The pituitary TGFβ1 system as a novel target for the treatment of resistant prolactinomas

M Victoria Recouvreux[1,2], M Andrea Camilletti[1], Daniel B Rifkin[3] and Graciela Díaz-Torga[1]

[1]Instituto de Biología y Medicina Experimental, Consejo Nacional de Investigaciones Científicas y Técnicas, Vuelta de Obligado 2490, 1428 Buenos Aires, Argentina
[2]Department of Medicine, Cedars Sinai Medical Center, Los Angeles, California 90048, USA
[3]Department of Cell Biology, New York University Medical Center, 550 First Avenue, New York, New York 10016, USA

Correspondence should be addressed to G Díaz-Torga
Email
gdiaz@ibyme.conicet.gov.ar

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 β1 (TGFβ1) is a known inhibitor of lactotroph cell proliferation and prolactin secretion, and it partly mediates dopamine inhibitory action. TGFβ1 is secreted to the extracellular matrix as an inactive latent complex, and its bioavailability is tightly regulated by different components of the TGFβ1 system including latent binding proteins, local activators (thrombospondin-1, matrix metalloproteases, integrins, among others), and TGFβ receptors. Pituitary TGFβ1 activity and the expression of different components of the TGFβ1 system are regulated by dopamine and estradiol. Prolactinomas (animal models and humans) present reduced TGFβ1 activity as well as reduced expression of several components of the TGFβ1 system. Therefore, restoration of TGFβ1 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 TGFβ1 important role as a local modulator of pituitary lactotroph function and to provide recent evidence of the restoration of TGFβ1 activity as an effective treatment in experimental prolactinomas.

Key Words
- resistant prolactinomas
- dopamine
- estradiol
- TGFβ1

Journal of Endocrinology (2016) **228**, R73–R83

Journal of Endocrinology

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 ~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

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DOI: 10.1530/JOE-15-0451

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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.* 2015*a*).

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 (amenorrea/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.* 2015*b*). 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

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angiogenesis, cellular atypia (multinucleated cells, irregular nuclei), and increased proliferation index measured by Ki67 staining, indicating an overall increase 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 pre-cabergoline 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 β1 (TGFβ1), 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 TGFβ system and biology

TGFβs 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 TGFβ 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 TGFβ have been identified (TGFβ1, 2, and 3).

The importance of TGFβ1 is clearly demonstrated by the fact that TGFβ1 null mutation causes excessive inflammatory response and early death (Kulkarni *et al.* 1993). On the contrary, an excess of TGFβ1 activity is associated to connective tissue diseases, fibrosis and inflammation, cirrhosis, arthritis and sclerosis, cardiovascular diseases, and cancer, making TGFβ an interesting target for therapeutic research (Pohlers *et al.* 2009, Akhurst & Hata 2012, Doyle *et al.* 2012).

Nearly all cell types are sensitive to TGFβ1, but TGFβ action is highly dependent on cell type, developmental stage, physiological–pathological conditions, interaction with components of the ECM and, once bond to its receptor, interaction with other signaling pathways.

The potent biological activity of TGFβ1 is tightly regulated at different levels, including its synthesis, secretion, storage, and activation. The three TGFβ isoforms are synthesized as homodimeric precursor molecules that contain a pro-peptide sequence, so-called latency-associated peptide (LAP), and the functional mature TGFβ sequence (Fig. 1, 1). After proteolytic processing by furin within the trans-Golgi, LAP remains associated with the mature TGFβ by non-covalent interactions in a small latent

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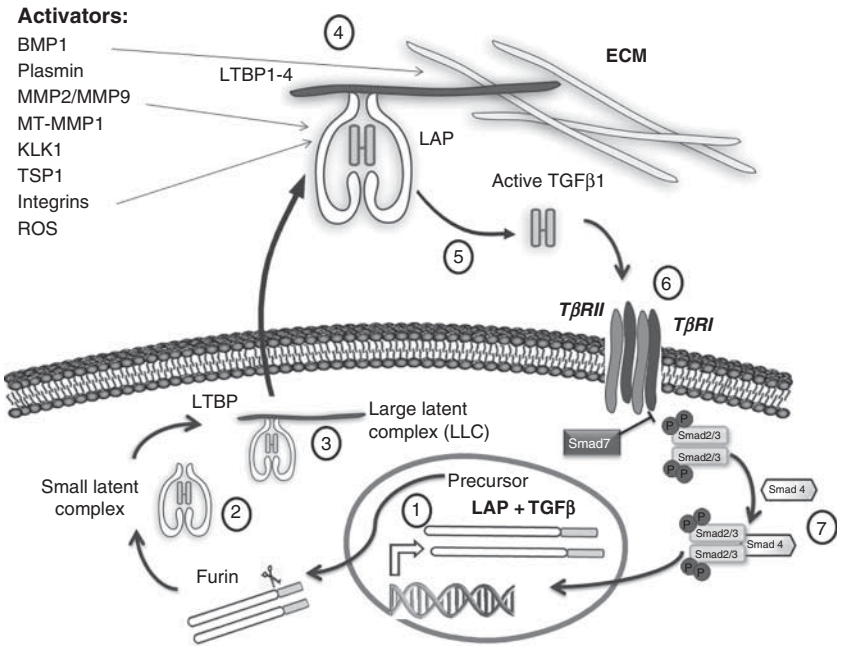

