

# Estrogen receptor antagonist fulvestrant inhibits proliferation and promotes apoptosis of prolactinoma cells by regulating the IRE1/XBP1 signaling pathway

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**Abstract.** The aim of the present study was to evaluate the effects of an estrogen receptor antagonist, fulvestrant, on proliferation and apoptosis of prolactinoma cells, and to reveal potential regulatory mechanisms. Prolactinoma GH3 cells were treated with  $10^{-6}$  mol/l fulvestrant for 2, 4, 8, 12 and 24 h. GH3 cell growth was observed under a microscope and cell viability was detected by MTT assay. Morphological changes of the nuclei in GH3 cells were observed by Hoechst 33258 staining and apoptotic rates were detected by flow cytometry. Preprolactin (PPL) and prolactin (PRL) secretion levels from GH3 cells were measured using ELISA. In addition, the protein expression levels of inositol-requiring enzyme 1 (IRE1), X-box binding protein (XBP)-1 and glucose-regulated protein, 78 kDa (GRP78) in GH3 cells were detected by western blot analysis. Cell density and cell viability of GH3 cells were significantly reduced in a time-dependent manner following treatment with fulvestrant ( $P<0.05$ ). GH3 cells treated with fulvestrant also acquired an apoptotic morphology and the apoptotic rate of GH3 cells was significantly increased by fulvestrant in a time-dependent manner ( $P<0.05$ ). PPL and PRL secretion levels were significantly reduced by fulvestrant treatment in a time-dependent manner ( $P<0.05$ ). The protein expression levels of IRE1, XBP1 and GRP78 were also significantly reduced in a time-dependent manner following treatment with fulvestrant ( $P<0.05$ ). Therefore, fulvestrant may inhibit proliferation and promote apoptosis of GH3 cells by downregulating the IRE1/XBP1 signaling pathway.

## Introduction

Prolactinoma is the most common hormone-secreting pituitary tumor, with an estimated prevalence of 10 per million adults (1). Prolactinoma may induce gonadal and sexual dysfunction related to hyperprolactinemia in addition to other symptoms related to tumor expansion (2). The clinical presentation of prolactinoma in females is usually more obvious compared with males, particularly owing to the manifestation of classical amenorrhea-galactorrhea syndrome (3). Dopamine agonists are commonly used to treat prolactinoma, as it normalizes serum prolactin (PRL) levels and reduces tumor size (4). For patients with drug resistant tumors, those that are pregnant or have malignant prolactinoma, transsphenoidal adenoma resection is considered a second-line therapy (5). However, the prognosis of patients undergoing surgery, particularly for those with invasive macroprolactinomas, is still poor (6). Therefore, novel therapies are required for the treatment of dopamine agonist-resistant prolactinomas.

Estrogen is an important hormone that serves a key role in regulation of physiological processes and cell growth (7). Because estrogen stimulates the proliferation of pituitary lactotrophs and promotes the synthesis and secretion of PRL, estrogen receptors (ERs) may also be involved in the progression of prolactinomas (8). ER $\alpha$  has been implicated in prolactinoma proliferation and PRL secretion by regulating various growth factors, including pituitary tumor transforming gene, basic fibroblast growth factor and transforming growth factor  $\beta$ 1 (TGF $\beta$ 1) (9). Therefore, ER inactivation may reduce PRL hypersecretion and control lactotroph adenomatous growth (10). Notably, a significant correlation between ER $\alpha$  and PRL, as well as tumor volume and TGF $\beta$ 1 expression, was observed in patients with prolactinoma (11). In addition, increased activity of estrogen-responsive genes was previously demonstrated to promote estrogen-regulated tumor proliferation and/or PRL secretion in patients with prolactinoma (12). Although ERs are considered a potential therapeutic target in prolactinoma, the regulatory mechanisms of ERs in prolactinoma are not yet fully understood.

