

## Review Article

# Function of G-Protein-Coupled Estrogen Receptor-1 in Reproductive System Tumors

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Received 27 January 2016; Revised 8 April 2016; Accepted 8 May 2016

Academic Editor: Bin Zhang

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The G-protein-coupled estrogen receptor-1 (GPER-1), also known as GPR30, is a novel estrogen receptor mediating estrogen receptor signaling in multiple cell types. The progress of estrogen-related cancer is promoted by GPER-1 activation through mitogen-activated protein kinases (MAPK), phosphoinositide 3-kinase (PI3K), and phospholipase C (PLC) signaling pathways. However, this promoting effect of GPER-1 is nonclassic estrogen receptor (ER) dependent manner. In addition, clinical evidences revealed that GPER-1 is associated with estrogen resistance in estrogen-related cancer patients. These give a hint that GPER-1 may be a novel therapeutic target for the estrogen-related cancers. However, preclinical studies also found that GPER-1 activation of its special agonist G-1 inhibits cancer cell proliferation. This review aims to summarize the characteristics and complex functions of GPER-1 in cancers.

## 1. Introduction

Estrogen is an important hormone in human beings, especially in females. It plays several important physiological and pathological roles in not only reproductive system but also other systems. Estrogen disorder results in various diseases, such as endometrial diseases, skeletal diseases, and reproductive system tumors. Increasing attention has been paid to revealing of the functions of estrogen in physiological and pathological conditions. Estrogen receptors (ER)  $\alpha$  and  $\beta$ , the two well established nuclear estrogen receptors, have different physiological functions depending upon their various distributions [1]. Meanwhile, the activity of ER $\beta$  is opposed to ER $\alpha$  in many systems. Lots of evidences show that estrogen induces the proliferation of cancer cells in breast, uterus, and ovarian cancer through ER $\alpha$ . On the contrary, activation of ER $\beta$  can reverse this effect. Notably, a novel transmembrane estrogen receptor, known as G-couple estrogen receptor (GPER), was found [2]. ER are involved in the initiation, migration, and progression of estrogen-related multiorgan cancers, such as breast cancer, ovarian cancer, prostate cancer, testicular cancer, liver cancer, and lung cancer as well [3]. Although increasing studied are

focused on the roles of GPER-1 in different types of cancers, the functions of GPER-1 in cancers remain unclear yet. Characteristics and functions of reproductive system cancer will be summarized and discussed in the present review.

## 2. The Characteristics of GPER-1

*2.1. The Structure and Distribution of GPER-1.* G-protein-coupled estrogen receptor-1 (GPER-1), a seven-transmembrane-domain receptor localized in cell surface, was first identified in 1996 [2]. GPER-1 is detected broadly in numerous human tissues, such as breast, prostate, ovary, placenta, subcutaneous adipose, visceral adipose, arteries, vessels, heart, liver, lung and intestine tissues. GPER-1 is a member of GPCR superfamily, which is structurally unrelated to the classical ER $\alpha$  and ER $\beta$ . There are four transcriptional variants encoding 375 amino acids composing seven transmembrane proteins [4].

Classical GPCR are cell membrane proteins which bind their ligands at cell surface. But GPER-1 binding domain exists inside the plasma membranes and the endoplasmic reticulum [5–8]. The biological functions of GPER-1 might be associated with cell types and its location. Estradiol, the

major type of estrogen, binds to GPER-1 with a high affinity to GPER-1 while the other two isoforms, estrone and estradiol, have very low binding affinities [5, 9]. Furthermore, numbers of environmental estrogens bind to GPER-1 and activate the downstream signaling pathways, such as bisphenol A, genistein, and nonylphenol [10]. GPER-1-specific compound 1 (G-1) is a specific agonist of GPER-1, which has no function of ER $\alpha$  and ER $\beta$  and was identified using virtual and biomolecular screening in 2006 [11]. G-1 has been widely used as a target tool to evaluate the function of GPER-1 in different cells and disease models.

**2.2. GPER-1 Signaling Pathways.** GPER-1 mediates both genomic and nongenomic response with its ligands. To date, GPER-1 signaling pathways have not been fully elucidated yet. The binding ligands of GPER-1, such as estrogen, G-1, tamoxifen, and ICI182,780, cross the plasma membrane and bind to the GPER-1 on endoplasmic reticulum where they activate its  $\beta$  and  $\gamma$  subunits and subsequently activate both Src and adenylyl cyclase (AC) leading to the intracellular cAMP production. The phosphorylation of Src induces matrix metalloproteinase (MMP) production, which cleaves pro-heparan-bound epidermal growth factor (pro-HB-EGF) releasing free HB-EGF. HB-EGF binds to the EGFR leading to activation of multiple molecules such as Ras, PI3K, AKT, and Erk1/2. The downstream signal of PI3K and AKT results in several nuclear receptors activation which is closely related the proliferation and migration of cancer cell. GPER-1 also binds to the G-couple protein  $\alpha$  subunit and activates the phospholipase C (PLC), AC, and CAMP. Activated PLC results in inositol triphosphate (IP<sub>3</sub>) production, which further binds to its receptor and leads to intracellular calcium mobilization.

