**Name of Journal:** *World Journal of Gastroenterology*

**Manuscript NO:** 47779

**Manuscript Type:** REVIEW

**G protein-coupled estrogen receptor in colon function, immune regulation and carcinogenesis**

Jacenik D *et al*. GPER in colon function and cancer

Damian Jacenik, Ellen J Beswick, Wanda M Krajewska, Eric R Prossnitz

**Damian Jacenik, Wanda M Krajewska,** Department of Cytobiochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Lodz 90-236, Poland

**Ellen J Beswick,** Division of Gastroenterology, Hepatology and Nutrition, Department of Internal Medicine, School of Medicine, University of Utah, Salt Lake City, UT 84132, United States

**Damian Jacenik, Eric R Prossnitz,** Department of Internal Medicine, School of Medicine, and UNM Comprehensive Cancer Center, University of New Mexico, Albuquerque, NM 87131, United States

**ORCID number:** Damian Jacenik (0000-0003-4563-2302); Ellen J Beswick (0000-0003-4147-5777); Wanda M Krajewska (0000-0002-1450-3712); Eric R Prossnitz (0000-0001-9190-8302).

**Author contributions:** Jacenik D conceived of this paper and performed the literature review; all authors contributed to writing, critical revisions, editing and approval of the final manuscript.

**Supported by** theGrants from the National Science Centre, Poland (2017/24/T/NZ5/00045 and 2015/17/N/NZ5/00336 to Damian Jacenik), the U.S. National Institutes of Health (NIH R01 CA163890 and CA194496 to Eric R. Prossnitz; R01 CA207051 to Ellen J. Beswick), the UNM Comprehensive Cancer Center (P30 CA118100), the Autophagy, Inflammation and Metabolism Center of Biomedical Research Excellence (P20 GM121176) and Dialysis Clinic, Inc. (to Eric R. Prossnitz).

**Conflict-of-interest statement:** Dr. Prossnitz discloses US patent 7,875,721 licensed to Sandia Biotech and Linnaeus Therapeutics, US patent 8,487,100 licensed to Linnaeus Therapeutics, and pending US patent applications 15/556,055 and 16/067,721.

**Open-Access:** This article is an open-access article which was selected byan in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Corresponding author: Eric R Prossnitz, PhD, Full Professor,** Department of Internal Medicine, School of Medicine, and UNM Comprehensive Cancer Center, University of New Mexico, 2325 Camino de Salud, Albuquerque, NM 87131, United States. [eprossnitz@salud.unm.edu](mailto:eprossnitz@salud.unm.edu)

**Telephone:** +1-505-272-5647

**Received:** March 28, 2019

**Peer-review started:** March 28, 2019

**First decision:** May 24, 2019

**Revised:** July 3, 2019

**Accepted:** July 5, 2019

**Article in press:** July 5, 2019

**Published online:** August 14, 2019

**Abstract**

Estrogens play important roles in the development and progression of multiple tumor types. Accumulating evidence points to the significance of estrogen action not only in tumors of hormonally regulated tissues such as the breast, endometrium and ovary, but also in the development of colorectal cancer (CRC). The effects of estrogens in physiological and pathophysiological conditions are mediated by the nuclear estrogen receptors α and β, as well as the membrane-bound G protein-coupled estrogen receptor (GPER). The roles of GPER in CRC development and progression, however, remain poorly understood. Studies on the functions of GPER in the colon have shown that this estrogen receptor regulates colonic motility as well as immune responses in CRC-associated diseases, such as Crohn’s disease and ulcerative colitis. GPER is also involved in cell cycle regulation, endoplasmic reticulum stress, proliferation, apoptosis, vascularization, cell migration, and the regulation of fatty acid and estrogen metabolism in CRC cells. Thus, multiple lines of evidence suggest that GPER may play an important role in colorectal carcinogenesis. In this review, we present the current state of knowledge regarding the contribution of GPER to colon function and CRC.

**Key words:** G protein-coupled estrogen receptor; Colorectal cancer; Proliferation; Migration; Colonic motility; Inflammatory bowel disease

© **The Author(s) 2019**. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** G protein-coupled estrogen receptor (GPER) is a membrane-bound estrogen receptor that participates in the rapid non-genomic actions of estrogens involving numerous downstream signaling pathways. GPER is expressed in the gastrointestinal tract and is engaged in physiological and pathophysiological processes in the colon. This review aims to assess the significance of GPER expression and estrogenic signaling in colorectal carcinogenesis.

Jacenik D, Beswick EJ, Krajewska WM, Prossnitz ER. G protein-coupled estrogen receptor in colon function, immune regulation and carcinogenesis. *World J Gastroenterol* 2019; 25(30): 4092-4104

**URL:** https://www.wjgnet.com/1007-9327/full/v25/i30/4092.htm

**DOI:** https://dx.doi.org/10.3748/wjg.v25.i30.4092

**INTRODUCTION**

The roles of estrogen (17β-estradiol) and its receptors in gastrointestinal (GI) diseases are complex. The lower incidence rate of colon cancer in women compared to men suggests a protective role for the female sex hormone estrogen[1,2]. However, because estrogen has at least three receptors, contradictory pro- and anti-tumorigenic mechanisms have been observed depending on the receptor-mediated mechanisms examined. The effects of estrogens are mediated by receptors including the nuclear estrogen receptors (*i.e*., ERα and ERβ)[3] and the G protein-coupled estrogen receptor (GPER, previously known as GPR30)[4]. GPER is a 7-transmembrane receptor cloned from the ER-positive MCF-7 cell line[5], among other sources[6-11]. As opposed to nuclear estrogen receptors that are primarily responsible for the genomic actions of estrogens, GPER initiates many rapid non-genomic actions of estrogen involving secondary messengers, which can also ultimately lead to secondary gene expression changes[4,12,13]. GPER activity is not only stimulated by 17β-estradiol, but also by numerous xeno- and phyto-estrogens (*e.g*., bisphenols and genistein), clinically relevant anti-hormonal therapeutic agents (*e.g*., tamoxifen and fulvestrant) and synthetic GPER-selective ligands (*e.g*., G-1,) as summarized in Table 1[4]. GPER expression and activity, the latter often defined employing GPER-selective ligands, have been linked to many aspects of normal physiology and pathophysiology[14-16].

Experimental evidence strongly suggests that estrogen, and therefore its receptors, plays important roles in neoplastic transformation of the colon[17,18]. One challenge in understanding these roles is the often-contradictory results regarding estrogen receptors and their roles in GI diseases. Although estrogen is generally thought to be anti-inflammatory, and inflammation typically contributes to carcinogenesis, the roles of individual estrogen receptors are complex, which may result in conflicting observations. Among the various estrogen receptors, ERβ, in particular, has been suggested to act as a tumor suppressor in colorectal cancer (CRC) and serves as a prognostic factor for CRC progression. Stevanato Filho *et al*[18] observed significantly lower ERβ levels in CRC patients with clinical stage III and IV disease compared to patients with stage I and II disease. The absence of ERβ in CRC is thus a poor prognostic factor associated with higher mortality [hazard ratio = 3, 95% confidence interval (CI) = 1.24-7.46]. However, in contrast to its established tumor-protective role, Cho *et al*[17] used ER-deficient mice to show that, in addition to ERβ, ERα is also crucial for enterocyte growth and differentiation. Both nuclear estrogen receptors appear to be important modulators of colon neoplastic transformation as ERβ or ERα deficiency was associated with tumor progression and abnormal mucosal histology in an APC-dependent tumorigenesis model employing C57BL/6-Min/+ mice[17]. However, in addition to the nuclear estrogen receptors, accumulating evidence suggests that GPER is also involved in many aspects of CRC cell pathophysiology. In this review, we summarize the evidence for GPER expression and function in the colon and in colorectal carcinogenesis.

**GPER MODULATES COLONIC MOTILITY**

Colonic motility regulates the frequency and timing of defecation as well as the consistency of stools, with the main symptoms of colon movement disorders being constipation and diarrhea. A large retrospective study demonstrated a positive association between chronic constipation and a higher prevalence, as well as risk, of benign neoplasm and CRC[19]. Moreover, incidence rate ratio analyses have shown that an increased risk of both benign and malignant colorectal neoplasms appears to be directly related to the severity of constipation. Additional evidence linked constipation, defined as fewer than three bowel movements per week, with an overall 2.4-fold increase of CRC development[20]. Interestingly, although women overall have a slightly lower risk of developing CRC than men, women with constipation appear to be more prone to CRC development (odds ratio = 2.7, 95%CI = 1.5-5.0) compared to men with constipation (odds ratio = 1.7, 95%CI = 0.6-4.9). Mechanistically, although colon motor dysfunction may increase exposure of the GI tract to carcinogens, evidence suggests that colonic transit time also influences bacterial metabolism and mucosal turnover, which may affect CRC development[21].