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The inositol-requiring enzyme 1 (IRE1)/X-box binding protein 1 (XBP1) signaling pathway is a conserved unfolded protein response pathway that is involved in endoplasmic reticulum stress (13). Under normal circumstances IRE1 binds to glucose-regulated protein, 78 kDa (GRP78) in the endoplasmic reticulum, whereas endoplasmic reticulum stress may inhibit this interaction. IRE1 activation facilitates the recovery of endoplasmic reticulum stress by removing an intron from the XBP1 mRNA. This results in a frame-shift in the XBP1 coding sequence, which leads to translation of the active XBP1 isoform (S) (14). The isoform encoded by the unspliced mRNA, XBP1(U), which is constitutively expressed, and thought to function as a negative feedback regulator of XBP1(S), which shuts off transcription of target genes during the recovery phase of ER stress (13). Previous studies have also demonstrated that the IRE1/XBP1 signaling pathway serves an important role in tumor progression. In the present study, the ER antagonist fulvestrant was used to treat the GH3 prolactinoma cell line, and cellular proliferation, apoptosis and preprolactin (PPL) and PRL secretion levels were monitored over a period of time. In addition, the protein expression levels of IRE1, XBP1 and GRP78 in fulvestrant-treated GH3 cells were measured. The results demonstrated that fulvestrant may have an inhibitory effect on GH3 prolactinoma cells by targeting the IRE1/XBP1 signaling pathway.

## Materials and methods

**Cell culture and treatment.** GH3 prolactinoma cell line was purchased from the cell culture center of Sun Yat-Sen University (Guangzhou, China). Cells were cultured in Dulbecco's modified Eagle's medium (BD Biosciences, Franklin Lakes, NJ, USA) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. The medium was replaced every 48 h and cells in the logarithmic growth phase were used in all subsequent treatments: According to preliminary results (data not shown) GH3 cells (1x10<sup>5</sup> cells/ml) were seeded in 96-well plates and treated with 10<sup>-6</sup> mol/l fulvestrant (ICI-182,780, Abmole bioscience, Hongkong, China; www.abmole.cn/search?q=fulvestrant). Cells were harvested at 2, 4, 8, 12 and 24 h post-treatment. Untreated cells (0 h) were used as controls.

**Cell growth and MTT viability assay.** Cell growth was observed using an Olympus CKX41 inverted microscope (Olympus Corporation, Tokyo, Japan). Cell viability was quantified by MTT assay (Shanghai Jiang Lai Biotechnology Co., Ltd., China), according to the manufacturer's protocol. Briefly, 100 µg/ml MTT solution was added to each well. Following 4 h of incubation, the supernatant was removed and 150 µl dimethylsulphoxide was added to dissolve the formazan crystals. Optical density was measured at 490 nm using an ultraviolet spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

**Hoechst 33258 staining.** Following fulvestrant treatment, morphological changes of the nuclei were observed over time (0–24 h) by Hoechst 33258 staining (Beyotime Institute of Biotechnology, Shanghai, China). Briefly, cells were washed

with PBS three times and fixed in 4% paraformaldehyde at room temperature (25°C) for 20 min. Subsequently, cells were permeabilized by 0.5% Triton X-100 for 15 min, followed by staining with Hoechst 33258 for 10 min in the dark. Samples were washed with PBS three times prior to mounting, and images were captured under an Olympus CX21 fluorescence microscope (Olympus Corporation, Tokyo, Japan).

**Cell apoptosis assay.** Following fulvestrant treatment, GH3 cell apoptosis was quantified by staining with 5 µl Annexin V-fluorescein isothiocyanate and 2.5 µl propidium iodide (Miltényi Biotec Inc., CA, USA). Following incubation on ice for 10 min in the dark, 400 µl 1X Annexin V binding buffer was added. Stained cells were detected using an Attune™ NxT version 2.5 flow cytometer (Thermo Fisher Scientific, Inc.; Attune NxT software version 2.5). Apoptosis rate was calculated as percentage of cells in Q2 which corresponds to early stage apoptosis.

