

# Estrogenic transmembrane receptor of GPR30 mediates invasion and carcinogenesis by endometrial cancer cell line RL95-2

Yin-Yan He · Gui-Qiang Du · Bin Cai ·  
Qin Yan · Long Zhou · Xiao-Yue Chen ·  
Wen Lu · Yi-Xia Yang · Xiao-Ping Wan

Received: 23 September 2011 / Accepted: 20 December 2011 / Published online: 24 January 2012  
© Springer-Verlag 2012

## Abstract

**Purpose** The mechanisms underlying the effects of estrogen on endometrial cancer remain undefined. Although the classical mechanism of the action of estrogen involves binding to the estrogen receptors  $\alpha$  and  $\beta$ , and transduction of the signal into the cell, G protein-coupled receptor (GPR) 30 has been shown to mediate nongenomic estrogen signaling. The goal of this study was to determine the role of GPR30 signal in the basic process such as invasion and carcinogenesis of endometrial cancer.

**Methods** We downregulated the expression of GPR30 in endometrial cancer cell line RL95-2 by transfection with shGPR30-pGFP-V-RS, a GPR30 antisense expression vector. The cells were then subjected to an MTT assay and a Transwell<sup>®</sup> migration assay. And an animal model was also used to investigate the influence of downregulation of GPR30 on oncogenesis.

**Results** Downregulation of GPR30 led to reduced growth and invasion by cells treated with  $17\beta$ -estradiol. And the capacity of transfected RL95-2 cells to promote tumorigenesis was weakened in vivo.

**Conclusions** Our data suggest that, for the endometrial cancer cell line RL95-2, GPR30 plays important roles in mediating the proliferative and invasive effects of estrogen and in tumorigenesis.

**Keywords** Endometrial cancer · GPR30 · Carcinogenesis · Estrogen · Estrogen receptor

## Abbreviations

GPR30	G protein-coupled receptor 30
E2	$17\beta$ -estradiol
MMP	Matrix metal-loproteinase
MAPK	Mitogen-activated protein kinase
ERK	Extracellular- signal-regulated kinase
MEK	MAPK/ERK kinase
ER	Estrogen receptors
ERE	Estrogen receptor elements
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra zolium
DMSO	Dimethyl sulphoxide
FBS	Fetal bovine serum
ShGPR30-pGFP-V-RS	Short hairpin RNA (shRNA) constructs against GPR30 in pGFP-V-RS
shiv-pGFP-V-RS vector	HuSH 29-mer noneffective against enhanced GFP vector
PTX	Pertussis toxin

Y-Y He and G-Q Du contributed equally to this work.

Y.-Y. He · B. Cai · Y.-X. Yang  
Department of Obstetrics and Gynecology, Shanghai First  
People's Hospital, Shanghai Jiao Tong University,  
Shanghai, China

G.-Q. Du · Q. Yan · L. Zhou · X.-Y. Chen · W. Lu ·  
X.-P. Wan (✉)  
Department of Obstetrics and Gynecology, The International  
Peace Maternity and Child Health Hospital of the China  
Welfare Institute Affiliated to Shanghai Jiao Tong  
University, 910, Hengshan Road, Shanghai, China  
e-mail: wanxiaoping61@126.com; wanxp61@live.com

## Introduction

Estrogen is a pleiotropic hormone with a broad spectrum of physiological functions ranging from regulation of the

menstrual cycle and reproduction to modulation of bone density, brain function, and cholesterol mobilization. As a mitogen, estrogen also promotes the development of endometrial cancer and breast cancer, which account for about 40% of cases of cancer in women (Shang 2007). The classical mechanism of estrogen action involves binding to the estrogen receptors (ERs)  $\alpha$  and  $\beta$ , which are members of the nuclear hormone receptor superfamily with modular structures and discrete domains for their specific functions as ligand-regulated transcription factors. The sequential activation of genes occurs via multiple mechanisms, either through direct binding at estrogen response elements in the promoter region of estrogen-responsive genes or through protein–protein interactions with sites that are canonical to other well-known transcription factors such as AP1, SP1, and nuclear factor  $\kappa$ B (Shang 2006; Pietras et al. 2005; Kumar and Chambon 1988). Following a large number of studies, G protein-coupled receptor (GPR) 30 is now widely recognized as an estrogen receptor that appears to alter gene expression in a rapid, “nongenomic” manner independently of the nuclear ERs and mediates a wide range of responses to estrogen in different cell types (Revankar et al. 2005).

GPR30 is a member of the seven-transmembrane GPR family, which signals through heterotrimeric guanine nucleotide-binding proteins to alter the activity of effector proteins. Cellular activation by GPR30 occurs through a mechanism involving transactivation of epidermal growth factor receptors (EGFRs) via a G protein-dependent pathway and activation of mitogen-activated protein kinase (MAPK), adenylyl cyclase, and phosphoinositide 3-kinase (PI3K) (Thomas et al. 2005; Filardo et al. 2000, 2002). GPR30 has been found to act in physiological processes such as the attenuation of liver injury and pubertal adrenal development (Wang et al. 2007; Baquedano et al. 2007) and has also been shown to mediate the proliferative effects of estrogen in a number of cancer cell lines (Vivacqua et al. 2005, 2006; Albanito et al. 2007). Furthermore, we have found that  $17\beta$ -estradiol (E2) and G1, a GPR30-specific ligand, stimulate the production and activity of matrix metalloproteinase (MMP), the proteolysis of which is involved in cancer metastasis, via the GPR30-mediated MEK/ERK MAPK pathway, and increased secretion of interleukin-6, the activation of which is associated with tumor growth (He et al. 2009). In the present study, we show that GPR30 mediates invasion induced by E2 in an endometrial cancer cell line and plays a crucial role in endometrial tumorigenesis. Our results provide new evidence for the role of GPR30 in endometrial carcinogenesis.