Figure 1

The biology of TGFβ system. TGFβ is synthesized as homodimeric precursor containing a pro-peptide sequence LAP (1) and then processed by furin. LAP remains associated with the mature TGFβ by non-covalent interactions in a small latent complex (2), which in turn is linked by disulfide bonds to one of the latent TGFβ-binding proteins (LTBP1–4) (3). TGFβ is secreted as part of this large latent TGFβ complex (LLC) (3), and it is incorporated as component of the extracellular matrix (ECM), which acts as a reservoir of the cytokine (4). TGFβ must undergo a highly regulated activation process by which mature cytokine is released (5) to enable binding to its receptor complex (TβRI and TβRII) (6) and signal through Smad2/Smad3 pathway (7). Known TGFβ 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 TGFβ-binding protein (LTBP) (Fig. 1, 3). LTBPs belong to a family of large secretory ECM glycoproteins. Although LTBPs are not required for maintenance of TGFβ latency, they facilitate the secretion, storage, and activation of the TGFβ–LAP complex (Rifkin 2005).

TGFβ is secreted as part of this large latent TGFβ 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, TGFβ 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 TGFβ 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 TGFβ1 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 αvβ6 and αvβ8, and reactive oxygen species or pH changes in the local environment, among others. However, their individual biological importance in releasing TGFβ1 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 TGFβ complex has been postulated as a 'sensor' of environment disturbances (Annes *et al.* 2003).

Once TGFβ1 is released from the ECM, the active cytokine binds to its transmembrane receptor, the type II TGFβ receptor (TβRII), a constitutively active kinase that recruits and phosphorylates type I TGFβ receptor (TβRI) forming a heterotetrameric complex of serine/threonine kinase receptors containing two type I and two type II subunits (Fig. 1, 6). Next, TβRI 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 TβRI, thereby exerting a negative effect on TGFβ/Smad signaling (Shi & Massague 2003, Han *et al.* 2015).

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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

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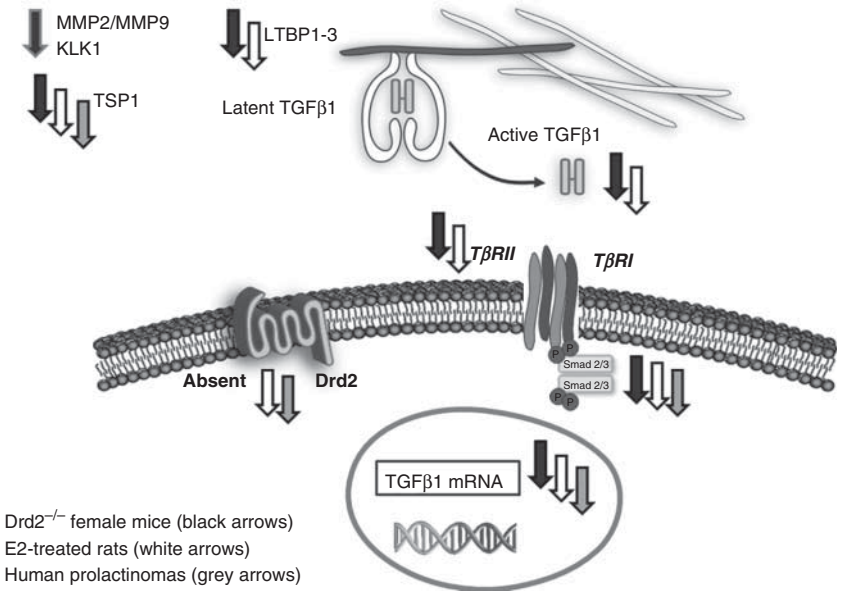

Figure 2
Alterations of TGFβ1 system in prolactinomas. Decreased expression of different components of pituitary TGFβ1 system in prolactinoma models are represented with down-pointed arrows. Grey arrows indicate findings in human prolactinoma specimens, black arrows indicate findings in Drd2$^{-/-}$ mice, and white arrows indicate findings in estradiol-induced prolactinomas in rats.