### 3. Functions of GPER-1 in Reproductive System Tumors

**3.1. Breast Cancer.** Breast cancer is the most common and deadly cancer in females worldwide [12]. Breast cancer is generally classified into estrogen receptor positive (ER+) and ER negative (ER-) [13]. In clinical practices, the endocrine treatments such as tamoxifen and aromatase are recommended in the ER positive breast cancers, while there is no benefit in the ER negative cancers [14]. GPER-1 is widely expressed in both of these breast cancer types and the primary breast cancers. Recent clinical study results showed that the expression of GPER-1 might correlate with clinical and pathological poor outcome biomarkers [15]. Other results also showed that the expression of GPER-1 was inversely correlated with the ER expression. Coexpression of GPER-1 and ER was found in almost 24% patients with inflammatory breast cancer, while 19% only express ER and 46% only express GPER-1 [16].

The GPER-1 mRNA levels were significant higher in ER positive breast cancer cells compared to ER negative cancer cells, and the expression of GPER-1 depends on ER $\alpha$  mRNA level. Interestingly, GPER-1 preformed a different proliferation manner in ER positive MCF-7 breast cancer cell line [17]. G-1 enhanced migration of MCF-7 breast cancer cells

by activating ERK1/2 and EGFR signaling pathway, which is tremendously attenuated by G15 [18]. The other evidences also approved that GPER-1 is an initiator of tamoxifen resistance in breast cancers [19–21]. The promotion roles of GPER-1 in cancer cells proliferation and migration may be correlated with the autolysis of calpain 1 [22], cleavage of cyclin E [18], or the expression of target gene. There are also some studies which found that GPER-1 inhibits the growth of ER positive MCF-7 cells, which is probably through G-couple  $\beta$  and  $\gamma$  subunits activating without CAMP signal activation [21–24]. However, combination treatment with G-1 and Her2 antibody Trastuzumab exerted an additive growth inhibitory effect on breast cancer cells [25]. Thus, GPER-1 inhibits ER positive breast cancers proliferation which is a potential target for ER positive breast cancers and drug-resistant breast cancer.

ER negative breast cancer cells are more aggressive than ER positive cancer cells. Deficiency of ER in breast cancer is correlated with poor response to endocrine therapy [26]. In ER negative breast cancers, GPER-1 stimulates the ERK1/2 through the EGFR/MAPK signal cascade, inducing target gene like c-fos expression, which is involved in the progressing of breast malignancies [27–29]. Estrogen and antiestrogens can also promote the production of the early growth response-1 (Egr-1), connective tissue growth factor (CTGF), and insulin-like growth factor 1 (IGF-1) through the GPER-1 [28, 30, 31]. GRP30 activation stimulated breast cancer cells migration through CTGH, CXC receptor (CXCR1), and notch pathways [28]. Furthermore, GPER-1 agonist G-1 promoted inflammation in breast cancers [32]. GPER-1 was reported to affect the deformation of breast glandular structure inducing the malignant transformation of breast tissue [33]. GPER-1 can also induce expression of cancer-associated fibroblasts (CAFs) in tumor microenvironment [34, 35]. On the contrary, a recent study showed that activation of GPER-1 by G-1 resulted in G2/M-phase arrest and induction of mitochondrial-related apoptosis [36]. The other studies also proved that G-1 treatment suppressed the growth of SKBr3 cancer cells and increased the survival rate by inducing the ERK1/2 signal activation [36, 37].

15–20% of breast cancers are included in triple negative breast cancers (TNBC), characterized by lack of ER $\alpha$ , progesterone receptor (PR), and EGFR2 (Her-2). A higher rate of recurrence and aggressive biological features were found in younger females [38, 39]. GPER-1 expression was found in majority of TNBCs patients [40]. In the GPER knockdown mice model, the proliferation of TNBCs, the activation of EGFR, and c-fos expression were reduced [41]. These findings suggest that GPER plays a key role in putative mechanism for TNBCs and GPER might be a therapeutic target for TNBCs.

Paradoxical debates still exist on the functions of GPER-1 in breast cancers. SNPs of GPER-1, histone acetylation, and transcription factor recruitment were significantly associated with tumor size and histological grading [42, 43]. The different results of GPER-1 in breast cancer were summarized in Table 1.

**3.2. Ovarian Tumors.** Estrogens play a crucial role in the development of ovarian cancers. GPER RNA as well as GPER-1 protein presents in both primary and malignant

TABLE 1: The effect of GPER-1 in reproductive system tumors.