Two independent studies have revealed that GPER activity influences colonic motility *in vivo*[22,23]. Li *et al*[22] demonstrated that GPER activity affects colonic motility in multiple phases of the estrous cycle in female mice. Colonic transit time, which was significantly longer during the proestrus and estrus phases (*vs* diestrus phase), was reduced by selective GPER inhibition by the GPER antagonist G-15[24,25]. Similarly, in ovariectomized mice, acute estrogen treatment increased colonic transit time, and was reversed by co-administration of G-15. *Ex vivo*, GPER activation with the GPER-selective agonist G-1[26] inhibited circular muscle strip contraction in a nitric oxide (NO)-dependent manner, and stimulated NO production in cultured myenteric nitrergic neurons, which together act to reduce colonic motor function. Further supporting this mechanism, Zielińska *et al*[23] and Li *et al*[22], demonstrated, using the colonic bead expulsion test, that GPER stimulation by G-1 or estrogen treatment prolongs colonic transit time in both male and female mice. The inhibitory effect of GPER activation on the number of fecal pellets excreted was further confirmed *in vivo* employing a mouse model of hypermotility. The potential mechanism by which GPER affects colonic motility appears to involve inhibition of muscle contractility, as determined by electrical field- and bethanechol-stimulated longitudinal smooth muscle contractions[23].

Further indirect evidence highlighting the importance of GPER in the regulation of colonic motor function is derived from studies of irritable bowel syndrome (IBS) patients[27,28]. Alterations in GPER mRNA and protein expression in the colonic mucosa, as well as serum estrogen levels, were reported in samples from IBS patients with constipation (IBS-C) or diarrhea (IBS-D)[27]. An increased number of GPER-positive colonic mast cells in IBS-D patients compared to either healthy control or IBS-C patients has also been observed[28]. GPER-positive cell staining in the colonic mucosa also correlated with increased abdominal pain severity in IBS-D patients and increased expression of GPER in the cytoplasm of mast cells, which are associated with abdominal bloating frequency and dysmotility-like dyspeptic symptom severity and frequency in IBS patients. These results suggest a possible role for GPER in mast cells and immune function at large, which play an important role in intestinal function and disease[29].

**GPER IN IMMUNE REGULATION AND COLONIC INFLAMMATION**

Crohn’s disease (CD) and ulcerative colitis (UC), the two most commonly diagnosed types of inflammatory bowel disease (IBD), are characterized by chronic colon inflammation and are associated with a higher risk of CRC development[27]. A meta-analysis performed by Flores *et al.* reported that patients with histologic inflammation are more prone to colorectal dysplasia and/or cancer development (odds ratio = 2.6, 95%CI = 1.5-4.5) compared to patients without mucosal inflammation[30]. For UC patients, the increased risk of CRC development appears to be related to disease duration[31]. A meta-analysis by von Roon *et al*[32] further revealed that patients with CD experience a higher relative risk of CRC development, related to both the anatomic localization of disease and patient age. CD patients with disease affecting ileocolic and colon segments experience relative risks of 4.6 (95%CI = 2.1-10.3) and 13.4 (95%CI = 5.7-13.2) for ileocolic and CRC development, respectively. Furthermore, population-based studies have shown that the presence of CD before the age of 25 and 30 correlates with very high relative risks of CRC development (21.5, 95%CI = 11.4-40.4 and 9.5, 95%CI = 3.1-23.2, before the age of 25 and 30, respectively)[32].

Immunomodulatory roles for estrogen and its receptors in the pathogenesis of IBD have been documented[17,33-36], but the specific mechanisms and roles of GPER in modulating immune responses remain elusive. In addition to the nuclear estrogen receptors, GPER is expressed at both the mRNA and protein levels in intestinal samples obtained from both IBD patient subtypes (*i.e*., CD and UC)[37]. In patients with CD, GPER protein content was increased in non-inflamed colon areas compared to the control group but lower in inflamed colon tissue compared to non-inflamed colon, suggesting the possibility of a complex protective role for GPER in colitis[37]. To date, although there is no direct evidence showing GPER regulates immune responses in CRC or the CRC microenvironment, several studies show that GPER is indeed expressed in multiple immune cells, including monocytes/macrophages, neutrophils, B and T cells[38-44]. Accumulating evidence suggests that GPER modulates cytokine and cytokine receptor expression in immune cells, cancer cells and cancer-associated fibroblasts (CAFs). In breast cancer, estrogen signaling through GPER regulates CAF-mediated progression of breast cancer and is associated with several signaling pathways leading to the modulation of gene expression[45-48]. Pro-tumorigenic effects of GPER activation result from receptor tyrosine kinase [*e.g*., insulin-like growth factor receptor I and epidermal growth factor receptor (EGFR)] modulation and downstream effector proteins such as AKT and extracellular signal-regulated kinase (ERK)[47]. GPER-mediated ERK activation in breast CAFs leads to expression of the proto-oncogenes *c-Fos*, cyclin D1 and connective tissue growth factor (CTGF)[45,46,48], which play an important role in many cancers including CRC[49].

GPER also plays an important role in the regulation of interleukin (IL)-1β and IL-1R expression in CAFs and breast cancer cells, leading to a gene profile associated with cancer cell invasiveness[50]. GPER’s immunomodulatory activity occurs not only in CAFs, but also in several immune cell types. In lipopolysaccharide (LPS)-stimulated primary human macrophages, GPER activation leads to inhibition of tumor necrosis factor-α (TNF-α), IL-6, IL-12 and C-C motif ligand (CCL) 5 secretion[38]. A similar effect was observed in the murine macrophage cell line RAW 264.7, where G-1 treatment inhibited TNF-α secretion from LPS-stimulated cells. The anti-inflammatory properties of GPER activation were confirmed in an animal model of multiple sclerosis (experimental autoimmune encephalomyelitis), where G-1 therapy reduced the severity of symptoms and the number of nervous system-infiltrating macrophages[38]. The ability of GPER to inhibit LPS-induced IL-6 expression in murine macrophages was shown to occur *via* NF-κB signaling[51]. Together, these data suggest a potentially central role for GPER in regulating inflammation during IBD.

GPER activity in macrophages may also be important in tumor development and progression of the GI tract. The importance of tumor-associated macrophages (TAMs), specifically in pancreatic cancer has been recently highlighted[41]. In a murine model of pancreatic cancer, treatment with tamoxifen, which acts as a GPER agonist (see Table 1), reduced the percentage of tissue macrophages as well as the polarization of TAMs to the M2 phenotype. In RAW 264.7 cells, GPER regulated focal adhesions, cell-extracellular matrix attachment and invasion of macrophages[41]. Macrophages, are associated with the development and progression of colitis and neoplastic transformation of the colon. Whereas M1 macrophages are elevated in intestinal samples from IBD patients where they promote inflammation, during CRC progression, increased M2/M1 ratios appear to correlate with increased liver metastasis[52-54]. Overall, these complex actions of GPER suggest a “protective” role in CRC development and progression.

IL-6 is an important mediator of cancer cell function and tumor development; however, conflicting data exist as to the role of GPER in its regulation. Bisphenol A (BPA), a non-selective ER/GPER agonist (Table 1) induces proliferation, migration and invasion of laryngeal cancer cells, as a result of increased IL-6 mRNA expression, potentially *via* GPER activation[55]. Furthermore, treatment with the GPER‑selective inhibitor G-15 and/or siRNA targeting of GPER attenuated cell proliferation, migration and IL-6 expression of laryngeal cancer cells. These processes involved signal transducer and activator of transcription 3 (STAT3) activation by GPER-IL-6 signaling as assessed by siRNA targeting of IL-6 or GPER[55]. As STAT3 is known to regulate both pro- and anti-inflammatory cytokine production, the exact role of GPER in these complex processes needs to be further investigated. It should be noted that BPA action may also depend on both the cell type and the nuclear estrogen receptor status of cells[56]. In contrast to these results, in a more “typical” anti-inflammatory estrogen response, GPER inhibited IL-6 expression *via* NF-κB inhibition, leading to reduced migration of triple negative MDA-MB-231 breast cancer cells[57]. As a result, in MDA-MB-231 xenograft tumors, GPER activation inhibited both angiogenesis and metastasis. GPER-mediated inhibition of IL-6 production was also demonstrated in TNFα-stimulated breast cancer cells and osteosarcoma cells where GPER inhibited IL-6 expression, suppressing migration and invasion[58,59]. Overall, these results suggest the effects of GPER expression and activity may be tumor type- and context-specific.

Accumulating evidence reveals that GPER is a crucial immunoregulatory factor regulating not only macrophages, but also granulocytes. GPER modulates multiple mediators of the immune response in fish acidophilic granulocytes[40], including *IL-1β*, *IL-10*, prostaglandin-endoperoxide synthase 2 and prostaglandin D2 synthase expression through activation of the cAMP/PKA/CREB signaling pathway. Granulocytes significantly impact IBD progression. These mechanisms are important in IBD[60] and thus should be further examined. Of interest in cancer, GPER activation, in a murine model of castration-resistant prostate cancer, led to increased neutrophil influx and tumor necrosis, suggesting a previously unrecognized role for GPER in men[61].

Multiple reports have highlighted that GPER also stimulates anti-inflammatory immune responses through the modulation of T cell function and cytokine expression, representing a potential therapeutic target for certain autoimmune diseases. In one study of autoimmune encephalomyelitis, splenocytes cultured from G-1-treated mice and stimulated with antigen, yielded lower IFN-γ, TNF-α, IL-17, CCL4 and CCL5 protein levels in supernatants compared to splenocytes from vehicle-treated mice[38]. In other studies, GPER activation increased production of IL-10 (an anti-inflammatory cytokine that inhibits several immune cell types) in CD4+ T cells under Th17 polarizing conditions, generating hybrid autoregulatory T cell populations[39]. This effect was abolished by GPER and ERK inhibitors, but not by p38 or Jun N-terminal kinase inhibitors, suggesting that GPER regulates IL-10 production through ERK signaling. GPER’s anti-inflammatory action may be mediated through specific immune cell types as a higher frequency of IL-10-producing CD4+ (but not CD8+) T cells is observed in mouse splenocytes following GPER activation[62]. Moreover, G-1-treated mice showed a significantly higher population of IL-10‑producing GPER+ CD4+ T cells, consistent with the ability of GPER to regulate IL-10 production.

