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The role of TGF-β/Smad signaling in dopamine agonist-resistant prolactinomas

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ABSTRACT

Background: Prolactinomas are the most common secretory pituitary adenomas. The first line of treatment involves dopamine agonists (DAs); however, a subset of patients is resistant to such therapy. Recent studies suggest that dopamine can up-regulate TGF-β1 synthesis in rat pituitary lactotrophs whereas estradiol down-regulates TGF-β1. To date, the role of TGF-β/Smad signaling in DAs-resistant prolactinomas has not been explored.
Methods: High-content screening (HCS) techniques, qRT-PCR, Western blot, immunofluorescence and ELISA, were performed to determine the role of TGF-β/Smad signaling in DAs-resistant prolactinomas.
Results: We reported a significant down-regulation of TGF-β/Smad signaling cascade in DAs-resistant prolactinomas compared to normal human anterior pituitaries. Following treatment with TGF-β1, the dopamine agonist, bromocriptine, and the estrogen antagonist (ER), fulvestrant in GH3 cells, we found that TGF-β1 and fulvestrant caused significant cytotoxicity in a dose- and time-dependent manner and activated Smad3 was detected following exposure to TGF-β1 and fulvestrant. In addition, treating GH3 cells with fulvestrant increased active TGF-β1 levels and decreased PRL levels in a dose-dependent manner.
Conclusion: TGF-β/Smad signaling pathway may play an important role in DA-resistant prolactinomas and has the potential to be a viable target for the diagnosis and treatment of prolactinomas, particularly in patients who are resistant to DAs.

1. Introduction

A prolactinoma is a prolactin-secreting pituitary adenoma and accounts for approximately 40–60% of all pituitary tumors (Colao and Savastano, 2011; Daly et al., 2006). These tumors secrete prolactin (PRL) in excess which leads to various health-related complications such as galactorrhea, hypogonadism, decreased libido, infertility, and osteoporosis (Gillam et al., 2006). Additionally, prolactinomas can cause headaches, visual dysfunction, and hypopituitarism. Dopamine agonists (DAs) such as bromocriptine and cabergoline are first-line therapies for prolactinomas. DAs have a higher affinity for dopamine D2 receptors (D2R), which suppresses the secretion of prolactin, inhibits tumor growth that leads to a decrease in the size of prolactin-secreting tumors (Colao and Savastano, 2011). However, nearly 10% prolactinomas cases do not respond to DA therapy (Oh and Aghi, 2011). Therefore, new medical treatments are needed for these patients.

Although it has been reported that the growth-inhibitory effect of dopamine is mediated in part by TGF-β1 (Sarkar et al., 2005), the role of TGF-β signaling in the development of prolactinomas has not been explored until now. Depending upon tumor stage and tumor type, TGF-β signaling can act as a tumor suppressor or tumor promoter (Wakefield and Roberts, 2002). The TGF-β signaling cascade is initiated by the binding of TGF-β1, TGF-β2, and TGF-β3 ligands to the type II TGF-β receptor (TGF-β RII), followed by recruitment and phosphorylation of the type I TGF-β receptor (TGF-β RI) to form a complex. Activated TGF-β RI propagates signaling by phosphorylating Smad2 and Smad3 (p-Smad2 and p-Smad3), which forms a heteromeric complex with the signal transducer, Smad4. Smad2/3 and Smad4 translocate into the nucleus to regulate gene expression of various transcription factors (Heldin et al., 1997; Massague, 2012).

It is well documented that dopamine increases TGF-β1 synthesis in rat pituitary lactotrophs to regulate cell growth and differentiation and elicits the same effect in prolactinomas (Recouvreux et al., 2011). However, most DAs-resistant prolactinomas have decreased expression of D2R, so DAs cannot stimulate TGF-β1 secretion in these prolactinomas and this may be one of the

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Table 1
Patient characteristics.

Patient ID	Gender/ Age	PRL levels (ng/ml)		Tumor size (mm)	Clinical symptoms
		Before DA treatment	Pre-operative		
1	F/48	562	341	17	Headache
2	M/35	1863	1149	37	Headache and visual impairment
3	F/36	326	243	21	Amenorrhoea and galactorrhoea
4	M/23	383	364	31	Gonadal dysfunction
5	F/36	386	268	28	Amenorrhoea and visual impairment
6	F/47	341	282	20	Headache and menstrual disorder
7	M/33	426	325	15	Headache
8	F/21	385	202	26	Amenorrhoea
9	F/42	227	192	28	Amenorrhoea and galactorrhoea
10	F/31	1063	742	29	Headache and menstrual disorder
11	F/34	1358	1326	35	Amenorrhoea and visual impairment
12	F/48	1725	928	40	Amenorrhoea and visual impairment

PRL, prolactin; DA, dopamine agonist.

Table 2
RT-PCR primer list.

