

The roles of estrogen and estrogen receptors in gastrointestinal disease (Review)

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Abstract. Estrogen is an important sex steroid hormone which serves an important role in the regulation of a number of biological functions, including regulating bone density, brain function, cholesterol mobilization, electrolyte balance, skin physiology, the cardiovascular system, the central nervous system and female reproductive organs. Estrogen exhibits various functions through binding to its specific receptors, estrogen receptor α , estrogen receptor β and G protein-coupled estrogen receptor 1. In recent years, researchers have demonstrated that estrogen and its receptors serve an important role in the gastrointestinal (GI) tract and contribute to the progression of a number of GI diseases, including gastroesophageal reflux, esophageal cancer, peptic ulcers, gastric cancer, inflammatory bowel disease, irritable bowel syndrome and colon cancer. The aim of this review is to provide an overview of estrogen and its receptors in GI disease, and highlight potential avenues for the prevention and treatment of GI diseases.

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1. Introduction

Estrogens, including estrone, estriol and the biologically active metabolite 17 β -estradiol (E2), have long been considered important regulators of female reproductive functions and are primarily produced in the ovaries. In addition to the ovaries, several extragonadal tissues, such as the mesenchymal cells in the adipose tissue of the breast, osteoblasts, chondrocytes, aortic smooth muscle cells, vascular endothelium and numerous parts of the brain also produce estrogens (1). Estrogens have been demonstrated to serve functions outside of the female reproductive system, including in the cardiovascular system and the central nervous system, and function to regulate bone density, brain function, cholesterol mobilization and electrolyte balance (2,3). In contrast to women, men are largely dependent on the local synthesis of estrogens in extragonadal target tissues. This local production of estrogens encompasses the signaling modality from endocrine to paracrine, autocrine and intracrine signaling (4). Both estrogen and estrogen receptors have numerous effects on various organs and diseases specific to the gastrointestinal (GI) tract. For example, estrogen and estrogen receptors have been demonstrated to serve pathophysiological roles in gastroesophageal reflux disease, esophageal cancer, peptic ulcer disease, gastric cancer, irritable bowel syndrome, inflammatory bowel disease and colon cancer (5-7).

2. Estrogen receptor

The estrogen receptor has three subtypes; estrogen receptor α (ER α), estrogen receptor β (ER β), which belong to nuclear receptors and membrane receptors, such as G protein-coupled

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estrogen receptor 1 (GPER1, also known as GPR30), which mediate all of estrogens effects, and the expression of each receptor is largely tissue-type specific (3) (Fig. 1).

ER α and ER β have similar structures, and were respectively identified and cloned in 1986 and 1996 (8). Both ER α and ER β have distinct cellular distributions and regulate separate sets of genes (9). ER α is encoded by estrogen receptor 1 which is located on chromosome 6q25.1, and ER β is encoded by estrogen receptor 2 or estrogen receptor 2, which is located on chromosome 14q22-24 (10). ER α is primarily expressed in female sex organs, such as the breast, uterus and ovaries. ER α has three known isoforms; two shorter ER α isoforms which lack the N-terminal domain, and a full-length ER α isoform. The truncated isoforms can heterodimerize with the full-length ER α isoform and repress ER α activity. ER β is expressed in different types of tissues and cells, and to a higher degree in females compared with males (11). ER β has at least five different isoforms; four shorter ER β isoforms and a full-length ER β isoform. The four shorter ER β isoforms exhibit reduced ligand binding activity (12). The ER β isoforms are neither homodimerizable nor transcriptionally active (12). However, they can preferentially dimerize with ER α . The ER α and ER β isoforms have different effects on estrogen signaling and target gene regulation (12) (Fig. 2).

GPER1 was first described in the 1990s (13) and it has been identified as one of the primary estrogen-sensitive receptors. Although GPER1 has a lower saturation, it possesses a high-affinity single binding site for estrogen, with a lower binding affinity for 17 β -estradiol (14). The binding and decomposition of the receptor and its ligand are completed within a few minutes (15). GPER1 mediates estrogen-dependent rapid signaling events, independent of classical estrogen nuclear receptors (14). GPER1 activates multiple downstream signaling pathways, resulting in the activation of adenylate cyclase and increasing cyclic AMP levels, which promote intracellular calcium mobilization and synthesis of phosphatidylinositol 3,4,5-trisphosphate within the nucleus (16-18). GPER1 regulates a diverse range of biological processes, including bone and nervous system development, metabolism, cognition, male fertility and uterine function (19).