## Materials and methods

### Cell culture

The human endometrial cell line RL95-2 was obtained from the Chinese Academy of Sciences Committee Type Culture Collection cell bank. The cells were grown in Dulbecco's modified Eagle's medium (DMEM)/F12 (11030; Gibco, Auckland, New Zealand) supplemented with 10% fetal bovine serum (FBS) (16000-44; Gibco, Carlsbad, CA), 100 units/mL penicillin, and 100  $\mu$ g/mL streptomycin in a humidified atmosphere of 5% CO<sub>2</sub>/95% air at 37°C.

### Stable transfection

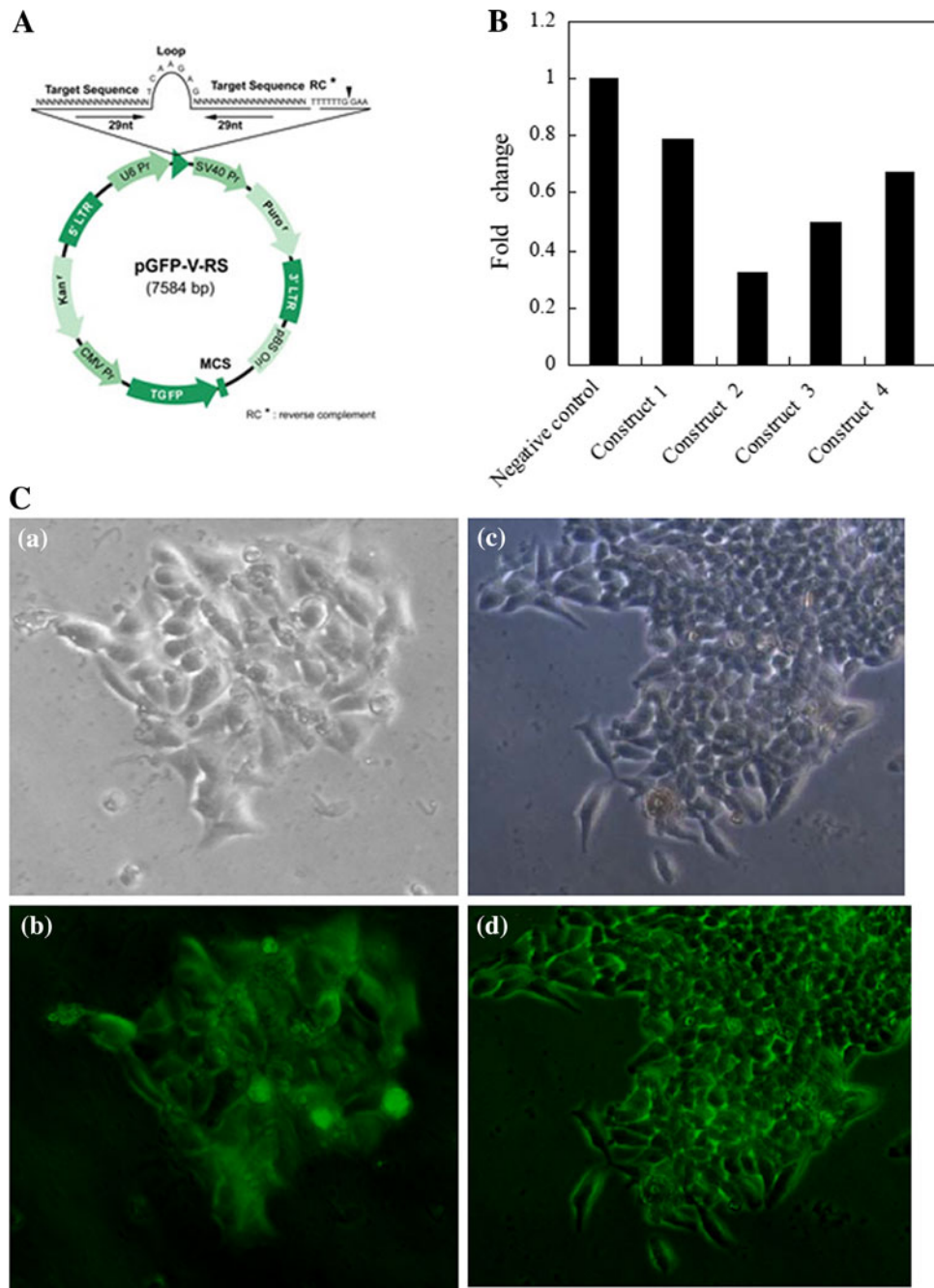
One hundred thousand cells were plated into 24-well dishes with 500  $\mu$ L regular growth medium per well on the day before transfection. The cells were transfected with short hairpin RNA (shRNA) constructs against GPR30 in pGFP-V-RS (ShGPR30-pGFP-V-RS vector, TG316565; OriGene Technologies, Rockville, MD) (Fig. 1) and the HuSH 29-mer non-effective against enhanced GFP vector (shiv-pGFP-V-RS vector, pRS.TR30003; OriGene Technologies) using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. To obtain a stable cell line, selection pressure was maintained with puromycin at a concentration of 0.5–1.0  $\mu$ g/mL in the growth medium for 6 weeks. Clonal populations of cells were selected by transferring well-isolated single clumps of cells (the clonal ancestor and cells divided from it) into a six-well plate. Growth of these cells was continued in the selection medium for two additional passages.

### Real-time RT-PCR

Total RNA was extracted from the transfected RL95-2 cells using TRI Reagent (TR118; Molecular Research Center, Cincinnati, OH). cDNA was generated with oligo(dT)18 primers using a Revert Aid First Strand cDNA Synthesis Kit (K1622; Fermentas Life Science, St. Leon-Rot, Germany). A 50- $\mu$ L PCR amplification of single-strand cDNA was performed with 35 cycles of denaturation (94°C) for 60 s, annealing (55°C) for 30 s, and elongation (72°C) for 30 s using PerfectShot Ex Taq (Loading Dye Mix) (DRR05TA; Takara, Dalian, China). The primer sequences are shown in Table 1.