**PPL and PRL assays.** Following treatment with fulvestrant, GH3 cells were seeded into 96-well plates (1x10<sup>5</sup> cells/well) and cultured for 24 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. The PPL and PRL concentrations in the cell culture supernatants of GH3 cells from different treatment groups were detected using rat PRL (rPRL; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), according to the manufacturer's protocol. PRL and PRL concentrations were calculated using a standard curve of known concentration.

**Western blot analysis.** Total protein was extracted from GH3 cells in the different treatment groups using RIPA cell lysis buffer (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). Following centrifugation at 10,000 x g for 15 min at 4°C, 40 µg proteins were separated by 12% SDS-PAGE and transferred onto polyvinylidene fluoride membranes (EMD Millipore, Billerica, MA, USA). Membranes were blocked with 5% skim milk for 1 h at 4°C and incubated with primary antibodies against IRE1 (cat. no. ab48187; 1:1,000), XBP1 (cat. no. ab37152; 1:500), GRP78 (cat. no. ab21685; 1:500) and β-actin (cat. no. ab8227; 1:1,000; all purchased from Abcam, Cambridge, UK) overnight at 4°C. Subsequently, membranes were washed with Tris-buffered saline containing 0.05% Tween-20 (TBST) three times and incubated with horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies (cat. no. A0208; 1:5,000; Beyotime Institute of Biotechnology, Haimen, China) for 1 h at 25°C. Membranes were washed a final time with TBST and the protein bands were visualized using the Enhanced Chemiluminescence Reagent (Beyotime Institute of Biotechnology). Protein expression levels were quantified using AlphaView software 3.2 CD in a FluorChem M Ultraviolet Gel Imaging System (ProteinSimple, Santa Clara, CA, USA).

**Statistical analysis.** All data are expressed as the mean ± standard deviation. Statistical analysis was performed using SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). Comparisons between different groups were determined by one-way analysis of variance followed by the Least Significant Difference post hoc test. P<0.05 was considered to indicate a statistically significant difference.

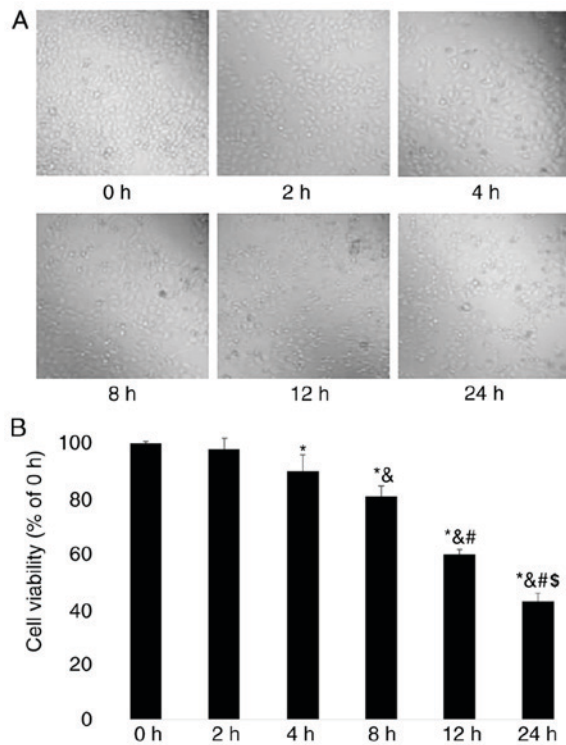


Figure 1. Fulvestrant-treated GH3 cell growth and viability. (A) Microscope images of GH3 cells treated with fulvestrant for 0, 2, 4, 8, 12 and 24 h (magnification, x200). (B) Viability of GH3 cells treated with fulvestrant for 0, 2, 4, 8, 12 and 24 h was determined by MTT assay. \* $P < 0.05$  vs. 2 h; & $P < 0.05$  vs. 4 h; # $P < 0.05$  vs. 8 h; &# $P < 0.05$  vs. 12 h.