Although, ER α , ER β and GPER1 possess a similar structure, they regulate divergent functions. In the present review, the role of estrogen and ERs in the physiology and pathology of the digestive system are explored.

3. Estrogen and estrogen receptor in gastrointestinal disease

Estrogen and estrogen receptors in esophageal diseases

Gastroesophageal reflux disease (GERD). GERD is a spectrum of reflux diseases of the gastroesophageal junction (20). GERD is a recurrent disease that has been defined in the Montreal Consensus Report as a chronic disease, in which the reflux of gastric contents into the esophagus in abnormal quantities causes clinical symptoms with or without mucosal erosions (21). GERD is influenced by multiple factors, including age, sex, and obesity, esophageal function (esophageal dysmotility), anatomical abnormalities (gastroesophageal hernias), *Helicobacter pylori* infection and environmental factors (including diet) (22). Epidemiological studies have

shown that reflux esophagitis is more prevalent in males (23). Kim *et al* (5) found that men are more likely to develop GERD compared with that in women, and the prevalence of GERD in women is significantly increased with age, particularly in women >50 years old. It indicates that the prevalence of GERD is closely associated with sex differences and highlighting the potential involvement of estrogen. Asanuma *et al* (24) showed that the severity and the prevalence of GERD appear to be closely related to the reproductive hormone status of women. In the postmenopausal period, the prevalence of GERD rapidly increased, whereas it was lower compared with that in men in the reproductive period, which could be responsible for the increased prevalence of GERD in younger men compared with that in women, which reflects the level of the sex hormone estrogen. This potential effect of estrogen could delay the development of GERD via its anti-inflammatory function and acquisition of epithelial resistance in the esophagus against causative refluxate. Thus demonstrating that estrogen in women could be responsible for GERD being more common in men compared with that in women. In addition, Iijima and Shimosegawa (25) demonstrated the role of estrogen in attenuating the esophageal tissue damage in NO-related esophageal damage. Furthermore this research could explain the well-recognized male predominance in the GERD spectrum in humans. Moreover, in female rats, estrogen binds the estrogen receptor and attenuates esophageal tissue damage (26). Masaka *et al* (26) reached a similar conclusion from an acid-related reflux esophagitis model that was produced by surgical operation on male and female rats. Boeckxstaens *et al* (27) demonstrated that the increased prevalence and severity of reflux esophagitis in women is associated with reduced levels of estrogen after menopause. Together, these studies highlight the sex differences in the severity of esophageal mucosa damage in GERD in animal models, highlighting the role of estrogen in controlling GERD with the relevant esophageal epithelial tissue injury.

However, contradictory studies have shown that estrogen and estrogen receptor agonists are associated with an increased risk of gastroesophageal reflux disease symptoms (28-31). Female sex hormones can relax the lower esophageal sphincter and increase the risk of gastroesophageal reflux symptoms (32). Previously, it has been demonstrated that there is a positive correlation between gastroesophageal reflux symptoms and postmenopausal hormone replacement therapy (HRT) (31).

Furthermore, women whom have never taken postmenopausal hormone therapies, have a lower risk of reflux symptoms compared with women who have or are still taking estrogen replacement therapy (28). There is a positive correlation between the risk of reflux symptoms, increased estrogen dose and increased duration of estrogen use. Jacobson *et al* (28) showed an odds ratio of 1.39 for reflux symptoms that used a selective estrogen receptor modulator, and an odds ratio of 1.37 for women who used over-the-counter hormone preparations (28). Therefore, it is important to understand the role of estrogen and estrogen receptors in the pathogenesis of GERD.