### Proliferation assay

RL95-2 cells were seeded in 96-well plates at  $2 \times 10^5$  cells/mL and cultured for 24 h. The cells were then



**Fig. 1** The pGFP-V-RS plasmid vector pattern and interferential efficiency of ShGPR30-pGFP-V-RS vectors. **a** The pGFP-V-RS plasmid vector was created with an integrated turboGFP element to readily verify transfection efficiency. It also incorporates both a kanamycin and puromycin resistance elements for greater selection capabilities. The shRNA expression cassette consists of a 29-nt GPR30-specific sequence, a 7-nt loop, and another 29-nt reverse complementary sequence, all under the control of the human U6 promoter. A termination sequence (TTTTTT) is located immediately downstream of the second 29-nt reverse complementary sequence to terminate the transcription by RNA Pol III. **b** The RNA expression levels of RL95-2 cells transfected with each of four different

ShGPR30-pGFP-V-RS vectors were determined using real-time RT-PCR. RNA expression of RL95-2 cells transfected with shiv-pGFP-V-RS (negative control) was set at 1, from which the RNA expression of the other cells was calculated. **c** RL95-2 cells were visualized by fluorescence microscopy after stable transfection with pGFP-V-RS(**b**). **a** Cells transfected with ShGPR30-pGFP-V-RS under *white light*. **b** Cells transfected with ShGPR30-pGFP-V-RS under fluorescence. **c** Cells transfected with shiv-pGFP-V-RS (negative control) under *white light*. **d** Cells transfected with shiv-pGFP-V-RS (negative control) under fluorescence. Magnification, (**a**, **b**)  $\times 200$ ; (**c**, **d**)  $\times 100$

**Table 1** Primer sequences of G protein-coupled receptor (GPR) 30 and  $\beta$ -actin used for real-time RT-PCR

mRNA	Size (bp)	Primer sequence	Position
GPR30 NM_001039966	217	Sense: 5'-GCCTGCCGCTGCAGGAAACATTTTC-3'	2323–2346
		Antisense: 5'-CTCGGCGGTGCGTAGCTAGCTCAG-3'	2539–2519
$\beta$ -Actin NM_001101	251	Sense: 5'-CAGCCATGTACGTTGCTATCCAGG-3'	462–485
		Antisense: 5'-AGGTCCAGACGCAGGATGGCATG-3'	612–590

switched to phenol red-free and serum-free medium (DMEM/F12, 11039; Gibco, Carlsbad, CA) and cultured for 24 h, followed by the addition of G1, a nonsteroidal GPR30-specific agonist ( $10^{-9} \sim 10^{-5}$  M) (371705; Calbiochem), or E2 ( $10^{-9} \sim 10^{-5}$  M) (E2758-250MG; Sigma Aldrich, St. Louis, MO), in the presence or absence of the MEK/ERK MAPK inhibitor U0126 (30  $\mu$ M) (9903; Cell Signaling Technology) or pertussis toxin (PTX) (200 ng/mL; 516560; Calbiochem). The cells were further cultured for 48 h with phenol red-free DMEM/F12 containing 5% dextran-coated charcoal-treated FBS (S181F; Biowest, Nuaille, France). Cell proliferation was evaluated using a Cell Counting Kit-8 (CK04-11; Dojindo Molecular Technologies, Gaithersburg, MD). The controls were treated with vehicle (0.1% dimethyl sulfoxide/phenol red-free DMEM/F12).

#### Transwell<sup>®</sup> assay

The Matrigel invasion assay was carried out as previously described with slight modification (Sato et al. 2002). The cell culture inserts (8  $\mu$ m pore size, 6.5 mm diameter; Corning, Corning, NY, USA) coated with 5  $\mu$ L pure Matrigel (356237, BD Biosciences) were placed in a 24-well plate. Before use, the Matrigel was rehydrated with 100  $\mu$ L warm DMEM for 2 h. The lower chamber was filled with 800  $\mu$ L DMEM with 1% FBS. The cells were allowed to invade for 12 h in 5% CO<sub>2</sub> at 37°C. RL95-2 cells were plated in the upper chamber. The cells were treated as in “Proliferation assay”. The lower chamber was filled with 800  $\mu$ L DMEM/F12 containing 5% dextran-coated charcoal-treated FBS. The cells were then incubated at 37°C for 48 h. After swabbing of noninvaded cells in the upper chambers, cells that migrated to the lower chambers were fixed with 3.7% paraformaldehyde in PBS and stained with hematoxylin. For quantification, the cells that had migrated to the lower surface were counted under a light microscope in five predetermined fields at a magnification of  $\times 200$ . The assay was repeated three times, and the results are expressed as a percentage to the mean number of three controls. Each experiment was carried out in triplicate and repeated three times.

#### Xenograft tumor formation assays

Intact, female athymic *nu/nu* mice of 5–6 weeks of age were obtained from the Shanghai Experimental Animal Center of the Chinese Academy of Science. The tumorigenicity of the cells was assessed by subcutaneously injecting  $10 \times 10^6$  cells bilaterally into five mice for each treatment; a total of 10 mice were thus used. Tumor measurement began one week after injection and was conducted weekly using digital calipers. Tumor volume was calculated as follows:  $(1/2 \times \text{length})(\text{width}^2)$ . All procedures were approved by the University of Shanghai Jiao Tong University Affiliated First People's Hospital Animal Care and Use Committee.

#### Statistics

Statistical analysis was performed using the *t* test or one way analysis of variance. All tests were performed using Statistical Product and Service Solutions software version 15.0 (SPSS Inc., Chicago, IL).  $P < 0.05$  was considered statistically significant.