In additional T cell studies employing a mouse model of asthma, G-1 reduced the level of Th2 cytokines, such as IL-5 and IL-13, in bronchoalveolar lavage fluid, suggesting negative regulation of acute asthma through IL-10-producing T cells[62]. In IBD, several intestinal T cell populations are dysregulated, with the extent of dysregulation correlating to disease severity. In clinical samples obtained from IBD patients with active disease, higher and lower levels of CD4+ T cells and CD8+ T cells, respectively, are present compared to healthy controls and IBD patients with inactive disease[63]. However, in CRC tumors, only an increased percentage of CD4+ T cell, but not CD8+ T cells was observed[64]. The significance of regulatory T cells in inflammation and CRC progression, together with the anti-inflammatory properties of GPER, indicates that GPER activation in CD4+ T cells may be a promising target to modulate colon immune responses. However, although GPER activity in T cells generally appears to promote anti-inflammatory responses, which would have a positive impact in inflammatory diseases, such responses may have a potentially negative impact in cancer, where immune monitoring is critical. Taken together, these results suggest that as an important regulator of colon inflammation and immune responses in CRC, GPER action may also be important in the context of long-term immune response deregulation, which is critical in CRC development associated with IBD.

**THE ROLES OF GPER IN CRC CELL PROLIFERATION AND APOPTOSIS**

Accumulating evidence reveals important roles for estrogen and its receptors, including GPER, as revealed by selective ligands such as G-1, in the regulation of cancer cell growth, survival and function[65]. GPER appears to regulate cancer cell proliferation and survival not only in estrogen-associated cancers such as breast[66], ovarian[67] or endometrial[68,69], but also in other cancer types not traditionally associated with estrogen, e.g. lung and CRC[47,70-77]. Several cellular mechanisms are regulated by GPER in cancer cells, including cell cycle, endoplasmic reticulum stress and apoptosis. In both HCT-116 and SW-480 CRC cells, GPER activation by G-1 led to cell cycle arrest and inhibition of proliferation[74]. The higher proportion of HCT-116 cells in the apoptotic sub-G1 phase, as well as lower mitochondrial membrane polarity, following GPER activation suggests that GPER activation promotes apoptosis in CRC cells. Consistent with this, G-1 treatment induced up-regulation of pro-apoptotic factors such as Bcl-2-associated X protein, cyclin-dependent kinase inhibitor 1 (p21), and cleaved caspase-3, with down-regulation of anti-apoptotic factors, such as B-cell lymphoma 2 (Bcl-2) and procaspase-3.

Cancer cell growth arrest and apoptosis are also regulated by endoplasmic reticulum stress signals (reviewed by Sano and Reed[78]). Among several factors modulating endoplasmic reticulum stress, protein expression of ATF4 and 6, XBP-1 and CHOP increased upon G-1 treatment of HCT-116 cells[74] (Figure 1). Elevated reactive oxygen species (ROS) and ERK1/2 phosphorylation, resulting from GPER-mediated signaling, also contributed to growth arrest of HCT-116 cells[74]. These pathways are particularly important in chronic inflammation, where immune cell-released ROS and cytokines activate the NF-κB pathway. GPER mediates inhibition of IκBα, which leads to phosphorylation of NFκB/p65 and nuclear translocation, as well as GSK-3β phosphorylation (Figure 1). Constitutive NF-κB activity in cancer cells drives neoplastic transformation and tumor progression, affecting cancer cell proliferation and survival[79-81] as well as tumor angiogenesis[82], metabolism[83,84], immune response[85] and metastatic potential of cancer cells[82].

GPER may also be a regulator of genetic transmission during cell division in CRC cells. In HT-29 cells, BPA up-regulated mRNA levels of CDCA8 (also known as Borealin), a crucial member of the chromosomal passenger complex that mediates several events during mitosis[86] (Figure 1). Although BPA is classically thought to function through the nuclear estrogen receptors, particularly ERα, it also binds and activates GPER (Table 1)[4]. Since HT-29 cells express only GPER and ERβ, the effects of BPA on CDCA8 expression are likely mediated, at least in part, by GPER[86]. GPER also regulates ataxia telangiectasia mutated (ATM), an important protein in carcinogenesis, through regulation of the cell cycle and DNA repair (reviewed by Branzei and Foiani[87]). In a human breast cancer study, ATM phosphorylation was positively correlated with the number of lymph-node metastasis cases[88]. However, in CRC and colon adenomas, increased *ATM* promoter methylation was observed compared to control tissue[89]. Estrogen, acting through GPER, represses *ATM* expression under both normoxic and hypoxic conditions in HT-29 cells[70], with the effect of estrogen on ATM expression being stronger under hypoxic conditions, suggesting an effect of oxygen levels on estrogen’s effects.

Hypoxia plays an important role in tumor progression, affecting tumor vascularization, epithelial-mesenchymal transition and metastasis as well as chemo- and radio-resistance[90]. Under hypoxic conditions, the pathways activated by hypoxia-inducible factor (HIF) control numerous cellular proteins, including vascular endothelial growth factor (VEGF), driving tumor growth. GPER mediates estrogen’s suppression or enhancement of HIF-1α and VEGFA expression under normoxic and hypoxic conditions, respectively[70]. Furthermore, GPER mediates opposing functions in HT-29 and DLD‑1 CRC cells, dependent on oxygen levels, with GPER agonists suppressing proliferation under normoxic conditions, but increasing proliferation under hypoxic conditions.

The production and presence of local estrogens may be important in the development and progression of CRC. The ability of GPER to regulate CRC cell proliferation may thus represent a mechanism through which the known effects of estrogen on CRC are mediated. *In vitro* and *in vivo* studies have shown that estrogen promotes CRC in part through the dysregulation of enzymes involved in estrogen metabolism. Specifically, in post-menopausal women and men with CRC, compared to age-matched control groups, higher colonic activity of the enzyme steroid sulfatase (STS), which converts circulating sulfated estrogens to the active forms, has been observed[91]. Furthermore, increased protein and mRNA levels of 17β-hydroxysteroid dehydrogenase (HSD17) B7 and B12 (responsible for the conversion of estrone to estrogen) are present in human CRC tissue samples, whereas mRNA and protein levels of the enzyme catalyzing conversion of estrogen to estrone (*i.e*., HSD17B2) are decreased. Experimental evidence strongly suggests that supplementation with estrogen or STS overexpression increases CRC cell proliferation *in vitro* and *in vivo*. The latter was demonstrated using a murine CRC xenograft model with STS overexpressing cells[91]. Activation of GPER by estrogen derived from these multiple sources results in the up-regulation of CTGF, EGR1 and ATF3, with CTGF up-regulation being required for enhanced cell proliferation, with estrone sulfate transport and GPER-stimulated STS activity producing a novel estrogen-generating positive feedback loop (Figure 1) that may play an important role in CRC progression[92]. Interestingly, in addition to estrogen and G-1, the breast cancer therapeutic agents, tamoxifen and fulvestrant (ICI 182.780), also increased STS activity (in a GPER-dependent manner), suggesting these drugs could have a negative impact on CRC development and progression.

**GPER REGULATES CRC CELL MIGRATION**

An increasing number of studies describe a modulatory effect of GPER activity on cancer cell migration and invasion in multiple cancer types, including breast (including triple-negative), endometrial, ovarian, lung, thyroid, kidney, and granulosa cell[57,71,77,93-105]. In previous work that demonstrated the dual role of GPER activation on CRC cell proliferation (see above), oxygen-dependent GPER-mediated effects on the migration of HT-29 and DLD-1 cells was also observed[70]. Scratch wound and Boyden chamber assays demonstrated that both G-1 and estrogen inhibited migration under normoxic conditions, whereas GPER stimulation enhanced migration of CRC cells under hypoxic conditions. Fatty acid synthase (FASN), a key lipogenic enzyme that affects neoplastic transformation of the breast, colon and liver, has been described as a metabolic oncogene[106-108] and is regulated by many factors, including estrogens[108,109]. GPER stimulation by G‑1 or estrogen increases expression and activity of FASN *via* EGFR/ERK/c-Fos/AP-1 signaling, resulting in increased growth and migration of CRC cells, which was in turn decreased by a FASN inhibitor[108]. Thus, GPER activity regulates cell motility through multiple complex mechanisms (Figure 1).

**CLINICAL SIGNIFICANCE OF GPER IN CRC**

There is conflicting evidence that GPER may act as a tumor suppressor as well as a tumor promoter in CRC. A seven-fold down-regulation of GPER mRNA in CRC samples compared to adjacent control tissue has been observed[74]. Immunohistochemical analysis confirmed a decrease in GPER protein expression in CRC patients that was associated with decreased survival. Lower GPER expression also correlated with tumor progression and lymph node metastasis. Bioinformatic analyses of multiple datasets (accession numbers: GSE2091 and GSE871) confirmed these results and showed statistically significant lower GPER mRNA levels in both colon and CRC samples compared to control samples. *In vitro* evidence further demonstrated that decreased GPER expression in HTC-8 and SW-480 CRC cell lines correlated with higher promoter methylation of the GPER gene as compared to LS147T CRC cells, which exhibit high GPER expression. Beyond promoter methylation, histone H3 acetylation may represent another mechanism regulating GPER expression in CRC cell lines and human tissues as down-regulation of GPER expression in HCT-8 and SW-480 cells was associated with decreased histone H3 acetylation. In contrast to the above results, based on Kaplan-Meier analyses and datasets (accession number: GSE39582), high GPER expression was associated with poor relapse-free survival in women with stage 3/4 (but not stage 1/2) CRC, but not in men with disease of any stage[70]. Overall, these results suggest that GPER plays a complex role in colorectal carcinogenesis that is further complicated by sexual dimorphism, potentially as a result of estrogen-dependent signaling in CRC.