Esophageal cancer (EC). EC is one of the deadliest malignancies of the GI tract and causes >400,000 deaths each year. The two most common histological subtypes are esophageal adenocarcinoma (EAC) and esophageal squamous cell

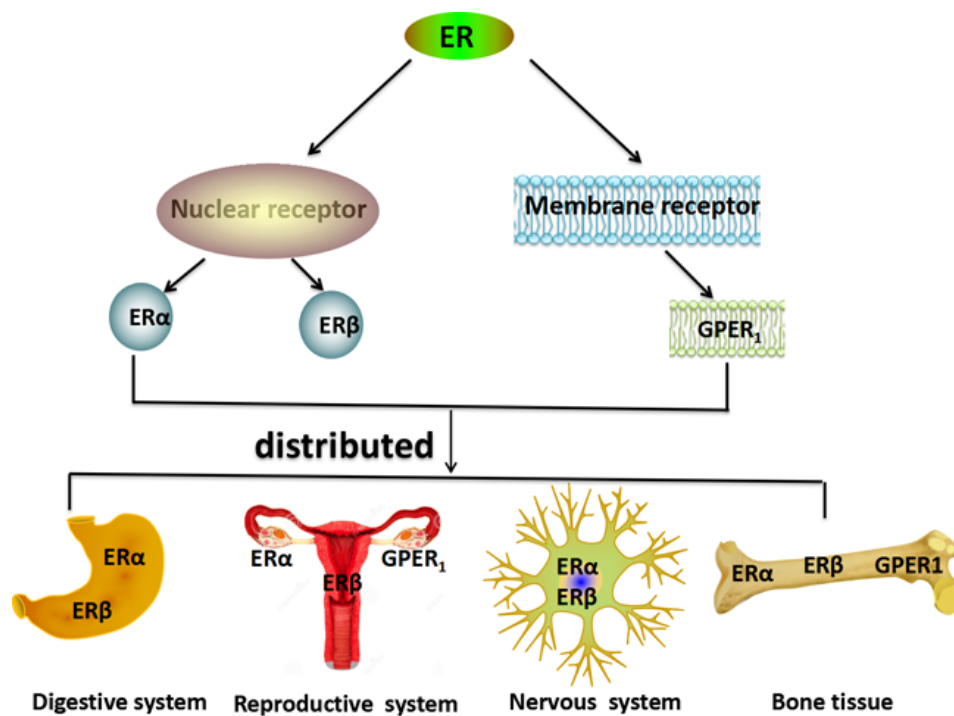


Figure 1. Distribution of estrogen and ERs in the human body. ERs include two broad categories: Nuclear receptors, which includes ER α and ER β and membrane receptors, which includes GPER1. ER α and ER β are primarily expressed in the digestive system and the nervous system. ER β , ER α and GPER1 are primarily expressed in bone tissue and the reproductive system. GPER1, G protein-coupled estrogen receptor 1; ER, estrogen receptor.

carcinoma (ESCC) (33). The incidence of EAC was 6-10x lower in women compared with men, and that ESCC incidence was 2-3x lower (34). Mathieu *et al* (35) analyzed the prevalence of EC by histology and gender differences between 1973 and 2008 in nine population-related cancer studies, and the incidence of EAC increased in both males and females over this time period. Furthermore, the ratio of EAC in male vs. female was highest in individuals aged 50-54. The risk of EAC age-based incidence rate in postmenopausal females aged 80 increased significantly, and this trend was not seen in similarly aged males. Overall, estrogen-related endocrine milieu in premenopausal and perimenopausal females serves as a protective factor in the prevention of EAC, and with loss of estrogen in the body or an increase in time without estrogen-mediated maintenance, the prevalence of EAC incidence increases in older postmenopausal females. A total of 16 independent studies were analyzed by Wang *et al* (36), and the results showed that estrogen can lower the risk of EC. The relative risks were pooled and they showed a negative correlation between the risk of EC and hormone replacement therapy. In addition, menopausal women were at an increased risk of EAC compared with EC (36). The serum levels of estradiol in a healthy cohort from a high-incidence area (HIA) and a low-incidence area for esophageal cancer, as well as that of patients with ESCC from a HIA in Hena, China were assessed, and it showed that lower serum levels of estradiol were associated with a higher predisposition for developing ESCC (37). Furthermore, Zhang *et al* (38) demonstrated that 17 β -E₂, but not 17 α -E₂, decreased proliferation of human ESCC cells in a dose-dependent manner, and this was attenuated by ICI1 82780 (an estrogen receptor antagonist). 17 β -E₂ promotes intracellular calcium mobilization and extracellular