## Results

#### Expression of GPR30 is stably downregulated by transfection with ShGPR30-pGFP-V-RS

The commercial plasmid pGFP-V-RS (Fig. 1a) contains an inserted sh sequence that can either suppress the expression of GPR30 (ShGPR30-pGFP-V-RS) or is invalid (shiv-pGFP-V-RS). Four designed and sequence-verified shRNA vectors (construct 1–4) are available for GPR30. The vector also contains the pCMV driven tGFP gene, which encodes the tGFP protein constitutively in mammalian cells. This feature makes it possible to monitor the efficiency of transfection.

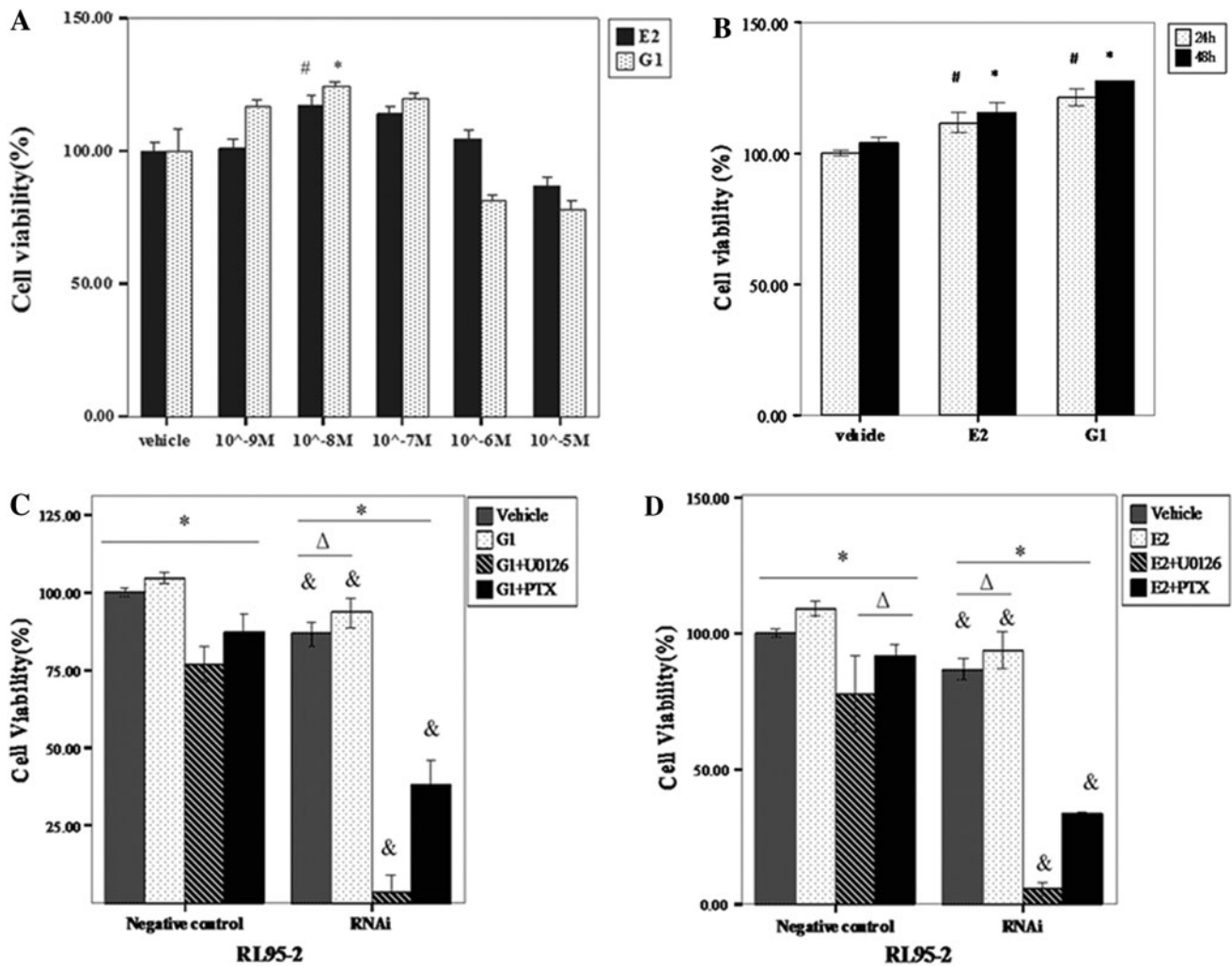
It took 42 days to obtain stably transfected RL95-2 cells. The interferential efficiency of the different vectors was then determined using real-time RT-PCR. Construct 2 showed the best interference effect (Fig. 1b). The successfully transfected cells were also examined under a

microscope, through which green fluorescence from GFP protein expressed by pGFP-V-RS could be seen (Fig. 1c).

GPR30 signaling stimulated proliferation of endometrial cancer cell line RL95-2

To determine whether GPR30 signaling could induce growth of endometrial cancer cells, proliferation assays were conducted. We carried out a proliferation assay to test whether GPR30 signaling could have effects on endometrial cancer cell proliferation. We found that E2 ( $10^{-8}$  M)

and G1 ( $10^{-8}$  M) increased the proliferation of endometrial cancer cell line RL95-2 (Fig. 2a). A longer duration of treatment (48 h vs. 24 h) had a greater proliferation-promoting effect (Fig. 2c), suggesting that the ability of G1 and E2 to promote proliferation is time dependent in RL95-2 cells. The stimulation of proliferation by E2 and G1 was disrupted by PTX, an inhibitor of G-protein signaling, and U0126, an inhibitor of MEK/ERK MAPK. When RL95-2 cells were transfected with ShGPR30-pGFP-V-RS, their proliferation declined even in the presence of E2 and G1, versus the negative control group (Fig. 2d). These results