## Results

**Fulvestrant inhibits GH3 cell viability.** The effects of fulvestrant on GH3 cell viability were evaluated. Microscopic observation revealed that the cell density of GH3 cells was reduced after 4 h of fulvestrant treatment (Fig. 1A); following 24 h of treatment, the density of GH3 cells was gradually reduced in time. The viability of GH3 cells was quantified using MTT assay, which demonstrated that cell viability was significantly reduced in a time-dependent manner between 8 and 24 h following fulvestrant treatment ( $P < 0.05$ ; Fig. 1B).

**Fulvestrant promotes apoptosis of GH3 cells.** As cell viability was significantly reduced following fulvestrant treatment, cell apoptosis was analyzed. Prior to treatment with fulvestrant, GH3 cells exhibited regular and full nuclei, uniformly colored chromatin, as determined by microscopic observation (Fig. 2A 0 h). However, obvious shrinkage and fragmentation of the nuclei were observed in GH3 cells at 8 h following fulvestrant treatment. As treatment time increased, the apoptotic morphology of GH3 cells became more apparent (Fig. 2A). Subsequently, the apoptotic rates of GH3 cells were determined by flow cytometry, which revealed that the apoptotic rates of GH3 cells treated with fulvestrant significantly increased in a time-dependent manner (Fig. 2B and C). At 8, 12 and 24 h of fulvestrant treatment, the early apoptotic rates of GH3 cells were 4.5, 10.2 and 16.4%, respectively.

**Fulvestrant reduces PPL and PRL secretion from GH3 cells.** The effects of fulvestrant treatment on PPL and PRL secretion

