A, Di Ieva A, Sav MA, Restrepo LM, Serna CA & Kovacs K 2015 Progress in the diagnosis and classification of pituitary adenomas. *Frontiers in Endocrinology* **6** 97–4898. (doi:10.3389/fendo.2015.00097)
- Vasilev V, Daly AF, Vroonen L, Zacharieva S & Beckers A 2011 Resistant prolactinomas. *Journal of Endocrinological Investigation* **34** 312–316. (doi:10.1007/BF03347092)
- Volker W, Gehring WG, Berning R, Schmidt RC, Schneider J & von zur MA 1982 Impaired pituitary response to bromocriptine suppression: reversal after bromocriptine plus tamoxifen. *Acta Endocrinologica* **101** 491–500.
- Vroonen L, Jaffrain-Rea ML, Petrossians P, Tamagno G, Chanson P, Vilar L, Borson-Chazot F, Naves LA, Brue T, Gatta B *et al.* 2012 Prolactinomas resistant to standard doses of cabergoline: a multicenter study of 92 patients. *European Journal of Endocrinology* **167** 651–662. (doi:10.1530/EJE-12-0236)
- Whitelaw BC, Dworakowska D, Thomas NW, Barazi S, Riordan-Eva P, King AP, Hampton T, Landau DB, Lipscomb D, Buchanan CR *et al.* 2012 Temozolomide in the management of dopamine agonist-resistant prolactinomas. *Clinical Endocrinology* **76** 877–886. (doi:10.1111/j.1365-2265.2012.04373.x)
- Wong A, Eloy JA, Couldwell WT & Liu JK 2015a Update on prolactinomas. Part 1: clinical manifestations and diagnostic challenges. *Journal of Clinical Neuroscience* **22** 1562–1567. (doi:10.1016/j.jocn.2015.03.058)
- Wong A, Eloy JA, Couldwell WT & Liu JK 2015b Update on prolactinomas. Part 2: treatment and management strategies. *Journal of Clinical Neuroscience* **22** 1568–1574. (doi:10.1016/j.jocn.2015.03.059)
- Yang Q, Tian Y, Liu S, Zeine R, Chlenski A, Salwen HR, Henkin J & Cohn SL 2007 Thrombospondin-1 peptide ABT-510 combined with valproic acid is an effective antiangiogenesis strategy in neuroblastoma. *Cancer Research* **67** 1716–1724. (doi:10.1158/0008-5472.CAN-06-2595)
- Ying SY, Becker A, Baird A, Ling N, Ueno N, Esch F & Guillemin R 1986 Type β transforming growth factor (TGF- β) is a potent stimulator of the basal secretion of follicle stimulating hormone (FSH) in a pituitary monolayer system. *Biochemical and Biophysical Research Communications* **135** 950–956. (doi:10.1016/0006-291X(86)91020-X)
- Yoshinaga K, Obata H, Jurukovski V, Mazzieri R, Chen Y, Zilberberg L, Huso D, Melamed J, Prijatelj P, Todorovic V *et al.* 2008 Perturbation of transforming growth factor (TGF)- β 1 association with latent TGF- β binding protein yields inflammation and tumors. *PNAS* **105** 18758–18763. (doi:10.1073/pnas.0805411105)
- Zaldivar V, Magri ML, Zarate S, Jaita G, Eijo G, Radl D, Ferraris J, Pisera D & Seilicovich A 2009 Estradiol increases the Bax/Bcl-2 ratio and induces apoptosis in the anterior pituitary gland. *Neuroendocrinology* **90** 292–300. (doi:10.1159/000235618)
- Zhenye L, Chuzhong L, Youtu W, Xiaolei L, Lei C, Lichuan H, Hongyun W, Yonggang W, Fei W & Yazhuo Z 2014 The expression of TGF- β 1, Smad3, phospho-Smad3 and Smad7 is correlated with the development and invasion of nonfunctioning pituitary adenomas. *Journal of Translational Medicine* **12** 71. (doi:10.1186/1479-5876-12-71)

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