**Fig. 2** G protein-coupled receptor 30 via the MEK/ERK mitogen-activated protein kinase (MAPK) pathway stimulates proliferation of an endometrial cancer cell line. **a** RL95-2 cells were treated with 17 $\beta$ -estradiol (E2,  $10^{-9}$  ~  $10^{-5}$ M) or G1 ( $10^{-9}$  ~  $10^{-5}$  M) for 24 h in medium containing 5% charcoal-stripped fetal bovine serum then analyzed on the indicated cultured times. #*P* < 0.05 versus the other five control groups, \**P* < 0.05 versus the other five RNAi groups; **b** RL95-2 cells were treated with E2 ( $10^{-8}$ M) and G1( $10^{-8}$ M) for 24 and 48 h. #*P* < 0.05 versus vehicle 24 h; \**P* < 0.05 versus vehicle 48 h. **c, d**: RL95-2 cells were treated with E2 ( $10^{-8}$  M) or G1 ( $10^{-8}$  M) with or without pertussis toxin (PTX) (200 ng/mL) or U0126 (30  $\mu$ M)

in medium containing 5% charcoal-stripped fetal bovine serum. Cells were further cultured for 24 h before evaluation of cell proliferation. Vehicle: 0.1% dimethyl sulfoxide/phenol red-free and serum-free Dulbecco's modified Eagle's medium/F12. Negative control: cells transfected with HuSH 29-mer noneffective against enhanced green fluorescent protein (shv-pGFP-V-RS vector). RNAi: cells transfected with HuSH 29-mer short hairpin RNA against GPR30 in pGFP-V-RS vector (ShGPR30-pGFP-V-RS vector). \**P* < 0.05;  $\Delta$ *P* > 0.05; &*P* < 0.05 versus the negative control. Medium was refreshed and treatments were renewed every 2 days. Each data point is the mean  $\pm$  SD of three independent experiments

suggest that GPR30 has a crucial signaling role in the proliferation of the endometrial cancer cell line RL95-2. Through the GPR30-mediated MEK/ERK MAPK pathway, G1 and E2 could stimulate the proliferation of RL95-2.

GPR30 signaling promoted invasion by endometrial cancer cell line RL95-2

The effect of E2 and G1 on RL95-2 invasion was determined by a Transwell<sup>®</sup> assay. To determine the role of GPR30 in endometrial cancer cell invasion induced by E2 and G1 in vitro, RL95-2 cells transfected with ShGPR30-pGFP-V-RS or shiv-pGFP-V-RS were treated with E2 ( $10^{-8}$ M) or G1 ( $10^{-8}$  M) with or without PTX or U0126. The results revealed that knockdown of GPR30 inhibited RL95-2 cell invasion induced by G1 or E2. Furthermore, incubation with PTX or U0126 markedly decreased cell invasion compared to the control (Fig. 3). These data suggest that GPR30 signaling promoted endometrial cancer cell invasion via the MEK/ERK MAPK pathway.

GPR30 signaling in endometrial carcinoma cells enhanced tumorigenic capacity

To determine whether GPR30 signaling has a direct effect on tumor cell proliferation in vivo, we implanted RL95-2 cells transfected stably with ShGPR30-pGFP-V-RS or shiv-pGFP-V-RS into female athymic mice using PBS as a vehicle. Significant differences were observed in the mean tumor volumes. Both cell lines formed palpable tumors in less than one week. However, compared with the control group, significant ( $P < 0.05$ ) decreases in volume were observed from week 4 to week 8 in tumors produced by RL95-2 cells transfected with ShGPR30-pGFP-V-RS, and this difference increased with time (Fig. 4). All animals survived for the duration of the study in apparent good health.

## Discussion

The classic or genomic mode of ER action is slow compared with rapid or nongenomic estrogen responses, which can occur within minutes and are mediated by two distinct mechanisms involving membrane ERs. One mechanism involves the localization of nuclear ERs at the plasma membrane (Pedram et al. 2006; Levin 2005; Harrington et al. 2006), whereas the second involves the receptor GPR30.