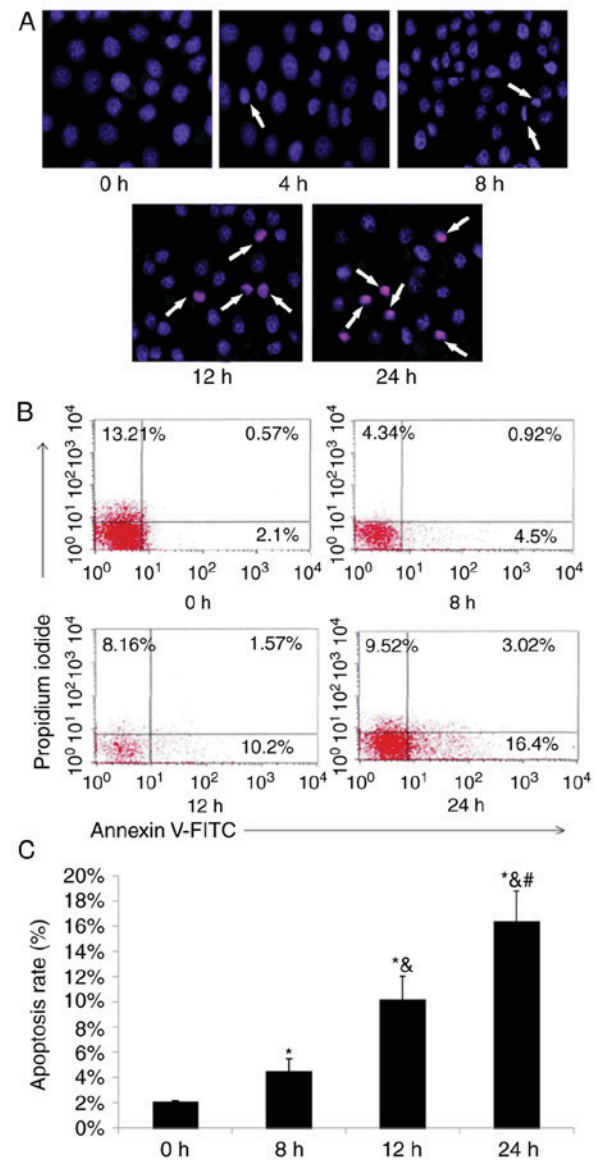


Figure 2. GH3 apoptosis following fulvestrant treatment. (A) Microscope images of Hoechst 33258-stained GH3 cells following 0, 2, 4, 8, 12 and 24 h of fulvestrant treatment (x400). (B and C) Apoptosis rate of GH3 cells treated with fulvestrant for 0, 2, 8, 12 and 24 h was determined by flow cytometry. \* $P < 0.05$  vs. 0 h; & $P < 0.05$  vs. 8 h; # $P < 0.05$  vs. 12 h. FITC, fluorescein isothiocyanate.

from GH3 cells were also evaluated. The results demonstrated that PPL and PRL secretion levels were significantly reduced in a time-dependent manner following treatment with fulvestrant ( $P < 0.05$ ; Fig. 3A and B, respectively).

**Fulvestrant reduces IRE1, XBP1 and GRP78 expression levels in GH3 cells.** To examine the potential mechanisms of action of fulvestrant on GH3 cells, the protein expressions levels of IRE1, XBP1 and GRP78 were detected following treatment (Fig. 4A); the expression levels of IRE1, XBP1 and GRP78 were significantly reduced by fulvestrant in a time-dependent manner (Fig. 4B).

## Discussion

Prolactinoma is a common type of hormone-secreting tumor in the pituitary gland (15). As significant correlations have been



identified between ERs and the occurrence, development and invasion of prolactinomas, ERs may be an effective therapeutic target for prolactinoma (11). Fulvestrant is an ER $\alpha$  antagonist that exhibits great potential for the treatment of cancer (16). It has been reported previously that fulvestrant inhibits tumor proliferation and PRL secretion by blocking ER $\alpha$  in a F344 rat model of prolactinoma (17). In addition, it was identified that fulvestrant significantly inhibits cell proliferation and PRL secretion in an MMQ prolactinoma cell line by inhibiting ER $\alpha$  (18). In the present study, fulvestrant treatment reduced the viability of GH3 cells and increased the apoptotic rate of GH3 cells. In addition, PPL and PRL secretion by GH3 cells were significantly reduced following fulvestrant treatment. These results suggested that fulvestrant may exhibit antitumor effects on prolactinoma. Binding of estrogen to the ER may induce prolactinoma by promoting pituitary lactotroph proliferation and PRL secretion (17). ER $\alpha$  activation has been demonstrated to promote prolactinoma cell proliferation by regulating the transcription of various target genes, including PRL, B-cell CLL/lymphoma 2 (Bcl-2), vascular endothelial growth factor and matrix metalloproteinase 9 (19). However, fulvestrant blocks the interaction between estrogen and ER, thereby inhibiting proliferation of prolactinoma cells. It was hypothesized that fulvestrant may be used for the effective treatment of prolactinoma in the future.

