Letter to the Editor

The Expression of Estrogen Receptor Subtypes in Prolactinomas and Their Relationship to Tumor Biological Behavior*

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Dopamine agonists (DA) are a first-line therapy for prolactinomas (PA). However, nearly 10% of prolactinomas do not respond to DA therapy. A considerable number of studies have shown that estrogen plays an important role in the development of prolactinomas. However, the expression of estrogen receptors (ER) in prolactinomas has not been fully explored. Accordingly, we examined the levels of ESR1 and its subtypes Δ5-Del-ESR1 and ESR2 mRNA in prolactinomas. In the present study, we found that ESR1 mRNA levels were significantly lower in women of childbearing age group compared to men (*P*<0.01) and post-menopausal women (*P*<0.05). In addition, ESR1 mRNA levels showed a significant positive correlation with the levels of prolactin (PRL). Moreover, ESR1 mRNA levels were significantly lower in noninvasive prolactinomas compared to invasive prolactinomas.

Prolactinomas are prolactin-secreting pituitary adenomas that account for approximately 40%-60% of all pituitary adenomas (PA)[1-2]. They secrete PRL in excess, which leads to various health-related complications such as galactorrhea, hypogonadism, infertility, osteoporosis, headaches, visual dysfunction, and hypopituitarism[3]. Dopamine agonists (DA) are a first-line therapy for prolactinomas. However, nearly 10% of these tumors do not respond to DA therapy.

A considerable number of studies have shown that estrogen plays an important role in the development of prolactinomas[4]. The biological effects of estrogen are mediated by two closely related receptor isoforms, estrogen receptor 1 (ESR1) and estrogen receptor 2 (ESR2), both of which belong to the nuclear receptor superfamily of steroid hormones[5]. However, the expression of ESR1 and ESR2 in prolactinomas has not been fully explored. Accordingly, in this study we examined the levels of ESR1 and its subtypes Δ5-Del-ESR1 and ESR2 mRNA in prolactinomas and aimed to understand the role of ERs in the development of prolactinomas.

Prolactinoma specimens were obtained from patients who underwent endoscopic transsphenoidal surgery between September 2007 and April 2008 at Beijing Tiantan Hospital. The specimens were stored in liquid nitrogen after harvesting. The diagnosis of a prolactinoma was confirmed by clinical manifestations, hormonal and magnetic resonance imaging (MRI) data, histopathological analysis, and immunohistochemical staining for all anterior pituitary hormones. This study was approved by the Ethics Committee of Beijing Tiantan Hospital and each patient provided informed consent. A total of 20 patients (37.6±9.8 years; range, 20-55 years) with prolactinomas were enrolled in the study, including 10 men and 10 women. Of the women, two were post-menopausal and eight were of childbearing age. The median PRL level was 928 ng/mL (range: 32-7410 ng/mL). Invasive PAs were classified as grade IV based on the Hardy-Wilson classification and/or grade III and IV based on the Knosp classification[6].

Total RNA was extracted from frozen tumor samples using Trizol (Invitrogen, Carlsbad, USA). First strand cDNA synthesis was performed using a kit according to manufacturer instructions (Invitrogen, Carlsbad, USA). PCR specificity was determined by dissociation curve analysis. All qRT-PCRs were performed in triplicate. The primers used for RT-PCR are listed in Table 1. Statistical analysis was performed with SPSS version 17.0 (SPSS Inc., Chicago, USA). Differences between groups were determined by one-way analysis of variance and the two independent samples *t*-test or the Mann-Whitney *U* test. Correlations of different genes were analyzed by stepwise regression method. *P*<0.05 was defined as statistically significant.

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There was a sex difference in the incidence of prolactinomas (female:male=3:1) and this difference could be greater among patients between 20 and 50 years of age[7-8]. In this study, we found that ESR1 mRNA levels were significantly lower in women of childbearing age compared to men (P<0.01) and post-menopausal women (P<0.05). In addition, ESR1 mRNA levels showed a significant positive correlation with PRL levels (LogPRL=0.326ESR1+ 1.996, P<0.01, R^2=0.665; Figure 1). Moreover, ESR1 mRNA levels were significantly lower in noninvasive prolactinomas compared to invasive prolactinomas (P<0.05). These results suggest that ESR1 may be involved in the regulation of PRL secretion and act as a tumor promoter in the development of prolactinomas; however, further studies are needed to confirm our findings.