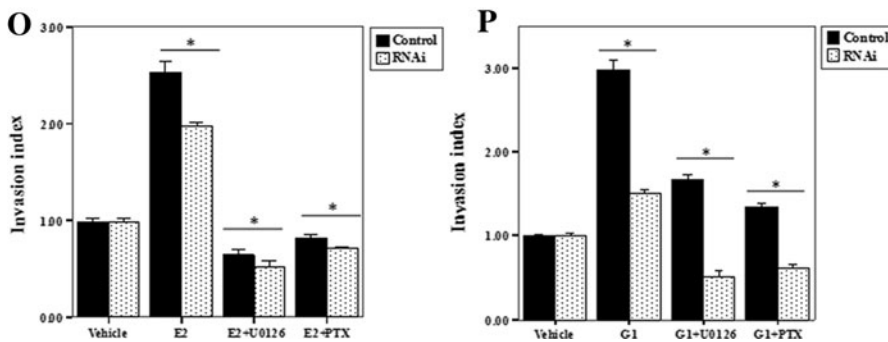
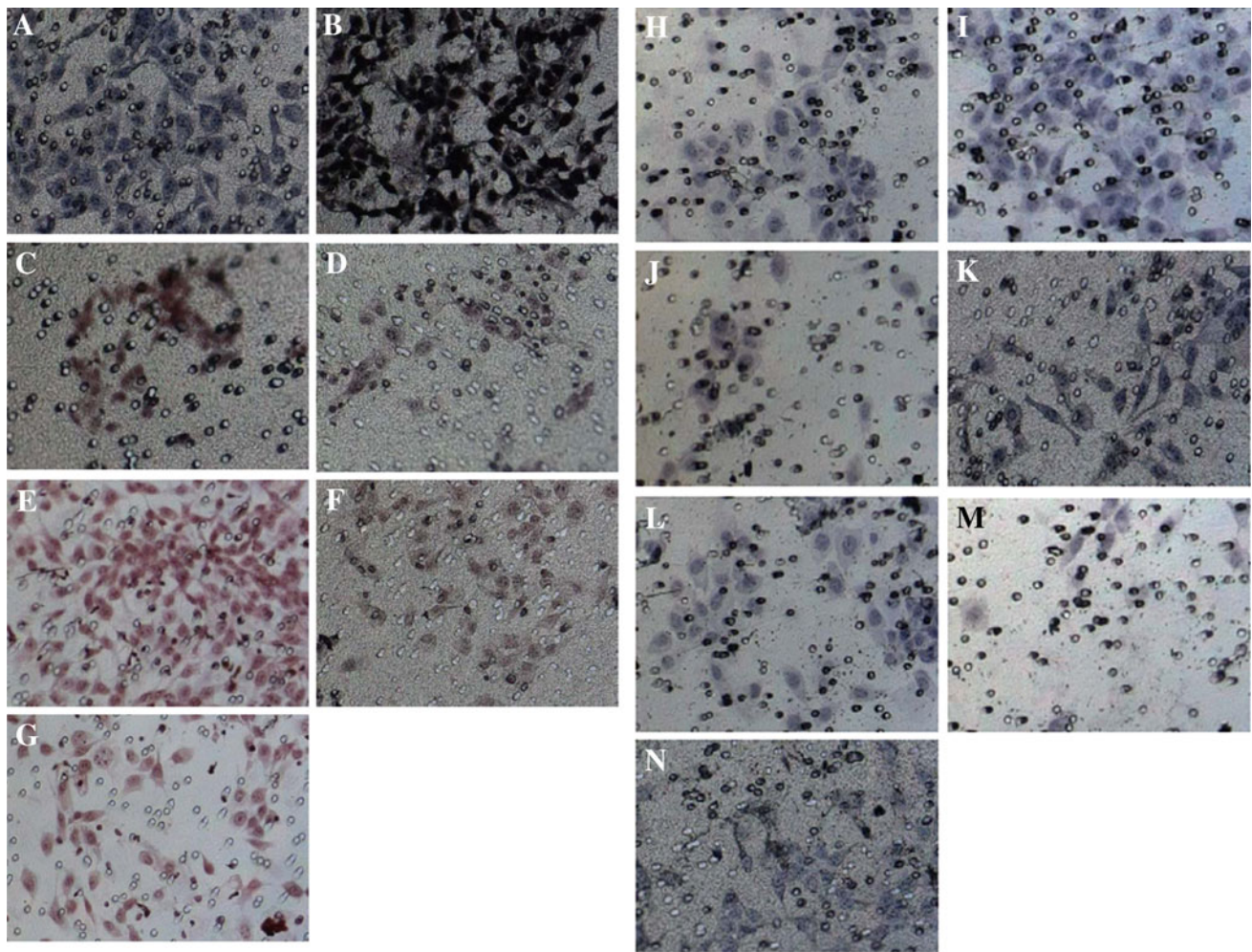
GPR30 transcripts are reported to be widely distributed in normal human tissues, with high levels found in the heart, lung, liver, intestine ovary, and brain. Accordingly, GPR30 has extensive functions. For example, in the

immunological system, studies of rat models have shown GPR30 to be involved in thymocyte apoptosis, and in the circulatory system, the reduction in blood pressure induced by E2 may be mediated by GPR30.

Expression of GPR30 has been observed in several cancer cell lines, and a number of reports have shown estrogen-mediated cell proliferation to be dependent on GPR30 (Vivacqua et al. 2005, 2006; Albanito et al. 2007). In the present study, we found that GPR30 signaling promotes proliferation of the endometrial cancer cell line RL95-2 via the MEK/ERK MAPK pathway. Invasion is a major marker of malignancy and prognosis. In our previous study, we showed that GPR30 signaling via the MEK/ERK MAPK pathway increased MMP production and activity in endometrial cancer cells. In the present study, a Transwell<sup>®</sup> assay showed that GPR30 signaling promoted endometrial cancer cell invasion through the MEK/ERK MAPK pathway. Together, these findings suggest that GPR30 plays a role in carcinogenesis.

Several studies have examined the expression of GPR30 in vivo. Filardo et al. (2006) performed immunohistochemistry for GPR30 in 361 breast carcinomas and found that overexpression of GPR30 was significantly associated with tumor size ( $>2$  cm), the presence of distant metastases, and HER-2/neu overexpression. Similar studies (Smith et al. 2007; Ignatov et al. 2010) have been conducted on tumors from patients with endometrial adenocarcinoma, and GPR30 overexpression has been found to occur more frequently in endometrial carcinomas exhibiting deep myometrial invasion, high grade, biologically aggressive histological subtype, advanced stage, and significantly worse overall survival rate. Interestingly, there was a significant correlation between G protein-coupled estrogen receptor expression and endometrial pathology in breast cancer patients receiving tamoxifen, an estrogen antagonist with antiproliferative properties in the breast but proliferative properties in the endometrium (Ignatov et al. 2010). In the present study, we have demonstrated for the first time that GPR30 signaling in endometrial carcinoma cells enhances their tumorigenic capacity. All the in vivo studies give strong support to an association between GPR30 and the occurrence and development of endometrial and breast cancer.