**CONCLUSION**

Estrogen signaling modulates cancer cell proliferation, invasion and migration, acting not only through the nuclear estrogen receptors α and β, but also through the GPER. GPER is without a doubt an important mediator of colorectal neoplastic transformation and progression. Clinical and experimental data are however, not unambiguous, which may result from the use of CRC cell lines with different GPER expression levels or GPER-mediated signaling pathways. On the other hand, recent studies have revealed the dual role of GPER in CRC development, potentially related to the modulation of both anti-tumorigenic and pro-tumorigenic effects, depending in part on oxygen levels in cancer cells. At the molecular level, GPER-mediated signaling in CRC cells regulates endoplasmic reticulum and mitochondrial functions, the metabolism of fatty acids and estrogens and the expression of genes directly involved in cell proliferation and survival (summarized in Table 2). In conclusion, GPER appears to be an important mediator of estrogenic actions in both neoplastic transformation of the colon and tumor progression, effects that need to be considered in the application and development of therapeutic strategies.

**ACKNOWLEDGEMENTS**

We thank Dr. Richard Pepermans for proofreading and editorial corrections.

**REFERENCES**

1 **Haziman AA**, Ravinderan S, Thangavelu T, Thomas W. A novel role for estrogen-induced signaling in the colorectal cancer gender bias. *Ir J Med Sci* 2019; **188**: 389-395 [PMID: 30014247 DOI: 10.1007/s11845-018-1867-1]

2 **Barzi A**, Lenz AM, Labonte MJ, Lenz HJ. Molecular pathways: Estrogen pathway in colorectal cancer. *Clin Cancer Res* 2013; **19**: 5842-5848 [PMID: 23965904 DOI: 10.1158/1078-0432.CCR-13-0325]

3 **Dahlman-Wright K**, Cavailles V, Fuqua SA, Jordan VC, Katzenellenbogen JA, Korach KS, Maggi A, Muramatsu M, Parker MG, Gustafsson JA. International Union of Pharmacology. LXIV. Estrogen receptors. *Pharmacol Rev* 2006; **58**: 773-781 [PMID: 17132854 DOI: 10.1124/pr.58.4.8]

4 **Prossnitz ER**, Arterburn JB. International Union of Basic and Clinical Pharmacology. XCVII. G Protein-Coupled Estrogen Receptor and Its Pharmacologic Modulators. *Pharmacol Rev* 2015; **67**: 505-540 [PMID: 26023144 DOI: 10.1124/pr.114.009712]

5 **Carmeci C**, Thompson DA, Ring HZ, Francke U, Weigel RJ. Identification of a gene (GPR30) with homology to the G-protein-coupled receptor superfamily associated with estrogen receptor expression in breast cancer. *Genomics* 1997; **45**: 607-617 [PMID: 9367686 DOI: 10.1006/geno.1997.4972]

6 **Feng Y**, Gregor P. Cloning of a novel member of the G protein-coupled receptor family related to peptide receptors. *Biochem Biophys Res Commun* 1997; **231**: 651-654 [PMID: 9070864 DOI: 10.1006/bbrc.1997.6161]

7 **Kvingedal AM**, Smeland EB. A novel putative G-protein-coupled receptor expressed in lung, heart and lymphoid tissue. *FEBS Lett* 1997; **407**: 59-62 [PMID: 9141481 DOI: 10.1016/S0014-5793(97)00278-0]

8 **O'Dowd BF**, Nguyen T, Marchese A, Cheng R, Lynch KR, Heng HH, Kolakowski LF Jr, George SR. Discovery of three novel G-protein-coupled receptor genes. *Genomics* 1998; **47**: 310-313 [PMID: 9479505 DOI: 10.1006/geno.1998.5095]

9 **Owman C**, Blay P, Nilsson C, Lolait SJ. Cloning of human cDNA encoding a novel heptahelix receptor expressed in Burkitt's lymphoma and widely distributed in brain and peripheral tissues. *Biochem Biophys Res Commun* 1996; **228**: 285-292 [PMID: 8920907 DOI: 10.1006/bbrc.1996.1654]

10 **Takada Y**, Kato C, Kondo S, Korenaga R, Ando J. Cloning of cDNAs encoding G protein-coupled receptor expressed in human endothelial cells exposed to fluid shear stress. *Biochem Biophys Res Commun* 1997; **240**: 737-741 [PMID: 9398636 DOI: 10.1006/bbrc.1997.7734]

11 **Barton M**, Filardo EJ, Lolait SJ, Thomas P, Maggiolini M, Prossnitz ER. Twenty years of the G protein-coupled estrogen receptor GPER: Historical and personal perspectives. *J Steroid Biochem Mol Biol* 2018; **176**: 4-15 [PMID: 28347854 DOI: 10.1016/j.jsbmb.2017.03.021]

12 **Revankar CM**, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science* 2005; **307**: 1625-1630 [PMID: 15705806 DOI: 10.1126/science.1106943]

13 **Prossnitz ER**, Hathaway HJ. What have we learned about GPER function in physiology and disease from knockout mice? *J Steroid Biochem Mol Biol* 2015; **153**: 114-126 [PMID: 26189910 DOI: 10.1016/j.jsbmb.2015.06.014]

14 **Meyer MR**, Fredette NC, Daniel C, Sharma G, Amann K, Arterburn JB, Barton M, Prossnitz ER. Obligatory role for GPER in cardiovascular aging and disease. *Sci Signal* 2016; **9**: ra105 [PMID: 27803283 DOI: 10.1126/scisignal.aag0240]

15 **Prossnitz ER**, Barton M. Estrogen biology: New insights into GPER function and clinical opportunities. *Mol Cell Endocrinol* 2014; **389**: 71-83 [PMID: 24530924 DOI: 10.1016/j.mce.2014.02.002]

16 **Sharma G**, Mauvais-Jarvis F, Prossnitz ER. Roles of G protein-coupled estrogen receptor GPER in metabolic regulation. *J Steroid Biochem Mol Biol* 2018; **176**: 31-37 [PMID: 28223150 DOI: 10.1016/j.jsbmb.2017.02.012]

17 **Cho NL**, Javid SH, Carothers AM, Redston M, Bertagnolli MM. Estrogen receptors alpha and beta are inhibitory modifiers of Apc-dependent tumorigenesis in the proximal colon of Min/+ mice. *Cancer Res* 2007; **67**: 2366-2372 [PMID: 17332369 DOI: 10.1158/0008-5472.CAN-06-3026]

18 **Stevanato Filho PR**, Aguiar Júnior S, Begnami MD, Ferreira FO, Nakagawa WT, Spencer RMSB, Bezerra TS, Boggiss PE, Lopes A. Estrogen Receptor β as a Prognostic Marker of Tumor Progression in Colorectal Cancer with Familial Adenomatous Polyposis and Sporadic Polyps. *Pathol Oncol Res* 2018; **24**: 533-540 [PMID: 28681123 DOI: 10.1007/s12253-017-0268-5]

19 **Guérin A**, Mody R, Fok B, Lasch KL, Zhou Z, Wu EQ, Zhou W, Talley NJ. Risk of developing colorectal cancer and benign colorectal neoplasm in patients with chronic constipation. *Aliment Pharmacol Ther* 2014; **40**: 83-92 [PMID: 24832002 DOI: 10.1111/apt.12789]

20 **Roberts MC**, Millikan RC, Galanko JA, Martin C, Sandler RS. Constipation, laxative use, and colon cancer in a North Carolina population. *Am J Gastroenterol* 2003; **98**: 857-864 [PMID: 12738468 DOI: 10.1111/j.1572-0241.2003.07386.x]

21 **Roager HM**, Hansen LB, Bahl MI, Frandsen HL, Carvalho V, Gøbel RJ, Dalgaard MD, Plichta DR, Sparholt MH, Vestergaard H, Hansen T, Sicheritz-Pontén T, Nielsen HB, Pedersen O, Lauritzen L, Kristensen M, Gupta R, Licht TR. Colonic transit time is related to bacterial metabolism and mucosal turnover in the gut. *Nat Microbiol* 2016; **1**: 16093 [PMID: 27562254 DOI: 10.1038/nmicrobiol.2016.93]

22 **Li Y**, Xu J, Jiang F, Jiang Z, Liu C, Li L, Luo Y, Lu R, Mu Y, Liu Y, Xue B. G protein-coupled estrogen receptor is involved in modulating colonic motor function via nitric oxide release in C57BL/6 female mice. *Neurogastroenterol Motil* 2016; **28**: 432-442 [PMID: 26661936 DOI: 10.1111/nmo.12743]

23 **Zielińska M**, Fichna J, Bashashati M, Habibi S, Sibaev A, Timmermans JP, Storr M. G protein-coupled estrogen receptor and estrogen receptor ligands regulate colonic motility and visceral pain. *Neurogastroenterol Motil* 2017; **29** [PMID: 28191706 DOI: 10.1111/nmo.13025]