excellent model to mimick 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 TGFβ1 levels, as well as TβRII and LTBP1 expression, are reduced in Drd2$^{-/-}$ pituitaries compared to controls (WT), highlighting the stimulatory role of dopamine on pituitary TGFβ1 system (Recouvreux *et al.* 2011) (Fig. 2). On the other hand, the impact of the chronic loss of dopaminergic tone on the TGFβ1 system was also stronger in females, evidenced by the down-regulation of several putative TGFβ1 activators (MMP2, MMP9, MT1–MMP, TSP1, and kallikrein) as well as the decreased expression of TGFβ1 target genes observed only in females (Drd2$^{-/-}$ vs their WT counterpart). In this model, we found sex differences in the regulation of the TGFβ1 system: males express higher levels of several components of the TGFβ1 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 TGFβ1 system could protect males from excessive lactotroph proliferation and prolactinoma development. Then, sex differences found in the regulation of the TGFβ1 system could explain sex differences found in the incidence of prolactinoma development in this model.

TGFβ1 alterations in human pituitary tumors

In humans, the expression of several components of the TGFβ 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 TGFβ1 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 TGFβ signaling would be limited during tumor development.

Recent work also described a significant down-regulation of the TGFβ1/Smad signaling cascade in 12 cases of DARPs compared to normal human anterior pituitaries. The authors showed that TGFβ1 mRNA levels,

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

Overall, decreased TGFβ1 activity and decreased expression of different components of the TGFβ1 system have been described in animal models of prolactinomas as well as in human prolactinomas. Taking into account that TGFβ1 inhibits lactotroph proliferation and PRL synthesis and secretion, we speculate that recovering local TGFβ1 activity could contribute to revert the adenoma development and to normalize hyperprolactinemia.

Recovery of local TGFβ1 activity: successful treatment in an experimental model of prolactinoma

TSP1 is one of the main physiologic latent TGFβ1 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 TGFβ1 activator, iv) TGFβ1 activity is also reduced during the development of prolactinomas, and v) TGFβ1 is an inhibitory factor of lactotroph proliferation and synthesis, we speculated that treatments that improve pituitary TSP1 and/or TGFβ1 activities could reduce the progression of prolactinomas.

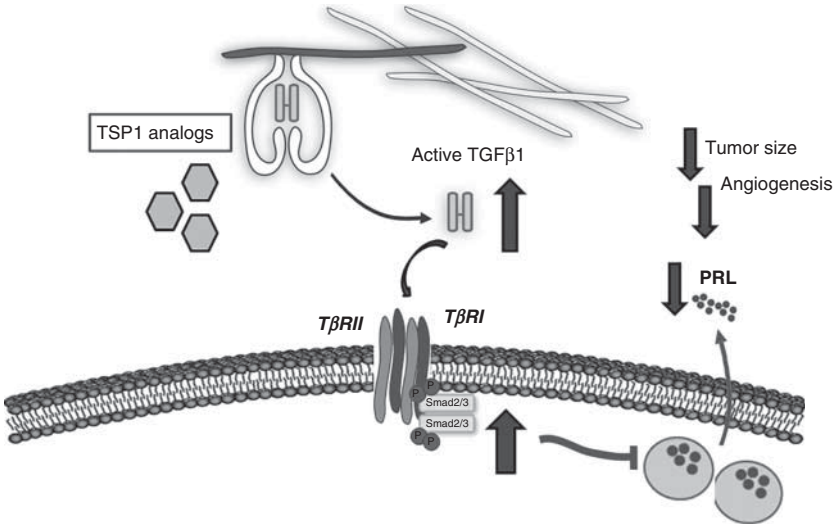

Figure 3
Recovery of TGFβ1 activity emerges as a novel therapeutic target for treatment of dopamine agonist resistant prolactinomas. Treatment with thrombospondin-1 analogs (ABT-510, ABT-898) recover pituitary TGFβ1 activity, reduce tumor size, tumor angiogenesis, and proliferation markers, as well as serum prolactin in estradiol-induced prolactinomas in female rats.

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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.

Funding
This work was supported by the Agencia Nacional de Promoción Científica y Técnica, Buenos Aires, Argentina (grant PICT 2013–2016 N2136 to G D-T), and the National Institutes of Health (grant R01 CA034282–25 to D B R).

Acknowledgements
We thank the National Institute of Diabetes and Digestive and Kidney Diseases National Hormone and Pituitary Program and Dr A F Parlow for prolactin RIA kits.

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Received in final form 10 December 2015
Accepted 22 December 2015
Accepted Preprint published online 23 December 2015

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-15-0451

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