The IRE1/XBP1 pathway is a conserved unfolded protein response pathway that is involved in endoplasmic reticulum stress and is considered to be an important pathway in tumor progression (13,20,21). It has been reported that the IRE1 $\alpha$ /XBP1 pathway promotes cell proliferation and progression of melanoma by activating interleukin 6/signal transducer and activator of transcription 3 signaling (22). The IRE1 $\alpha$ /XBP1 signaling pathway is also involved in the development of multiple myeloma and is closely associated with the effects of treatment and prognosis (23). IRE1, XBP1 and GRP78 are three key components in the IRE1/XBP1 pathway, and their abnormal expression is commonly associated with tumor progression. For example, ER $\beta$ -induced downregulation of IRE1 $\alpha$  and XBP1 was reported to be associated with decreased survival of breast cancer cells (24), whereas increased expression of IRE1 $\alpha$  was demonstrated to promote cell proliferation and invasion of colorectal carcinoma cells (25). GRP78 is a binding target of IRE1 in the endoplasmic reticulum, and the GRP78-specific monoclonal antibody MAb159 has been identified to inhibit tumor growth and metastasis by regulating the phosphoinositide 3-kinase pathway (26). In the present study, significantly decreased expression levels of IRE1, XBP1 and GRP78 were observed in GH3 cells treated with fulvestrant, which occurred in a time-dependent manner. These findings are consistent with previous studies, and they further demonstrated that the inhibitory effects of fulvestrant on GH3 cells were associated with the IRE1/XBP1 signaling pathway. As previously described, upregulation of wild-type ER $\beta$ 1 or treatment with ER $\beta$  agonists enhances apoptosis of breast cancer cells in the presence of pharmacological inducers of endoplasmic reticulum stress. However, targeting Bcl-2 to the endoplasmic reticulum of ER $\beta$ 1-expressing cells prevents apoptosis induced by endoplasmic reticulum stress (27). Therefore, the present study hypothesized that fulvestrant

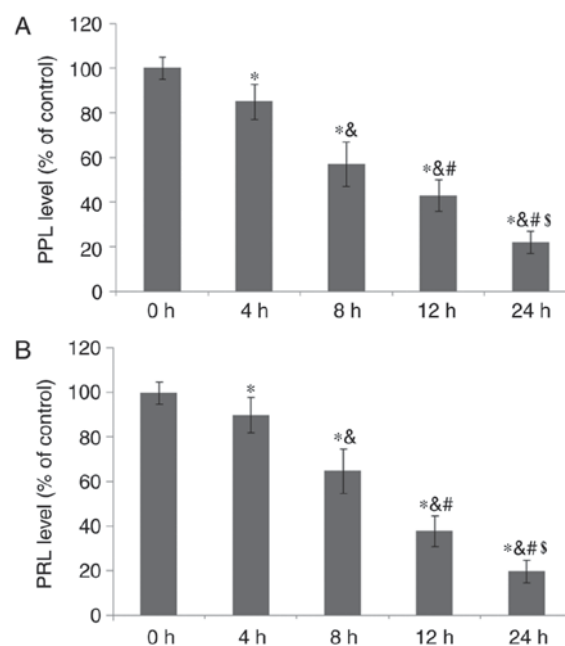


Figure 3. Effects of fulvestrant treatment on PPL and PRL secretion in GH3 cells. (A) PPL and (B) PRL levels in the cell culture supernatant of GH3 cells treated with fulvestrant for 0, 2, 4, 8, 12 and 24 h. \* $P < 0.05$  vs. 0 h;  $^{\&}$  $P < 0.05$  vs. 4 h;  $^{\#}$  $P < 0.05$  vs. 8 h;  $^s$  $P < 0.05$  vs. 12 h. PPL, prolactin; PRL, prolactin.