Several ESR mRNA variants have been identified in breast cancer by the alternative splicing of mRNA and they play an important role in the process of tumorigenesis and/or hormone resistance[9]. Δ5-Del-ESR1 mRNA is a subtype of ESR1 mRNA and is highly expressed in tamoxifen-resistant breast cancer cells[10]. In the present study, we found no significant associations between the expression of Δ5-Del-ESR1 mRNA and patient sex (P=0.208) or PRL level (P=0.636). However, the Δ5-Del-ESR1 mRNA levels of the patients with PRL≥1000 ng/mL were significantly higher than that of patients with PRL<1000 ng/mL. Surprisingly, there was no significant difference in the Δ5-Del-ESR1 mRNA levels between invasive and noninvasive prolactinomas. This finding needs further investigation.

Table 1. ABI Primers and Probes List

Gene	Primers and Probes (Item or Sequence)	Reference Sequence
ESR1	Hs01046815_m1	NM_000125.231
ESR2	Hs00230957_m1	NM_001040275.1
β-actin	4333762F	NM_001101.2
Δ5-Del-ESR1	Forward: 5'ACATGATCAAC TGGGCGAAGA3'	NM_000125.2
	Reverse: 5'ACCATGCCCTCT ACACATTTTCC3'	

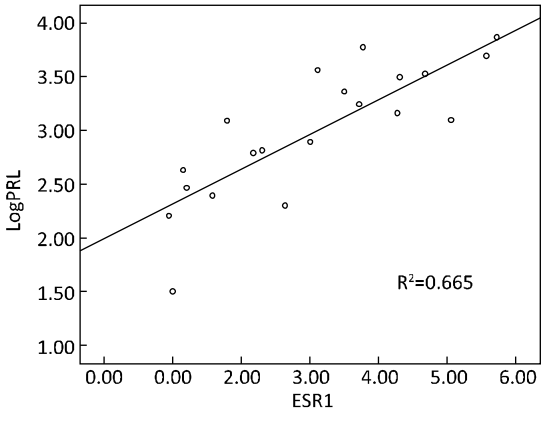

Figure 1. Univariate linear regression analysis of ESR1 mRNA and LogPRL. A positive correlation between ESR1 expression levels and LogPRL is observed; the linear equation is LogPRL=0.326ESR1+1.996, P=0.000, R^2=0.665.

We also evaluated the expression of ESR2 mRNA and found that there was no difference in the expression levels of ESR2 mRNA between invasive and noninvasive prolactinomas (P=0.721). Additionally, there were no significant associations between the expression of ESR2 mRNA and patient sex (P=0.263) or PRL level (P=0.566). These data suggest that ESR2 may not play a role in the development of prolactinomas.

It has been reported that estrogen receptor antagonists have been used in cases of hyperprolactinemia in which neither surgical resection nor radiotherapy could induce remission[4]. In this study, we found that ESR1 mRNA was expressed in prolactinomas, especially among men and post-menopausal women; ESR1 may play an important role in the development of prolactinomas. We believe that ESR1 represents a promising new therapeutic target in the treatment of prolactinomas.

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REFERENCES

1. Daly AF, Rixhon M, Adam C, et al. High prevalence of pituitary adenomas: a cross-sectional study in the province of Liege, Belgium. J Clin Endocrinol Metab, 2006; 91, 4769-75.
2. Fernandez A, Karavitaki N, Wass JA. Prevalence of pituitary adenomas: a community-based, cross-sectional study in Banbury (Oxfordshire, UK). Clin Endocrinol (Oxf), 2010; 72, 377-82.
3. Colao A, Lombardi G. Growth-hormone and prolactin excess. Lancet, 1998; 352, 1455-61.
4. Oh MC, Aghi MK. Dopamine agonist-resistant prolactinomas. J Neurosurg, 2011; 114, 1369-79.
5. Mosselman S, Polman J, Dijkema R. ER beta: identification and

characterization of a novel human estrogen receptor. FEBS Lett, 1996; 392, 49-53.
6. Zhenye L, Chuzhong L, Youtu W, et al. The expression of TGF-beta1, Smad3, phospho-Smad3 and Smad7 is correlated with the development and invasion of nonfunctioning pituitary adenomas. J Transl Med, 2014; 12, 71.
7. Colao A, Sarno AD, Cappabianca P, et al. Gender differences in the prevalence, clinical features and response to cabergoline in hyperprolactinemia. Eur J Endocrinol, 2003; 148, 325-31.
8. Mindermann T, Wilson CB. Age-related and gender-related occurrence of pituitary adenomas. Clin Endocrinol (Oxf), 1994; 41, 359-64.
9. Gotteland M, Desauty G, Delarue JC, et al. Human estrogen receptor messenger RNA variants in both normal and tumor breast tissues. Mol Cell Endocrinol, 1995; 112, 1-13.
10. Fuqua SA, Fitzgerald SD, Chamness GC, et al. Variant human breast tumor estrogen receptor with constitutive transcriptional activity. Cancer Res, 1991; 51, 105-9.