In summary, our findings demonstrate that GPR30 signaling promotes the proliferation of endometrial cancer cells and increases their potential for invasion through the MEK/ERK MAPK pathway, providing further evidence for the crucial role of GPR30 in endometrial carcinogenesis. Recently, several groups have provided more details of the mechanism of GPR30 signaling. Lin et al. (2009) found a novel signaling paradigm that is initiated by activation of GPR30 by estrogen, with signal transduction cascades (PI3K and MAPK) that converge on nuclear hormone



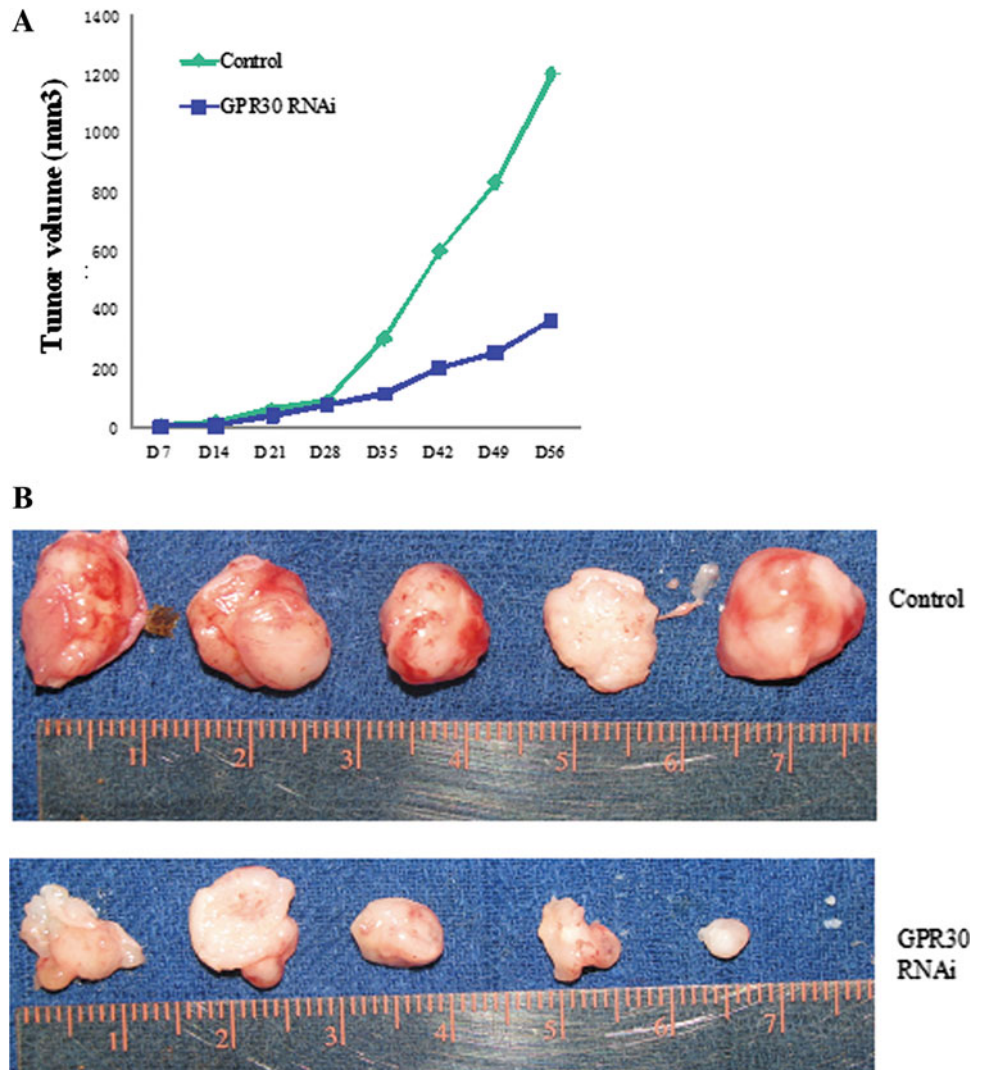
**Fig. 3** G protein-coupled receptor 30 stimulates invasion by an endometrial cancer cell line via the MEK/ERK mitogen-activated protein kinase (MAPK) pathway. RL95-2 cells stably transfected with shiv-pGFP-V-RS (negative control; **a–g**) or ShGPR30-pGFP-V-RS (**h–n**) were treated as follows. **a, h** Control group (vehicle 0.1% dimethyl sulfoxide/phenol-free Dulbecco’s modified Eagle’s medium/F12; **b, i** 17β-estradiol (E2) ( $10^{-7}$  M); **c, j** E2 ( $10^{-7}$  M) with U0126 (30 μM); **d, k** E2 ( $10^{-7}$  M) with pertussis toxin (PTX) (200 ng/mL);

**e, l** G1 ( $10^{-8}$  M); **f, m** G1 ( $10^{-8}$  M) with U0126 (30 μM); **g, n** G1 ( $10^{-8}$  M) with PTX (200 ng/mL). Cell invasion was then assessed and photomicrographs taken. Quantitation (**o, p**) and representative photomicrographs (**a–n**) show inhibition of invasion by RL95-2 cells transfected with ShGPR30-pGFP-V-RS vector (RNAi) compared with the negative control (shiv-pGFP-V-RS). Data are expressed as the mean ± SD. \* $P < 0.05$  compared with the negative control

receptors (steroidogenic factor-1/liver receptor homolog-1) to modulate their transcriptional output. GPR30 expression has been found to be induced by EGF and transforming

growth factor through the EGFR/ERK transduction pathway in endometrial and tamoxifen-resistant breast cancer cells (Vivacqua et al. 2009). Considering that GPR30

**Fig. 4** G protein-coupled receptor 30 (GPR30) signaling in endometrial carcinoma cells enhances their tumorigenic capacity. **a** Volume of tumors produced by RL95-2 cells stably transfected with shiv-pGFP-V-RS (control) or ShGPR30-pGFP-V-RS (GPR30 RNAi) in nude mice over 56 days and **b** tumors taken from nude mice 56 days after inoculation of RL95-2 cells. Data are expressed as the mean  $\pm$  SD



might activate the EGFR/ERK pathway through the release of heparin-binding EGF, we could assume that a positive feedback loop occurs within the ligand/EGFR/GPR30 signaling network leading to the stimulation of estrogen-sensitive tumors. Like the ER signaling network, the GPR30 network must be complicated and needs further research.