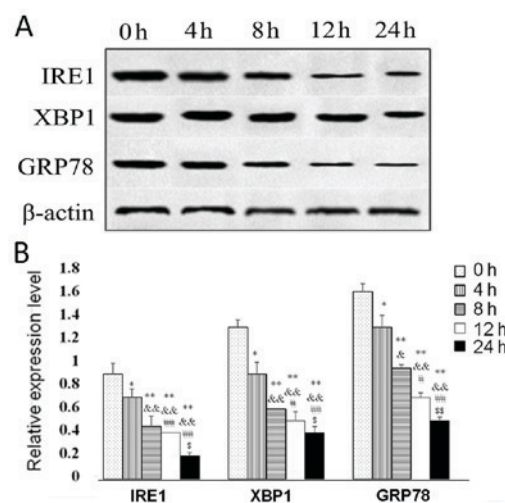


Figure 4. Protein expression levels of IRE1, XBP1 and GRP78 in GH3 cells treated with fulvestrant for 0, 2, 4, 8, 12 and 24 h. (A) Representative western blot images of IRE1, XBP1 and GRP78 protein expression levels;  $\beta$ -actin was used as a loading control. (B) Quantification of protein expression level from (A). \* $P < 0.05$  vs. 0 h;  $^{\&}$  $P < 0.05$  vs. 4 h;  $^{\#}$  $P < 0.05$  vs. 8 h;  $^s$  $P < 0.05$  vs. 12 h. \*\* $P < 0.01$  vs. 0 h;  $^{\&\&}$  $P < 0.01$  vs. 4 h;  $^{\#\#}$  $P < 0.01$  vs. 8 h;  $^{ss}$  $P < 0.01$  vs. 12 h. GRP78, glucose-regulated protein, 78 kDa; IRE1, inositol-requiring enzyme 1; XBP1, X-box binding protein 1.

promotes endoplasmic reticulum stress-regulated apoptosis of GH3 cells by regulating the IRE1/XBP1 signaling pathway.

In conclusion, fulvestrant inhibited proliferation and promoted apoptosis of GH3 cells by downregulating the IRE1/XBP1 signaling pathway. However, the present study was limited to *in vitro* experiments. Further studies on the anti-prolactinoma effects of fulvestrant in an *in vivo* model are required to better understand its regulatory mechanisms.

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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Authors' contributions

CW and YW designed the study. CW, MB, XW, CT, DZ and LC performed the cell experiments. CW, GL and LX analyzed and interpreted the data. JS performed the morphological examination. CW drafted the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