calcium entry into ESCC cells, and estrogen exerted an anti-proliferative effect on human ESCC cells, likely through an estrogen receptor-calcium signaling pathway. According to Hennessy *et al* (39), the antiproliferative effects of 17 β -E₂ may occur through the ER β estrogen receptor. Zuguchi *et al* (40) examined the expression status of both ER α and ER β in 90 Japanese patients with ESCC and demonstrated that both ER α and ER β were upregulated in ECGI-10 cells (an ESCC cell line). Additionally, the status of ER β in ESCC was closely associated with unfavorable outcomes, possibly through increasing proliferation of carcinoma cells.

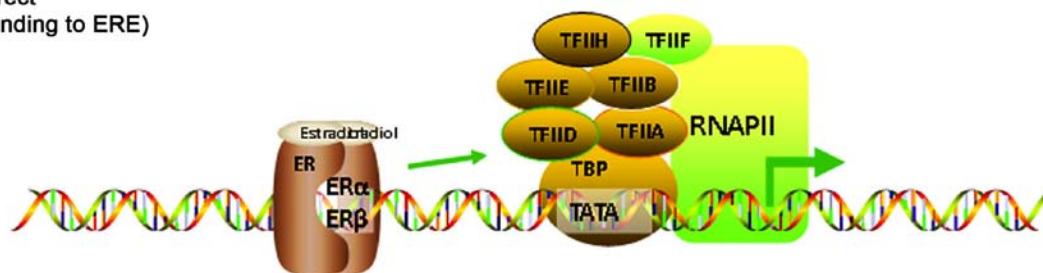
Taken together, these results indicate that estrogen and estrogen receptors inhibit growth of esophageal cancer by estradiol (41). Furthermore, estrogen replacement in postmenopausal women serves as a protective factor against esophageal cancer by reducing the degree of damage to esophageal tissues caused by gastric acid (42). Estrogen can reduce the risk of esophageal cancer. Therefore, a reduction or lack of estrogen may be an important factor in the high incidence of esophageal cancer in men and postmenopausal women.

Estrogen and estrogen receptors in gastric diseases

Peptic ulcers. Peptic ulcers include both gastric and duodenal ulcers, and complications include upper GI bleeding, GI perforation and gastric outlet obstruction (43). Peptic ulcer disease is a multifactorial and complex digestive disease, and its pathogenesis is unclear (44). Gastric protective factors include mucous, endogenous bicarbonate, prostaglandins and antioxidant agents; whereas, pepsin, gastric acid, bile acids and endogenous oxidant agents are recognized as risk factors that could cause damage to the stomach (45). Acid secretion, and the pH values of the stomach and duodenum did not differ

Two mechanisms of ER transcriptional activation

Direct (binding to ERE)



Indirect (binding to other TF's)