24 **Dennis MK**, Burai R, Ramesh C, Petrie WK, Alcon SN, Nayak TK, Bologa CG, Leitao A, Brailoiu E, Deliu E, Dun NJ, Sklar LA, Hathaway HJ, Arterburn JB, Oprea TI, Prossnitz ER. In vivo effects of a GPR30 antagonist. *Nat Chem Biol* 2009; **5**: 421-427 [PMID: 19430488 DOI: 10.1038/nchembio.168]

25 **Dennis MK**, Field AS, Burai R, Ramesh C, Petrie WK, Bologa CG, Oprea TI, Yamaguchi Y, Hayashi S, Sklar LA, Hathaway HJ, Arterburn JB, Prossnitz ER. Identification of a GPER/GPR30 antagonist with improved estrogen receptor counterselectivity. *J Steroid Biochem Mol Biol* 2011; **127**: 358-366 [PMID: 21782022 DOI: 10.1016/j.jsbmb.2011.07.002]

26 **Bologa CG**, Revankar CM, Young SM, Edwards BS, Arterburn JB, Kiselyov AS, Parker MA, Tkachenko SE, Savchuck NP, Sklar LA, Oprea TI, Prossnitz ER. Virtual and biomolecular screening converge on a selective agonist for GPR30. *Nat Chem Biol* 2006; **2**: 207-212 [PMID: 16520733 DOI: 10.1038/nchembio775]

27 **Jacenik D,** Cygankiewicz AI, Krajewska WM. Risk factors in colorectal cancer. In: Fichna J, editor. Introduction to gastrointestinal diseases. Springer International Publishing, 2017: 113-128

28 **Qin B**, Dong L, Guo X, Jiang J, He Y, Wang X, Li L, Zhao J. Expression of G protein-coupled estrogen receptor in irritable bowel syndrome and its clinical significance. *Int J Clin Exp Pathol* 2014; **7**: 2238-2246 [PMID: 24966932 DOI: 10.7314/APJCP.2014.15.11.4733]

29 **Cremon C**, Gargano L, Morselli-Labate AM, Santini D, Cogliandro RF, De Giorgio R, Stanghellini V, Corinaldesi R, Barbara G. Mucosal immune activation in irritable bowel syndrome: Gender-dependence and association with digestive symptoms. *Am J Gastroenterol* 2009; **104**: 392-400 [PMID: 19174797 DOI: 10.1038/ajg.2008.94]

30 **Flores BM**, O'Connor A, Moss AC. Impact of mucosal inflammation on risk of colorectal neoplasia in patients with ulcerative colitis: A systematic review and meta-analysis. *Gastrointest Endosc* 2017; **86**: 1006-1011.e8 [PMID: 28750838 DOI: 10.1016/j.gie.2017.07.028]

31 **Castaño-Milla C**, Chaparro M, Gisbert JP. Systematic review with meta-analysis: The declining risk of colorectal cancer in ulcerative colitis. *Aliment Pharmacol Ther* 2014; **39**: 645-659 [PMID: 24612141 DOI: 10.1111/apt.12651]

32 **von Roon AC**, Reese G, Teare J, Constantinides V, Darzi AW, Tekkis PP. The risk of cancer in patients with Crohn's disease. *Dis Colon Rectum* 2007; **50**: 839-855 [PMID: 17308939 DOI: 10.1007/s10350-006-0848-z]

33 **Armstrong CM**, Allred KF, Weeks BR, Chapkin RS, Allred CD. Estradiol Has Differential Effects on Acute Colonic Inflammation in the Presence and Absence of Estrogen Receptor β Expression. *Dig Dis Sci* 2017; **62**: 1977-1984 [PMID: 28573506 DOI: 10.1007/s10620-017-4631-x]

34 **Bábíčková J**, Tóthová Ľ, Lengyelová E, Bartoňová A, Hodosy J, Gardlík R, Celec P. Sex Differences in Experimentally Induced Colitis in Mice: A Role for Estrogens. *Inflammation* 2015; **38**: 1996-2006 [PMID: 25962374 DOI: 10.1007/s10753-015-0180-7]

35 **Linares PM**, Algaba A, Urzainqui A, Guijarro-Rojas M, González-Tajuelo R, Garrido J, Chaparro M, Gisbert JP, Bermejo F, Guerra I, Castellano V, Fernández-Contreras ME. Ratio of Circulating Estrogen Receptors Beta and Alpha (ERβ/ERα) Indicates Endoscopic Activity in Patients with Crohn's Disease. *Dig Dis Sci* 2017; **62**: 2744-2754 [PMID: 28823012 DOI: 10.1007/s10620-017-4717-5]

36 **Pierdominici M**, Maselli A, Varano B, Barbati C, Cesaro P, Spada C, Zullo A, Lorenzetti R, Rosati M, Rainaldi G, Limiti MR, Guidi L, Conti L, Gessani S. Linking estrogen receptor β expression with inflammatory bowel disease activity. *Oncotarget* 2015; **6**: 40443-40451 [PMID: 26497217 DOI: 10.18632/oncotarget.6217]

37 **Włodarczyk M**, Sobolewska-Włodarczyk A, Cygankiewicz AI, Jacenik D, Piechota-Polańczyk A, Stec-Michalska K, Krajewska WM, Fichna J, Wiśniewska-Jarosińska M. G Protein-Coupled Receptor 30 (GPR30) Expression Pattern in Inflammatory Bowel Disease Patients Suggests its Key Role in the Inflammatory Process. A Preliminary Study. *J Gastrointestin Liver Dis* 2017; **26**: 29-35 [PMID: 28338111 DOI: 10.15403/jgld.2014.1121.261.gpr]

38 **Blasko E**, Haskell CA, Leung S, Gualtieri G, Halks-Miller M, Mahmoudi M, Dennis MK, Prossnitz ER, Karpus WJ, Horuk R. Beneficial role of the GPR30 agonist G-1 in an animal model of multiple sclerosis. *J Neuroimmunol* 2009; **214**: 67-77 [PMID: 19664827 DOI: 10.1016/j.jneuroim.2009.06.023]

39 **Brunsing RL**, Prossnitz ER. Induction of interleukin-10 in the T helper type 17 effector population by the G protein coupled estrogen receptor (GPER) agonist G-1. *Immunology* 2011; **134**: 93-106 [PMID: 21722102 DOI: 10.1111/j.1365-2567.2011.03471.x]

40 **Cabas I**, Rodenas MC, Abellán E, Meseguer J, Mulero V, García-Ayala A. Estrogen signaling through the G protein-coupled estrogen receptor regulates granulocyte activation in fish. *J Immunol* 2013; **191**: 4628-4639 [PMID: 24062489 DOI: 10.4049/jimmunol.1301613]

41 **Cortes E**, Sarper M, Robinson B, Lachowski D, Chronopoulos A, Thorpe SD, Lee DA, Del Río Hernández AE. GPER is a mechanoregulator of pancreatic stellate cells and the tumor microenvironment. *EMBO Rep* 2019; **20**: pii: e46556 [PMID: 30538117 DOI: 10.15252/embr.201846556]

42 **Jacenik D**, Cygankiewicz AI, Krajewska WM. The G protein-coupled estrogen receptor as a modulator of neoplastic transformation. *Mol Cell Endocrinol* 2016; **429**: 10-18 [PMID: 27107933 DOI: 10.1016/j.mce.2016.04.011]

43 **Rettew JA**, McCall SH 4th, Marriott I. GPR30/GPER-1 mediates rapid decreases in TLR4 expression on murine macrophages. *Mol Cell Endocrinol* 2010; **328**: 87-92 [PMID: 20654686 DOI: 10.1016/j.mce.2010.07.017]

44 **Wang C**, Dehghani B, Magrisso IJ, Rick EA, Bonhomme E, Cody DB, Elenich LA, Subramanian S, Murphy SJ, Kelly MJ, Rosenbaum JS, Vandenbark AA, Offner H. GPR30 contributes to estrogen-induced thymic atrophy. *Mol Endocrinol* 2008; **22**: 636-648 [PMID: 18063692 DOI: 10.1210/me.2007-0359]

45 **Albanito L**, Lappano R, Madeo A, Chimento A, Prossnitz ER, Cappello AR, Dolce V, Abonante S, Pezzi V, Maggiolini M. Effects of atrazine on estrogen receptor α- and G protein-coupled receptor 30-mediated signaling and proliferation in cancer cells and cancer-associated fibroblasts. *Environ Health Perspect* 2015; **123**: 493-499 [PMID: 25616260 DOI: 10.1289/ehp.1408586]

46 **Madeo A**, Maggiolini M. Nuclear alternate estrogen receptor GPR30 mediates 17beta-estradiol-induced gene expression and migration in breast cancer-associated fibroblasts. *Cancer Res* 2010; **70**: 6036-6046 [PMID: 20551055 DOI: 10.1158/0008-5472.CAN-10-0408]

47 **Pisano A**, Santolla MF, De Francesco EM, De Marco P, Rigiracciolo DC, Perri MG, Vivacqua A, Abonante S, Cappello AR, Dolce V, Belfiore A, Maggiolini M, Lappano R. GPER, IGF-IR, and EGFR transduction signaling are involved in stimulatory effects of zinc in breast cancer cells and cancer-associated fibroblasts. *Mol Carcinog* 2017; **56**: 580-593 [PMID: 27341075 DOI: 10.1002/mc.22518]