Gene name	Forward sequence (5′–3′)	Reverse sequence (5′–3′)
Smad2	ATCCTAACAGAACTTCCGCC	CTCAGCAAAAACTTCCCCAC
Smad3	GGAGAAATGGTGCGAGAAGG	GAAGGCGAACTCACACAGC
TGF-β1	CCCTGGACACCAACTATTGC	TGCGGAAGTCAATGTACAGC
GAPDH	GAAGGTCGGAGTCAACGGATT	CGCTCCTGGAAGATGGTGAT

mechanisms of DAs resistance (Oh and Aghi, 2011). Estrogen receptor antagonists have been used in cases where surgical resection and radiotherapy have not induced remission of hyperprolactinemia (Oh and Aghi, 2011). In our previously published work, we found that fulvestrant, a novel ER antagonist, can inhibit proliferation of MMQ rat prolactinoma cells, promote apoptosis and necrosis, and inhibit prolactin secretion (Cao et al., 2014; Lv et al., 2011). However, no reports have studied the relationship between estrogen receptor antagonists and TGF-β signaling in DAs-resistant prolactinomas. Therefore, in this study, we examined the expression of Smad2, p-Smad2, Smad3, and p-Smad3 in normal human anterior pituitaries and DA-resistant prolactinomas. Furthermore, we studied the anti-tumor effect of fulvestrant on GH3 cells, and to explore whether fulvestrant could regulate cell proliferation in DAs-resistant prolactinomas via TGF-β/Smad signaling.

2. Materials and methods

2.1. Patients and prolactinoma samples

DAs-resistant prolactinomas were obtained from 12 patients who underwent endoscopic transsphenoidal surgery between January 2011 and March 2012 at Beijing Tiantan Hospital. DAs-resistant patients were defined as patients whose serum PRL levels remained abnormally high (>150 ng/ml) after at least 3 months of treatment with a daily dose of 15 mg bromocriptine. The diagnosis of a prolactinoma was confirmed by clinical manifestation, hormonal and magnetic resonance imaging (MRI) data, histopathological analysis, and immunohistochemical staining for all anterior pituitary hormones. Patients who had received previous radiation therapy or had recurrence were excluded from this study. We received six normal human anterior pituitaries from organ donors that died of non-neurological and non-endocrine diseases. All normal anterior pituitaries were histologically examined using immunocytochemistry to exclude the possibility of incidental pathologies. This study

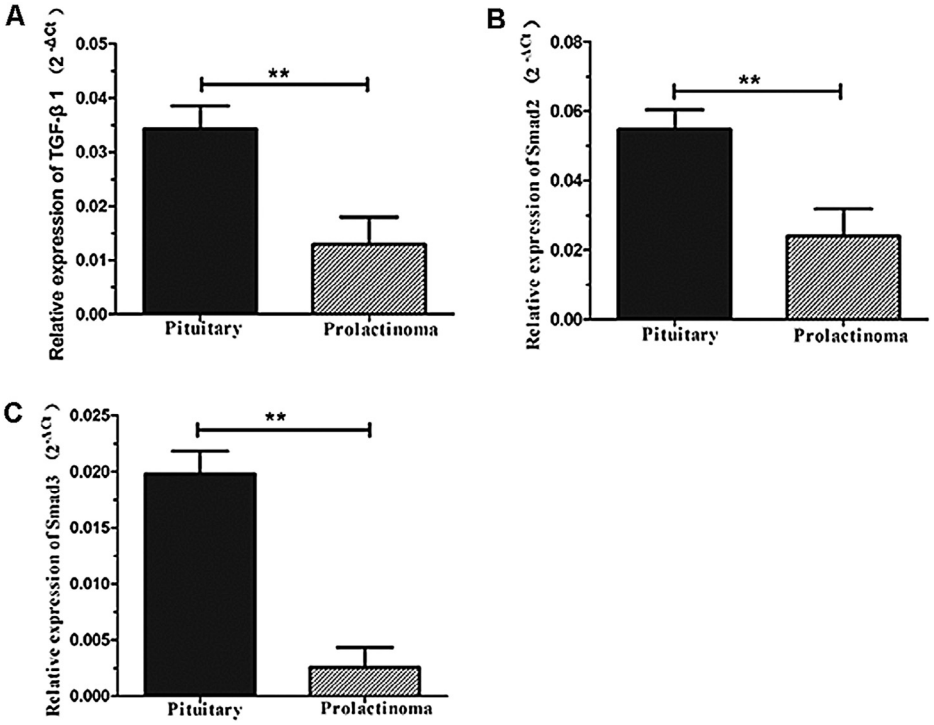

Fig. 1. TGF-β1, Smad2/3 levels are down-regulated in DA-resistant prolactinomas. TGF-β1 (panel A), Smad2 (panel B) and Smad3 (panel C) mRNA levels are significantly lower in DA-resistant prolactinomas compared to normal anterior pituitaries ($^{*}P < 0.05$; $^{**}P < 0.01$).