Cancer types	ER positive breast cancer	ER negative breast cancer	Triple negative breast cancer	Ovarian cancer	Testicular cancer	Prostate cancer
Proliferation	+24, 31 -17, 25, 52	+28, 31 -23, 36, 37	+40, 41	+47, 48, 49, 50 -51, 52	+55, 56, 57	-61, 62
Migration and metastasis	+18, 21, 22, 31, 15, 32	+27, 28, 29	+41	+48, 49	/	/
Poor survival	+20, 16	-36	+38, 39, 40	+46	/	/
Drug resistance	+19, 20, 21	+26	/	/	/	/

ovarian tumor tissues [44]. The expression of GPER-1 was significantly increased in ovarian carcinomas compared to pericarcinomatous tissues independent with the expression of EGFR, ER $\alpha$ , and ER $\beta$  [45]. Further investigation showed that the expression of GPER-1 was associated with lower survival rates [46]. Estrogen and G-1 induce ovarian cancer cell growth responses via EGFR-MAPK signaling pathways. This procedure required coexpression of ER $\alpha$  [44, 47]. Furthermore, GPER-1 promoted the migration and invasion of ovarian cancer cells OVCAR5 which is characterized by negative ER $\alpha$  and positive GPER by increasing the expression MMP-9 [48, 49]. Atrazine, one of the most common pesticide contaminants, promoted ovarian cancer cells proliferation via induction of Erk and expression of estrogen target gene through GPER-1 pathway [50]. But other studies results showed that G-1 suppressed proliferation and induced apoptosis of human ovarian cancer cells probably through inhibition of cell cycle progression in G2/M-phase in ovarian carcinomas [51, 52].

**3.3. Testicular Cancers.** GPER-1 has been shown to be involved in a variety of hormone-dependent cancers. It is well understood that estrogens play a critical role in pathological germ cell proliferation in testicular germ cell tumors. GPER-1 seems to be involved in modulating the growth of estrogen dependent testicular cancer cells [53]. Estrogen induces the high expression of GPER-1 correlated with low levels of ER $\beta$  in human testicular carcinoma in situ and seminomas [53, 54]. Bisphenol A, a common environmental estrogen, can also promote the proliferation of testicular seminomas cells through GPER-1 [55]. The above findings suggested that GPER-1 may be a potential therapeutic target [56, 57].

**3.4. Prostate Cancer.** Estrogen has an efficacy for advanced prostate cancer (PC) via the mediation of the classical estrogen receptors [58]. The effects of ER on PC growth and metastases have different mechanisms in different cellular microenvironments [59]. The expression of GPER-1 is higher in the preneoplastic lesions and normal areas of benign prostate than the basal epithelial cells [60]. G-1, the selectively activating GPER-1, inhibited the growth of multiple PC cells in vitro and in vivo through Erk1/2 and c-jun/c-fos signaling pathways, which indicates that the G-1 may be a new option for PC through targeting GPER-1 [61]. G-1

inhibited castration-resistant phase but had no effect on androgen-sensitive tumors. The antitumor effect of G-1 on CR tumors was related to necrosis (approximately 65%) accompanied with neutrophils infiltration. G-1 can also upregulate neutrophil-related chemokines and inflammation-mediated cytokines in the CR tumors. In one word, GPER-1 is an androgen-repressed target. The antitumor effect of G-1 was neutrophil-infiltration-associated necrosis [62].

#### 4. GPER-1 in Other Tumors

Overexpression of GPER-1 was detected in various reproductive system cancers. Studies showed that the activation of GPER-1 signaling pathways leads to tumor. There are other studies which proved that GPER-1 induced proliferation, differentiation, and drug resistance of lung cancers [63, 64], thyroid cancers [65], bladder cancers [66], and oral squamous carcinomas [67]. More studies to reveal the functions and mechanisms of GPER-1 in the other system cancers are warranted.

#### 5. Conclusion

GPER-1 activation by estrogen induces nongenomic signaling pathways and regulates certain gene transcriptions. Majority of the study results addressed that activation of GPER-1 by estrogen and G-1 results in the downstream signals and target genes activation, which promotes the proliferation, migration, and invasion of cancer cells. And this effect is in nonclassical ER expression dependent manner in most cancers except for ovarian cancers. It is interesting that several other studies showed that G-1, the special agonist of GPER-1, promoted the expression of GPER-1 and inhibited the proliferation of ER negative breast cancer cells, ovarian cancer cells, and prostate cancer cells. The opposite effects of GPER-1 in cancer cells may be associated with the epigenetic of GPER-1, such as the SNPs and histone acetylation. The different cell types, tumor microenvironment, and hormonal level may also affect the functions of GPER-1. Controversies still exist on the GPER-1 localization and related signaling pathways, in particular the potential action as proapoptotic mediator. Since the function and mechanisms of GPER-1 are still unclear, more researches and clinical studies are strongly warranted to clarify the different function and mechanisms in different cancer types and conditions.

## Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

## Acknowledgments

This work is supported by the National Nature Science Foundation of China (no. 81471534).

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