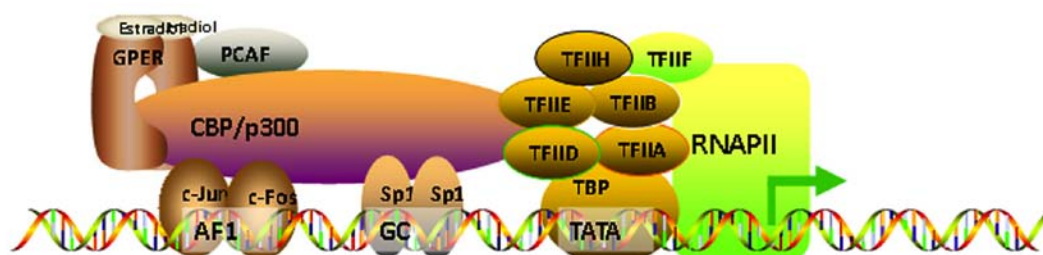


Figure 2. Mechanisms of estrogen signaling. Direct pathway: When estrogen binds to the nuclear receptor, it forms an estrogen-receptor complex, which changes the conformation of the receptor, forming a dipolymer and exposing the DNA binding region. The complex enters the nucleus through the nuclear pore in the form of a dipolymer. Activated ER α / β can directly combine with ERE and subsequently interacts with the target gene or with other transcription factors, forming a complex, which promotes activity of regulatory proteins located at the promoter sites of target genes. This results in an increase in the mRNA expression and thus potentially protein expression levels of the target gene. Indirect pathway: GPER is rapidly activated by intracellular PCAF combining with estrogen-like substances. Activated GPER directly associates with CBP/P300 and interacts with the target gene or with other transcription factors, forming complexes such as the proto-oncogene Fos/jun and SP-1, which promotes activity of regulatory proteins located at the promoter sites of target genes. ER, estrogen receptor; GPER, G protein-coupled estrogen receptor; CBP/P300, transcription complex auxiliary activation factor; PCAF, P300/CBP-related factors; ERE, estrogen response element.

between males and females (46). Peptic ulcers are relatively rare during pregnancy, and estrogen exhibits a protective effect against the incidence and severity of peptic ulcers, and the risk of ulcers is lower in women compared with men (6,47). Okada *et al* (48) found that individuals >70 years in age, had an increased prevalence of ulcers and this was also true in postmenopausal women. The decrease in the serum levels of estrogen induced a reduction in gastric mucosal defenses. Additionally, another study suggested that estrogen exerts an antioxidant effect, which directly scavenges free oxygen radicals, activates antioxidant enzymes, represses the production of superoxides and reduces the formation of peptic ulcers (49).

Therefore, estrogen exhibits a protective effect from peptic ulcers, which may be achieved through its antioxidant effects. However, the specific mechanisms underlying its protective effects require further study.

Gastric cancer (GC). GC is a malignant tumor and the fifth highest incidence and third highest mortality rates in the world (50). Epidemiological studies have suggested that the prevalence of gastric cancer is higher in men than women with a ratio of 1.2:1.0 male:female. However, the differences between male and females becomes negligible when compared with postmenopausal women (51). Tokunaga *et al* (52) first

reported the relationship between hormone receptors and GC, and they highlighted the fact that estrogens may serve a protective role against gastric cancer. Lindblad *et al* (53) found that the probability of developing gastric cancer was not increased in patients with prostate cancer. In addition, Furukawa *et al* (54) found that female, castrated male and estrogen-treated male rats had a lower incidence of gastric cancer with lower histological differentiation compared with that in non-treated male rats after administration of the carcinogen, N-methyl-N0-nitro-N-nitrosoguanidine, and untreated male rats had increased rates of morbidity as a result of gastric cancer compared with castrated or estrogen-treated male rats.

The expression of ER α and ER β in gastric cancer has been previously demonstrated (55). It has been hypothesized that ERs serve an important role in the occurrence and development of gastric cancer (56). Studies have demonstrated that ER β , but not ER α , is abundantly expressed in GC (57-61). However, other researchers have demonstrated the expression of both receptors in GC (62-64). Zhou *et al* (65) found that the expression of β -catenin was reduced when ER α was overexpressed, and this resulted in a decrease in growth and proliferation of GC cells, and an increase in the apoptotic rate by preventing entry into the G₁/G₀ phase. ER- α is considered a rare subtype of estrogen receptor ER α , which is associated with increased