**Acknowledgments** The study was supported by National Natural Science Foundation of China (NO. 30872760 and NO. 81001154), China Postdoctoral Science Foundation (Special program, NO. 200902242), Project of the Shanghai “Post-Qi-Ming-Xing Plan” for Young Scientist (11QA1405200) and the Bureau grant of Shanghai Municipal Health Bureau (2005ZD002).

## References

- Albanito L, Madeo A, Lappano R, Vivacqua A, Rago V, Carpino A, Oprea TI, Prossnitz ER, Musti AM, Andò S, Maggiolini M (2007) G protein-coupled receptor 30 (GPR30) mediates gene expression changes and growth response to 17 $\beta$ -estradiol and selective GPR30 ligand G-1 in ovarian cancer cells. *Cancer Res* 67:1859–1866
- Baquedano MS, Saraco N, Berensztein E, Pepe C, Bianchini M, Levy E, Goñi J, Rivarola MA, Belgorosky A (2007) Identification and developmental changes of aromatase and estrogen receptor expression in prepubertal and pubertal human adrenal tissues. *J Clin Endocrinol Metab* 92:2215–2222
- Filardo EJ, Quinn JA, Bland KI, Frackelton AR Jr (2000) Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. *Mol Endocrinol* 14:1649–1660
- Filardo EJ, Quinn JA, Frackelton AR Jr, Bland KI (2002) Estrogen action via the G protein-coupled receptor, GPR30: stimulation of adenylyl cyclase and cAMP-mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis. *Mol Endocrinol* 16:70–84
- Filardo EJ, Graeber CT, Quinn JA, Resnick MB, Giri D, DeLellis RA, Steinhoff MM, Sabo E (2006) Distribution of GPR30, a Seven Membran $\bar{C}$ Spanning estrogen receptor, in primary breast cancer and its association with clinicopathologic determinants of tumor progression. *Clin Cancer Res* 12:6359–6366
- Harrington WR, Kim SH, Funk CC, Madak-Erdogan Z, Schiff R, Katzenellenbogen JA, Katzenellenbogen BS (2006) Estrogen



- dendrimer conjugates that preferentially activate extranuclear, nongenomic versus genomic pathways of estrogen action. *Mol Endocrinol* 20:491–502
- He Y–Y, Cai B, Yang Y–X, Liu XL, Wan XP (2009) Estrogenic G protein-coupled receptor 30 signaling is involved in regulation of endometrial carcinoma by promoting proliferation, invasion potential, and interleukin-6 secretion via the MEK/ERK mitogen-activated protein kinase pathway. *Cancer Sci* 100:1051–1061
- Ignatov T, Eggemann H, Semczuk A, Smith B, Bischoff J, Roessner A, Costa SD, Kalinski T, Ignatov A (2010) Role of GPR30 in endometrial pathology after tamoxifen for breast cancer. *Am J Obstet Gynecol* 203:595.e9–595.e16
- Kumar V, Chambon P (1988) The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer. *Cell* 55:145–156
- Levin ER (2005) Integration of the extranuclear and nuclear actions of estrogen. *Mol Endocrinol* 19:1951–1959
- Lin BC, Suzawa M, Blind RD, Tobias SC, Bulun SE, Scanlan TS, Ingraham HA (2009) Stimulating the GPR30 estrogen receptor with a novel tamoxifen analogue activates SF-1 and promotes endometrial cell proliferation. *Cancer Res* 69:5415–5423
- Pedram A, Razandi M, Levin ER (2006) Nature of functional estrogen receptors at the plasma membrane. *Mol Endocrinol* 20:1996–2009
- Pietras RJ, Levin ER, Szego CM (2005) Estrogen receptors and cell signaling. *Science* 310:51–53
- Revankar CM, Cimino DF, Sklar LA et al (2005) A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science* 307:1625–1630
- Sato Y, Fujiwara H, Yoshioka S, Tatsumi K, Maeda M, Fujii S (2002) Involvement of dipeptidyl peptidase IV in extravillous trophoblast invasion and differentiation. *J Clin Endocrinol Metab* 87:4287–4296
- Shang Y (2006) Molecular mechanisms of oestrogen and SERMs in endometrial carcinogenesis. *Nat Rev Cancer* 6:360–368
- Shang Y (2007) Hormones and cancer. *Cell Res* 17:277–279
- Smith HO, Leslie KK, Singh M, Qualls CR, Revankar CM, Joste NE, Prossnitz ER (2007) GPR30: a novel indicator of poor survival for endometrial carcinoma. *Am J Obstet Gynecol* 196:386.e1–386.e11
- Thomas P, Pang Y, Filardo E, Dong J (2005) Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology* 146:624–632
- Vivacqua A, Bonofiglio D, Recchia AG, Musti AM, Picard D, Andò S, Maggiolini M (2005) The G protein-coupled receptor GPR30 mediates the proliferative effects induced by 17-estradiol and hydroxytamoxifen in endometrial cancer cells. *Mol Endocrinol* 20:631–646
- Vivacqua A, Bonofiglio D, Albanito L, Madeo A, Rago V, Carpino A, Musti AM, Picard D, Andò S, Maggiolini M (2006) 17 $\beta$ -Estradiol, genistein, and 4-hydroxytamoxifen induce the proliferation of thyroid cancer cells through the g protein-coupled receptor GPR30. *Mol Pharmacol* 70:1414–1423
- Vivacqua A, Lappano R, De Marco P, Sisci D, Aquila S, De Amicis F, Fuqua SA, Andò S, Maggiolini M (2009) G protein-coupled receptor 30 expression is up-regulated by EGF and TGF  $\alpha$  in estrogen receptor { $\alpha$ }-positive cancer cells. *Mol Endocrinol* 23:1815–1826
- Wang C, Prossnitz ER, Roy SK (2007) Expression of G protein-coupled receptor 30 in the hamster ovary: differential regulation by gonadotropins and steroid hormones. *Endocrinology* 148:4853–4864