48 **Pupo M**, Pisano A, Lappano R, Santolla MF, De Francesco EM, Abonante S, Rosano C, Maggiolini M. Bisphenol A induces gene expression changes and proliferative effects through GPER in breast cancer cells and cancer-associated fibroblasts. *Environ Health Perspect* 2012; **120**: 1177-1182 [PMID: 22552965 DOI: 10.1289/ehp.1104526]

49 **Ubink I**, Verhaar ER, Kranenburg O, Goldschmeding R. A potential role for CCN2/CTGF in aggressive colorectal cancer. *J Cell Commun Signal* 2016; **10**: 223-227 [PMID: 27613407 DOI: 10.1007/s12079-016-0347-5]

50 **De Marco P**, Lappano R, De Francesco EM, Cirillo F, Pupo M, Avino S, Vivacqua A, Abonante S, Picard D, Maggiolini M. GPER signalling in both cancer-associated fibroblasts and breast cancer cells mediates a feedforward IL1β/IL1R1 response. *Sci Rep* 2016; **6**: 24354 [PMID: 27072893 DOI: 10.1038/srep24354]

51 **Okamoto M**, Suzuki T, Mizukami Y, Ikeda T. The membrane-type estrogen receptor G-protein-coupled estrogen receptor suppresses lipopolysaccharide-induced interleukin 6 via inhibition of nuclear factor-kappa B pathway in murine macrophage cells. *Anim Sci J* 2017; **88**: 1870-1879 [PMID: 28722236 DOI: 10.1111/asj.12868]

52 **Platt AM**, Bain CC, Bordon Y, Sester DP, Mowat AM. An independent subset of TLR expressing CCR2-dependent macrophages promotes colonic inflammation. *J Immunol* 2010; **184**: 6843-6854 [PMID: 20483766 DOI: 10.4049/jimmunol.0903987]

53 **Zigmond E**, Varol C, Farache J, Elmaliah E, Satpathy AT, Friedlander G, Mack M, Shpigel N, Boneca IG, Murphy KM, Shakhar G, Halpern Z, Jung S. Ly6C hi monocytes in the inflamed colon give rise to proinflammatory effector cells and migratory antigen-presenting cells. *Immunity* 2012; **37**: 1076-1090 [PMID: 23219392 DOI: 10.1016/j.immuni.2012.08.026]

54 **Cui YL**, Li HK, Zhou HY, Zhang T, Li Q. Correlations of tumor-associated macrophage subtypes with liver metastases of colorectal cancer. *Asian Pac J Cancer Prev* 2013; **14**: 1003-1007 [PMID: 23621176 DOI: 10.7314/APJCP.2013.14.2.1003]

55 **Li S**, Wang B, Tang Q, Liu J, Yang X. Bisphenol A triggers proliferation and migration of laryngeal squamous cell carcinoma via GPER mediated upregulation of IL-6. *Cell Biochem Funct* 2017; **35**: 209-216 [PMID: 28466560 DOI: 10.1002/cbf.3265]

56 **Kurosawa T**, Hiroi H, Tsutsumi O, Ishikawa T, Osuga Y, Fujiwara T, Inoue S, Muramatsu M, Momoeda M, Taketani Y. The activity of bisphenol A depends on both the estrogen receptor subtype and the cell type. *Endocr J* 2002; **49**: 465-471 [PMID: 12402979 DOI: 10.1507/endocrj.49.465]

57 **Liang S**, Chen Z, Jiang G, Zhou Y, Liu Q, Su Q, Wei W, Du J, Wang H. Activation of GPER suppresses migration and angiogenesis of triple negative breast cancer via inhibition of NF-κB/IL-6 signals. *Cancer Lett* 2017; **386**: 12-23 [PMID: 27836733 DOI: 10.1016/j.canlet.2016.11.003]

58 **Okamoto M**, Mizukami Y. GPER negatively regulates TNFα-induced IL-6 production in human breast cancer cells via NF-κB pathway. *Endocr J* 2016; **63**: 485-493 [PMID: 26888479 DOI: 10.1507/endocrj.EJ15-0571]

59 **Zha Z**, Su A, Huo S. Activation of GPER suppresses the malignancy of osteosarcoma cells via down regulation of IL-6 and IL-8. *Arch Biochem Biophys* 2018; **660**: 149-155 [PMID: 30385323 DOI: 10.1016/j.abb.2018.10.018]

60 **Shen M**, Hu P, Donskov F, Wang G, Liu Q, Du J. Tumor-associated neutrophils as a new prognostic factor in cancer: A systematic review and meta-analysis. *PLoS One* 2014; **9**: e98259 [PMID: 24906014 DOI: 10.1371/journal.pone.0098259]

61 **Lam HM**, Ouyang B, Chen J, Ying J, Wang J, Wu CL, Jia L, Medvedovic M, Vessella RL, Ho SM. Targeting GPR30 with G-1: A new therapeutic target for castration-resistant prostate cancer. *Endocr Relat Cancer* 2014; **21**: 903-914 [PMID: 25287069 DOI: 10.1530/ERC-14-0402]

62 **Itoga M**, Konno Y, Moritoki Y, Saito Y, Ito W, Tamaki M, Kobayashi Y, Kayaba H, Kikuchi Y, Chihara J, Takeda M, Ueki S, Hirokawa M. G-protein-coupled estrogen receptor agonist suppresses airway inflammation in a mouse model of asthma through IL-10. *PLoS One* 2015; **10**: e0123210 [PMID: 25826377 DOI: 10.1371/journal.pone.0123210]

63 **Smids C**, Horjus Talabur Horje CS, Drylewicz J, Roosenboom B, Groenen MJM, van Koolwijk E, van Lochem EG, Wahab PJ. Intestinal T Cell Profiling in Inflammatory Bowel Disease: Linking T Cell Subsets to Disease Activity and Disease Course. *J Crohns Colitis* 2018; **12**: 465-475 [PMID: 29211912 DOI: 10.1093/ecco-jcc/jjx160]

64 **Chirica M**, Le Bourhis L, Lehmann-Che J, Chardiny V, Bouhidel F, Foulboeuf L, Gornet JM, Lourenco N, Dulphy N, Toubert A, Allez M. Phenotypic analysis of T cells infiltrating colon cancers: Correlations with oncogenetic status. *Oncoimmunology* 2015; **4**: e1016698 [PMID: 26405567 DOI: 10.1080/2162402X.2015.1016698]

65 **Zekas E**, Prossnitz ER. Estrogen-mediated inactivation of FOXO3a by the G protein-coupled estrogen receptor GPER. *BMC Cancer* 2015; **15**: 702 [PMID: 26470790 DOI: 10.1186/s12885-015-1699-6]

66 **Filardo EJ**. A role for G-protein coupled estrogen receptor (GPER) in estrogen-induced carcinogenesis: Dysregulated glandular homeostasis, survival and metastasis. *J Steroid Biochem Mol Biol* 2018; **176**: 38-48 [PMID: 28595943 DOI: 10.1016/j.jsbmb.2017.05.005]

67 **Smith HO**, Arias-Pulido H, Kuo DY, Howard T, Qualls CR, Lee SJ, Verschraegen CF, Hathaway HJ, Joste NE, Prossnitz ER. GPR30 predicts poor survival for ovarian cancer. *Gynecol Oncol* 2009; **114**: 465-471 [PMID: 19501895 DOI: 10.1016/j.ygyno.2009.05.015]

68 **Smith HO**, Leslie KK, Singh M, Qualls CR, Revankar CM, Joste NE, Prossnitz ER. GPR30: A novel indicator of poor survival for endometrial carcinoma. *Am J Obstet Gynecol* 2007; **196**: 386.e1-9; discussion 386.e9-11 [PMID: 17403429 DOI: 10.1016/j.ajog.2007.01.004]

69 **Petrie WK**, Dennis MK, Hu C, Dai D, Arterburn JB, Smith HO, Hathaway HJ, Prossnitz ER. G protein-coupled estrogen receptor-selective ligands modulate endometrial tumor growth. *Obstet Gynecol Int* 2013; **2013**: 472720 [PMID: 24379833 DOI: 10.1155/2013/472720]

70 **Bustos V**, Nolan ÁM, Nijhuis A, Harvey H, Parker A, Poulsom R, McBryan J, Thomas W, Silver A, Harvey BJ. GPER mediates differential effects of estrogen on colon cancer cell proliferation and migration under normoxic and hypoxic conditions. *Oncotarget* 2017; **8**: 84258-84275 [PMID: 29137421 DOI: 10.18632/oncotarget.20653]

71 **Hao J**, Bao X, Jin B, Wang X, Mao Z, Li X, Wei L, Shen D, Wang JL. Ca2+ channel subunit α 1D promotes proliferation and migration of endometrial cancer cells mediated by 17β-estradiol via the G protein-coupled estrogen receptor. *FASEB J* 2015; **29**: 2883-2893 [PMID: 25805831 DOI: 10.1096/fj.14-265603]

72 **Huff MO**, Todd SL, Smith AL, Elpers JT, Smith AP, Murphy RD, Bleser-Shartzer AS, Hoerter JE, Radde BN, Klinge CM. Arsenite and Cadmium Activate MAPK/ERK via Membrane Estrogen Receptors and G-Protein Coupled Estrogen Receptor Signaling in Human Lung Adenocarcinoma Cells. *Toxicol Sci* 2016; **152**: 62-71 [PMID: 27071941 DOI: 10.1093/toxsci/kfw064]