- Gillam MP, Molitch ME, Lombardi G and Colao A: Advances in the treatment of prolactinomas. *Endocr Rev* 27: 485-534, 2006.
- Hu J, Zheng X, Zhang W and Yang H: Current drug withdrawal strategy in prolactinoma patients treated with cabergoline: A systematic review and meta-analysis. *Pituitary* 18: 745-751, 2015.
- Colao A, Sarno AD, Cappabianca P, Briganti F, Pivonello R, Somma CD, Faggiano A, Biondi B and Lombardi G: Gender differences in the prevalence, clinical features and response to cabergoline in hyperprolactinemia. *Eur J Endocrinol* 148: 325-331, 2003.
- Beran RG: 'Prolactinoma: Are dopamine agonists still first choice?' *Intern Med J* 41: 757, 2011.
- Casanueva FF, Molitch ME, Schlechte JA, Abs R, Bonert V, Bronstein MD, Brue T, Cappabianca P, Colao A, Fahlbusch R, *et al*: Guidelines of the pituitary society for the diagnosis and management of prolactinomas. *Clin Endocrinol (Oxf)* 65: 265-273, 2006.
- Klibanski A: Clinical practice. Prolactinomas. *N Engl J Med* 362: 1219-1226, 2010.
- Jameera Begam A, Jubie S and Nanjan MJ: Estrogen receptor agonists/antagonists in breast cancer therapy: A critical review. *Bioorg Chem* 71: 257-274, 2017.
- Gorski J, Wendell D, Gregg D and Chun TY: Estrogens and the genetic control of tumor growth. *Prog Clin Biol Res* 396: 233-243, 1997.
- Lv H, Li C, Gui S, Sun M, Li D and Zhang Y: Effects of estrogen receptor antagonist on biological behavior and expression of growth factors in the prolactinoma MMQ cell line. *J Neurooncol* 102: 237-245, 2011.
- Kansra S, Yamagata S, Sneade L, Foster L and Ben-Jonathan N: Differential effects of estrogen receptor antagonists on pituitary lactotroph proliferation and prolactin release. *Mol Cell Endocrinol* 239: 27-36, 2005.
- Lv H, Li C, Gui S and Zhang Y: Expression of estrogen receptor  $\alpha$  and growth factors in human prolactinoma and its correlation with clinical features and gender. *J Endocrinol Invest* 35: 174-180, 2012.
- Chaidarun SS, Swearingen B and Alexander JM: Differential expression of estrogen receptor-beta (ER beta) in human pituitary tumors: Functional interactions with ER $\alpha$  and a tumor-specific splice variant. *J Clin Endocrinol Metab* 83: 3308-3315, 1998.
- Hay MP, Jiang D, Kozak M, Niwa M and Koong AC: Inhibition of the IRE1 $\alpha$ -XBP1 pathway: A new approach to targeting the tumour microenvironment. *Journal* 2015.
- Chen Y and Brandizzi F: IRE1: ER stress sensor and cell fate executor. *Trends Cell Biol* 23: 547-555, 2013.
- Rigante M, Massimi L, Parrilla C, Galli J, Caldarelli M, Di Rocco C and Paludetti G: Endoscopic transsphenoidal approach versus microscopic approach in children. *Int J Pediatr Otorhinolaryngol* 75: 1132-1136, 2011.
- Nagykalnai T, Landherr L, Laczó I and Piko B: Fulvestrant (Faslodex ®) for hormone sensitive breast cancer. A review. *Magy Onkol* 59: 251-257, 2015 (In Hungarian).
- Cao L, Gao H, Gui S, Bai G, Lu R, Wang F and Zhang Y: Effects of the estrogen receptor antagonist fulvestrant on F344 rat prolactinoma models. *J Neurooncol* 116: 523-531, 2014.
- Leng L and Zhang Y: Effects of an estrogen receptor antagonist on proliferation, prolactin secretion and growth factor expression in the MMQ pituitary prolactinoma cell line. *J Clin Neurosci* 18: 1694-1698, 2011.
- Leng L and Zhang Y: Effects of an estrogen receptor antagonist on proliferation, prolactin secretion and growth factor expression in the MMQ pituitary prolactinoma cell line. *J Clin Neurosci* 18: 1694-1698, 2011.
- Koong AC, Chauhan V and Romero-Ramirez L: Targeting XBP-1 as a novel anti-cancer strategy. *Cancer Biol Ther* 5: 756-759, 2006.
- Henkel A and Green RM: The unfolded protein response in fatty liver disease. *Semin Liver Dis* 33: 321-329, 2013.
- Chen C and Zhang X: IRE1 $\alpha$ -XBP1 pathway promotes melanoma progression by regulating IL-6/STAT3 signaling. *J Transl Med* 15: 42, 2017.
- Chen L, Li Q, She T, Li H, Yue Y, Gao S, Yan T, Liu S, Ma J and Wang Y: IRE1 $\alpha$ -XBP1 signaling pathway, a potential therapeutic target in multiple myeloma. *Leuk Res* 49: 7-12, 2016.
- Rajapaksa G, Nikolos F, Bado I, Clarke R, Gustafsson JA and Thomas C: ER $\beta$  decreases breast cancer cell survival by regulating the IRE1/XBP-1 pathway. *Oncogene* 34: 4130-4141, 2015.
- Jin C, Jin Z, Chen NZ, Lu M, Liu CB, Hu WL and Zheng CG: Activation of IRE1 $\alpha$ -XBP1 pathway induces cell proliferation and invasion in colorectal carcinoma. *Biochem Biophys Res Commun* 470: 75-81, 2016.
- Liu R, Li X, Gao W, Zhou Y, Wey S, Mitra SK, Krasnoperov V, Dong D, Liu S, Li D, *et al*: Monoclonal antibody against cell surface GRP78 as a novel agent in suppressing PI3K/AKT signaling, tumor growth, and metastasis. *Clin Cancer Res* 19: 6802-6811, 2013.
- Rajapaksa G, Nikolos F, Bado I, Clarke R, Gustafsson JÅ and Thomas C: ER $\beta$  decreases breast cancer cell survival by regulating the IRE1/XBP-1 pathway. *Oncogene* 34: 4130-4141, 2015.