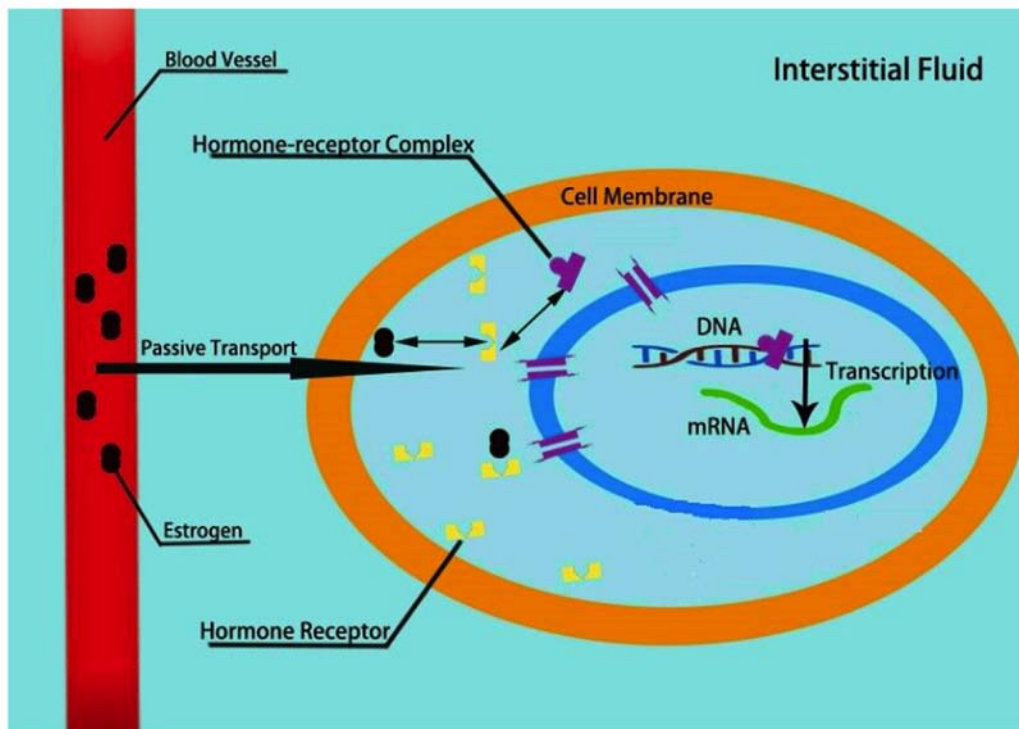


Figure 3. Estrogen and the estrogen receptor-mediated signaling pathway. Estrogen is transported into the cell and combines with the estrogen receptor, forming a hormone-receptor complex. The combined complex enters the nucleus, and regulates the transcription process as described in Fig. 2. Nuclear estrogen receptor is a transcription factor that regulates the function of estrogen complexes and can modulate gene expression by interacting with other proteins and receptors.

lymph node metastasis and invasion in GC. Non-genomic estrogen signaling mediated by ER- α was involved in the c-Src signaling pathway in SGC7901 GC cells (66). A recent study found that ER α expression in gastric cancer cells was increased by low concentrations of 17 β -estradiol, which in turn resulted in increased proliferation by activating mitogen-activated protein kinase signaling pathway (67). In addition, knock down of ER α did not affect the proliferation, migration and invasion of gastric cancer cells (67). Compared with expression of ER α , expression of ER β in noncancerous tissues was significantly higher in female rats compared with male rats (68). Ryu *et al* (61) evaluated the presence of ER β in gastric cancer and showed that ER β was likely not a contributing factor for the invasiveness of gastric cancer.

Therefore, investigating the roles and mechanisms of ER and its receptors may highlight potential mechanisms to improve management of the disease (Fig. 3).

Estrogen and estrogen receptors in intestinal diseases

Irritable bowel syndrome (IBS). Irritable bowel syndrome is one of the most common GI disorders, and is typically characterized by disorderly bowel movements and chronic abdominal pain (69). Based on epidemiological studies (70-72), irritable bowel syndrome is more prevalent in women than men, with a ratio range of 2-4:1, highlighting the possibility of the involvement of estrogen serving a role in the pathophysiology of IBS (7). IBS symptoms were determined to be associated with hormonal status, and the role of sex steroid hormones in the pathophysiology of IBS is gaining increasing attention (73). Studies have demonstrated that estrogen participates in modulating visceral sensitivity and regulating motor and

sensory functions in IBS animal models (74,75). Additionally, Jacenik *et al* (76) determined the estrogen receptor engagement in the IBS subtypes, constipation predominant IBS and diarrhea predominant IBS (IBS-D). The authors analyzed whether estrogen signaling was accompanied by alterations in the expression of pro-inflammatory and anti-inflammatory cytokines and microRNAs, which regulate genes associated with the immune response. Both ER α and GPER expression were upregulated in IBS. There was a correlation between the expression of GPER in patients with IBS-D and the severity of abdominal pain, and an association between the GPER-mediated estrogenic effects on IBS pathogenesis and activation of mast cells in the colon, thus highlighting a novel avenue for understanding the pathogenesis of sex differences in IBS (77). GPER-mediated estrogenic effects were involved in the regulation of visceral pain and GI motility (78).