73 **Liu C**, Liao Y, Fan S, Fu X, Xiong J, Zhou S, Zou M, Wang J. G-Protein-Coupled Estrogen Receptor Antagonist G15 Decreases Estrogen-Induced Development of Non-Small Cell Lung Cancer. *Oncol Res* 2019; **27**: 283-292 [PMID: 28877783 DOI: 10.3727/096504017X15035795904677]

74 **Liu Q**, Chen Z, Jiang G, Zhou Y, Yang X, Huang H, Liu H, Du J, Wang H. Epigenetic down regulation of G protein-coupled estrogen receptor (GPER) functions as a tumor suppressor in colorectal cancer. *Mol Cancer* 2017; **16**: 87 [PMID: 28476123 DOI: 10.1186/s12943-017-0654-3]

75 **Santolla MF**, Avino S, Pellegrino M, De Francesco EM, De Marco P, Lappano R, Vivacqua A, Cirillo F, Rigiracciolo DC, Scarpelli A, Abonante S, Maggiolini M. SIRT1 is involved in oncogenic signaling mediated by GPER in breast cancer. *Cell Death Dis* 2015; **6**: e1834 [PMID: 26225773 DOI: 10.1038/cddis.2015.201]

76 **Sathya S**, Sudhagar S, Lakshmi BS. Estrogen suppresses breast cancer proliferation through GPER / p38 MAPK axis during hypoxia. *Mol Cell Endocrinol* 2015; **417**: 200-210 [PMID: 26432358 DOI: 10.1016/j.mce.2015.09.032]

77 **Yan Y**, Jiang X, Zhao Y, Wen H, Liu G. Role of GPER on proliferation, migration and invasion in ligand-independent manner in human ovarian cancer cell line SKOV3. *Cell Biochem Funct* 2015; **33**: 552-559 [PMID: 26526233 DOI: 10.1002/cbf.3154]

78 **Sano R**, Reed JC. ER stress-induced cell death mechanisms. *Biochim Biophys Acta* 2013; **1833**: 3460-3470 [PMID: 23850759 DOI: 10.1016/j.bbamcr.2013.06.028]

79 **Bassères DS**, Ebbs A, Levantini E, Baldwin AS. Requirement of the NF-kappaB subunit p65/RelA for K-Ras-induced lung tumorigenesis. *Cancer Res* 2010; **70**: 3537-3546 [PMID: 20406971 DOI: 10.1158/0008-5472.CAN-09-4290]

80 **Meylan E**, Dooley AL, Feldser DM, Shen L, Turk E, Ouyang C, Jacks T. Requirement for NF-kappaB signalling in a mouse model of lung adenocarcinoma. *Nature* 2009; **462**: 104-107 [PMID: 19847165 DOI: 10.1038/nature08462]

81 **Xia Y**, Yeddula N, Leblanc M, Ke E, Zhang Y, Oldfield E, Shaw RJ, Verma IM. Reduced cell proliferation by IKK2 depletion in a mouse lung-cancer model. *Nat Cell Biol* 2012; **14**: 257-265 [PMID: 22327365 DOI: 10.1038/ncb2428]

82 **Huang S**, Pettaway CA, Uehara H, Bucana CD, Fidler IJ. Blockade of NF-kappaB activity in human prostate cancer cells is associated with suppression of angiogenesis, invasion, and metastasis. *Oncogene* 2001; **20**: 4188-4197 [PMID: 11464285 DOI: 10.1038/sj.onc.1204535]

83 **Kawauchi K**, Araki K, Tobiume K, Tanaka N. p53 regulates glucose metabolism through an IKK-NF-kappaB pathway and inhibits cell transformation. *Nat Cell Biol* 2008; **10**: 611-618 [PMID: 18391940 DOI: 10.1038/ncb1724]

84 **Mauro C**, Leow SC, Anso E, Rocha S, Thotakura AK, Tornatore L, Moretti M, De Smaele E, Beg AA, Tergaonkar V, Chandel NS, Franzoso G. NF-κB controls energy homeostasis and metabolic adaptation by upregulating mitochondrial respiration. *Nat Cell Biol* 2011; **13**: 1272-1279 [PMID: 21968997 DOI: 10.1038/ncb2324]

85 **Iliopoulos D**, Hirsch HA, Struhl K. An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell* 2009; **139**: 693-706 [PMID: 19878981 DOI: 10.1016/j.cell.2009.10.014]

86 **Ribeiro-Varandas E**, Viegas W, Sofia Pereira H, Delgado M. Bisphenol A at concentrations found in human serum induces aneugenic effects in endothelial cells. *Mutat Res* 2013; **751**: 27-33 [PMID: 23142537 DOI: 10.1016/j.mrgentox.2012.10.007]

87 **Branzei D**, Foiani M. Regulation of DNA repair throughout the cell cycle. *Nat Rev Mol Cell Biol* 2008; **9**: 297-308 [PMID: 18285803 DOI: 10.1038/nrm2351]

88 **Sun M**, Guo X, Qian X, Wang H, Yang C, Brinkman KL, Serrano-Gonzalez M, Jope RS, Zhou B, Engler DA, Zhan M, Wong ST, Fu L, Xu B. Activation of the ATM-Snail pathway promotes breast cancer metastasis. *J Mol Cell Biol* 2012; **4**: 304-315 [PMID: 22923499 DOI: 10.1093/jmcb/mjs048]

89 **Bai AH**, Tong JH, To KF, Chan MW, Man EP, Lo KW, Lee JF, Sung JJ, Leung WK. Promoter hypermethylation of tumor-related genes in the progression of colorectal neoplasia. *Int J Cancer* 2004; **112**: 846-853 [PMID: 15386372 DOI: 10.1002/ijc.20485]

90 **Muz B**, de la Puente P, Azab F, Azab AK. The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. *Hypoxia (Auckl)* 2015; **3**: 83-92 [PMID: 27774485 DOI: 10.2147/HP.S93413]

91 **Gilligan LC**, Rahman HP, Hewitt AM, Sitch AJ, Gondal A, Arvaniti A, Taylor AE, Read ML, Morton DG, Foster PA. Estrogen Activation by Steroid Sulfatase Increases Colorectal Cancer Proliferation via GPER. *J Clin Endocrinol Metab* 2017; **102**: 4435-4447 [PMID: 28945888 DOI: 10.1210/jc.2016-3716]

92 **Gilligan LC**, Gondal A, Tang V, Hussain MT, Arvaniti A, Hewitt AM, Foster PA. Estrone Sulfate Transport and Steroid Sulfatase Activity in Colorectal Cancer: Implications for Hormone Replacement Therapy. *Front Pharmacol* 2017; **8**: 103 [PMID: 28326039 DOI: 10.3389/fphar.2017.00103]

93 **Castillo Sanchez R**, Gomez R, Perez Salazar E. Bisphenol A Induces Migration through a GPER-, FAK-, Src-, and ERK2-Dependent Pathway in MDA-MB-231 Breast Cancer Cells. *Chem Res Toxicol* 2016; **29**: 285-295 [PMID: 26914403 DOI: 10.1021/acs.chemrestox.5b00457]

94 **Chen ZJ**, Wei W, Jiang GM, Liu H, Wei WD, Yang X, Wu YM, Liu H, Wong CK, Du J, Wang HS. Activation of GPER suppresses epithelial mesenchymal transition of triple negative breast cancer cells via NF-κB signals. *Mol Oncol* 2016; **10**: 775-788 [PMID: 26842883 DOI: 10.1016/j.molonc.2016.01.002]

95 **Deng Q**, Jiang G, Wu Y, Li J, Liang W, Chen L, Su Q, Li W, Du J, Wong CKC, Chen Z, Wang H. GPER/Hippo-YAP signal is involved in Bisphenol S induced migration of triple negative breast cancer (TNBC) cells. *J Hazard Mater* 2018; **355**: 1-9 [PMID: 29758456 DOI: 10.1016/j.jhazmat.2018.05.013]

96 **François CM**, Wargnier R, Petit F, Goulvent T, Rimokh R, Treilleux I, Ray-Coquard I, Zazzu V, Cohen-Tannoudji J, Guigon CJ. 17β-estradiol inhibits spreading of metastatic cells from granulosa cell tumors through a non-genomic mechanism involving GPER1. *Carcinogenesis* 2015; **36**: 564-573 [PMID: 25823895 DOI: 10.1093/carcin/bgv041]

97 **Guan BZ**, Yan RL, Huang JW, Li FL, Zhong YX, Chen Y, Liu FN, Hu B, Huang SB, Yin LH. Activation of G protein coupled estrogen receptor (GPER) promotes the migration of renal cell carcinoma via the PI3K/AKT/MMP-9 signals. *Cell Adh Migr* 2018; **12**: 109-117 [PMID: 25588050 DOI: 10.4161/19336918.2014.990781]

98 **Henic E**, Noskova V, Høyer-Hansen G, Hansson S, Casslén B. Estradiol attenuates EGF-induced rapid uPAR mobilization and cell migration via the G-protein-coupled receptor 30 in ovarian cancer cells. *Int J Gynecol Cancer* 2009; **19**: 214-222 [PMID: 19395996 DOI: 10.1111/IGC.0b013e31819bcb75]

99 **Jiang QF**, Wu TT, Yang JY, Dong CR, Wang N, Liu XH, Liu ZM. 17β-estradiol promotes the invasion and migration of nuclear estrogen receptor-negative breast cancer cells through cross-talk between GPER1 and CXCR1. *J Steroid Biochem Mol Biol* 2013; **138**: 314-324 [PMID: 23907016 DOI: 10.1016/j.jsbmb.2013.07.011]

100 **Li Y**, Chen Y, Zhu ZX, Liu XH, Yang L, Wan L, Lei TW, Wang XD. 4-Hydroxytamoxifen-stimulated processing of cyclin E is mediated via G protein-coupled receptor 30 (GPR30) and accompanied by enhanced migration in MCF-7 breast cancer cells. *Toxicology* 2013; **309**: 61-65 [PMID: 23624423 DOI: 10.1016/j.tox.2013.04.012]

101 **Shang D**, Li Z, Zhu Z, Chen H, Zhao L, Wang X, Chen Y. Baicalein suppresses 17-β-estradiol-induced migration, adhesion and invasion of breast cancer cells via the G protein-coupled receptor 30 signaling pathway. *Oncol Rep* 2015; **33**: 2077-2085 [PMID: 25672442 DOI: 10.3892/or.2015.3786]

102 **Yan Y**, Liu H, Wen H, Jiang X, Cao X, Zhang G, Liu G. The novel estrogen receptor GPER regulates the migration and invasion of ovarian cancer cells. *Mol Cell Biochem* 2013; **378**: 1-7 [PMID: 23580092 DOI: 10.1007/s11010-013-1579-9]

103 **Zhang KS**, Chen HQ, Chen YS, Qiu KF, Zheng XB, Li GC, Yang HD, Wen CJ. Bisphenol A stimulates human lung cancer cell migration via upregulation of matrix metalloproteinases by GPER/EGFR/ERK1/2 signal pathway. *Biomed Pharmacother* 2014; **68**: 1037-1043 [PMID: 25312822 DOI: 10.1016/j.biopha.2014.09.003]

104 **Zhu G**, Huang Y, Wu C, Wei D, Shi Y. Activation of G-Protein-Coupled Estrogen Receptor Inhibits the Migration of Human Nonsmall Cell Lung Cancer Cells via IKK-β/NF-κB Signals. *DNA Cell Biol* 2016; **35**: 434-442 [PMID: 27082459 DOI: 10.1089/dna.2016.3235]

105 **Zhu P**, Liao LY, Zhao TT, Mo XM, Chen GG, Liu ZM. GPER/ERK&amp;AKT/NF-κB pathway is involved in cadmium-induced proliferation, invasion and migration of GPER-positive thyroid cancer cells. *Mol Cell Endocrinol* 2017; **442**: 68-80 [PMID: 27940299 DOI: 10.1016/j.mce.2016.12.007]

106 **Che L**, Pilo MG, Cigliano A, Latte G, Simile MM, Ribback S, Dombrowski F, Evert M, Chen X, Calvisi DF. Oncogene dependent requirement of fatty acid synthase in hepatocellular carcinoma. *Cell Cycle* 2017; **16**: 499-507 [PMID: 28118080 DOI: 10.1080/15384101.2017.1282586]

107 **Corominas-Faja B**, Vellon L, Cuyàs E, Buxó M, Martin-Castillo B, Serra D, García J, Lupu R, Menendez JA. Clinical and therapeutic relevance of the metabolic oncogene fatty acid synthase in HER2+ breast cancer. *Histol Histopathol* 2017; **32**: 687-698 [PMID: 27714708 DOI: 10.14670/HH-11-830]

108 **Santolla MF**, Lappano R, De Marco P, Pupo M, Vivacqua A, Sisci D, Abonante S, Iacopetta D, Cappello AR, Dolce V, Maggiolini M. G protein-coupled estrogen receptor mediates the up-regulation of fatty acid synthase induced by 17β-estradiol in cancer cells and cancer-associated fibroblasts. *J Biol Chem* 2012; **287**: 43234-43245 [PMID: 23135268 DOI: 10.1074/jbc.M112.417303]

109 **Van de Sande T**, Roskams T, Lerut E, Joniau S, Van Poppel H, Verhoeven G, Swinnen JV. High-level expression of fatty acid synthase in human prostate cancer tissues is linked to activation and nuclear localization of Akt/PKB. *J Pathol* 2005; **206**: 214-219 [PMID: 15880754 DOI: 10.1002/path.1760]

**P-Reviewer:** Caruso R, Soriano-Ursúa MA **S-Editor:** Yan JP

**L-Editor:** A **E-Editor:** Zhang YL

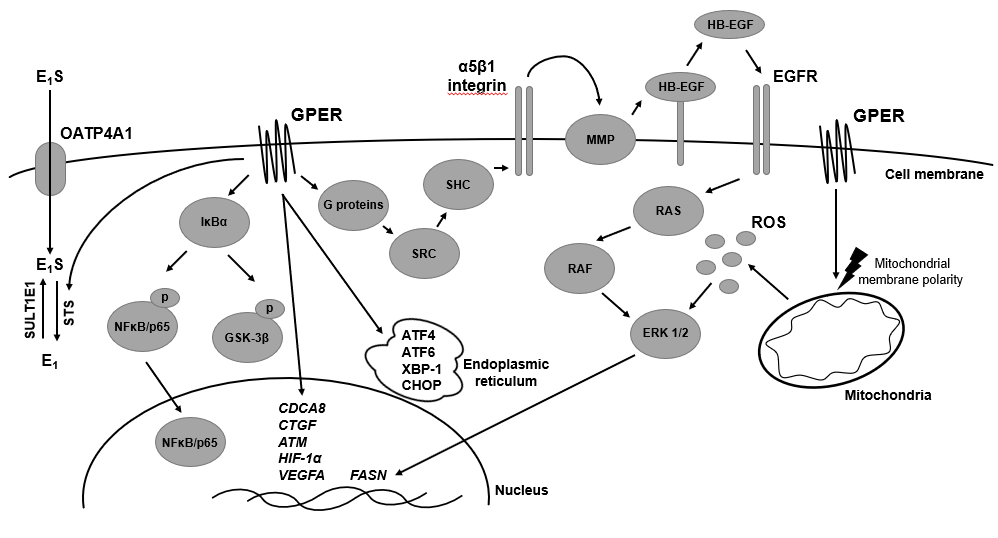
**Specialty type:** Gastroenterology and hepatology   
**Country of origin:** United States  
**Peer-review report classification**  
**Grade A (Excellent):** 0  
**Grade B (Very good):** B  
**Grade C (Good):** 0  
**Grade D (Fair):** D **Grade E (Poor):** 0

**Table 1 G protein-coupled estrogen receptor ligands**

|  |  |
| --- | --- |
| **Agonist** | **Type** |
| Estrogen (17β-estradiol) | Natural steroid |
| Bisphenol A | Synthetic xenoestrogen |
| Genistein | Natural phytoestrogen |
| Tamoxifen | Synthetic therapeutic |
| Fulvestrant (ICI 182.780) | Synthetic therapeutic |
| G-1 | Synthetic selective ligand |
| **Antagonist** | **Type** |
| G-15 | Synthetic selective ligand |
| G-36 | Synthetic selective ligand |

**Table 2 Processes modulated by G protein-coupled estrogen receptor and their importance in neoplastic transformation of the colon**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Features of cancer cells** | **Process** | **Anti-tumorigenic** | **Pro-tumorigenic** | **Ref.** |
| Proliferation and tumor growth | Apoptosis | Yes | No | [73] |
| Cell cycle | Yes | No | [73] |
| DNA repair | No | Yes | [64] |
| Endoplasmic reticulum stress | Yes | No | [73] |
| Estrogen metabolism | No | Yes | [90,91] |
| Mitochondrial membrane polarity | Yes | No | [73] |
| Oxygen level | Yes under normoxia | Yes under hypoxia | [64] |
| Migration | Fatty acid metabolism | No | Yes | [107] |
| Oxygen level | Yes under normoxia | Yes under hypoxia | [64] |



**Figure 1 Signaling pathways modulated by G protein-coupled estrogen receptor in colorectal cancer cells.** ATF4: Activating transcription factor 4; ATF6: Activating transcription factor 6; ATM: Ataxia telangiectasia mutated; CDCA8: Cell division cycle A8; CHOP: C/EBP-homologous protein; CTGF: Connective tissue growth factor; E1: Estrone; E1S: Estrone sulfate; EGFR: Epithelial growth factor receptor; ERK 1/2: Extracellular signal-regulated kinase 1/2; FASN: Fatty acid synthase; GPER: G protein-coupled estrogen receptor; HB-EGF: Heparin-binding epidermal growth factor; HIF-1α: Hypoxia-inducible factor-1α; IκBα: NFκB inhibitor α; MMP: Matrix metalloproteinase; NFκB/p65: Nuclear factor κ-light-chain-enhancer of activated B cells; OATP4A1: Organic anion transporter polypeptide 4A1; P: Phosphorylation; RAF: Rapidly accelerated fibrosarcoma, serine-threonine kinase; RAS: Rat sarcoma, small GTPase; ROS: Reactive oxygen species; SHC: Adapter protein containing SRC homology 2 domain; SRC: Non-receptor tyrosine kinase; STS: Steroid sulfatase; SULT1E1: Sulfotransferase family 1E member 1; VEGFA: Vascular endothelial growth factor A; XBP-1: X-box binding protein 1.