Inflammatory bowel disease (IBD). IBD is an intestinal inflammatory disease, which is incompletely understood. There is a lack of clear understanding of the pathogenesis of IBD and established effective treatments (79). Previously, patients with IBD were diagnosed primarily in North America and Europe (80). As lifestyle, environment and diets of individuals has changed overtime, the prevalence of IBD has increased worldwide, particularly in children and adult populations (81). IBD includes both ulcerative colitis (UC) and Crohn's disease (CD). The differences in cancer risk between male and female mice were evaluated for patients with IBD, and the results showed that IBD conferred a higher risk of developing colorectal cancer (CRC) in males compared with females. Colitis is hypothesized to be associated with the development of IBD (82).

ER β is the predominant ER subtype expressed in colon tissues, and it maintains a normal epithelial architecture protecting against chronic colitis (83-85). Men present with a higher risk of developing colitis than women, implicating estrogen as a protective factor against developing colitis. Armstrong *et al* (86) found that E2 treatment reduced inflammation in the colon in control mice. The expression of interleukins (ILs; particularly IL-6, IL-12 and IL-17), granulocyte-macrophage colony-stimulating factor, interferon- γ , monocyte chemotactic proteins-1, macrophage inflammatory protein-1 α and tumor necrosis factor- α were not significantly increased in control mice following treatment with E2. The extent of damage was higher in the control ER β knockout mice compared with the E2-treated ER β knockout mice. Additionally, ER β mRNA expression levels were decreased in a colitis mouse model of intestinal inflammation (87). ER β knockout mice presented with colitis of increased severity compared with the wild-type group (88). Therefore, E2 may protect against acute colitis through the activation of ER β .

Colon cancer. Colon cancer is one of the most common types of malignant tumor of the GI tract and the second leading cause of cancer-associated death worldwide. An epidemiological study of colon cancer prevalence found that females exhibited a higher prevalence of colon cancer. However, women aged 18-44 with colon cancer had an improved prognosis compared with men of the same age and women >50 years (89). Upregulated expression of ER β 1 in colon cancer is associated with an improved survival outcome (90). Similarly, downregulated expression of ER β 1 is associated with poorer survival outcome (90). Numerous studies have demonstrated that hormone replacement therapy (HRT) in postmenopausal women did not serve a protective role (91,92), contradicting previous studies (93,94). The Women's Health Initiative showed that the prevalence of colon cancer decreased by 30% following treatment with HRT in postmenopausal women (95).

Interestingly, Bustos *et al* (89) found that estrogen receptors (ER α and GPER) are activated by 17 β -E2 under anoxic conditions, when ER β expression was reduced/absent in patients with colon cancer. An E2-related gene (ataxia telangiectasia mutated) was inhibited in anoxic conditions through GPER signaling.

E2 treatment reinforced hypoxia-associated migration and proliferation of colon cancer cells, whereas in an aerobic environment, cell migration and proliferation were decreased by E2 treatment (89). The effects of E2 on the cellular responses in an aerobic environment and anoxic conditions were mediated by GPER. Therefore, in order to fully predict the estrogenic response in patients with colon cancer, it is necessary to understand not only the status of estrogen receptor expression in tumor cells, but also the aerobic/anoxic conditions of the local tumor microenvironment (89).

4. Conclusions

Estrogen is a sex hormone that regulates the development and function of the reproductive systems in all mammalian species, and increasing evidence demonstrates the multifaceted nature of its effects on non-reproductive organs during physiological and pathophysiological conditions. Understanding the effects

of estrogen and estrogen receptor function may provide an important theoretical basis for improving clinical treatments of GI disease.

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Availability of data and materials

Data sharing is not applicable to the present study, as no data-sets were generated or analyzed during the current study.

Authors' contributions

CMC, XG, XXY, XHS, QD, QSL and RX conceived, wrote and revised the paper. YSC and JYX wrote and revised the paper. All authors approved the final version of the manuscript for submission.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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