REVIEW

The role of estrogen receptors in intestinal homeostasis and disease

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Estrogen has a pivotal role in many biological functions in both reproductive and non-reproductive organs, mediating actions through its receptors, estrogen receptor α (ERα) and ERβ. The expression of ERs is widespread in the body and is implicated in normal physiological processes as well as in disease conditions, including intestinal diseases. Immunohistochemical and functional analyses have revealed that ERβ is the predominant ER type in intestinal tract, but not ERα. The ERβ mediates to provide protection against duodenal ulcer, inflammatory bowel disease and colon cancer but may also contribute to the progression of constipation. In this review, we summarize the recent findings regarding estrogen and its receptors and their role in intestinal diseases. Based on these findings, it is possible to drive the pathogenesis of intestinal diseases using ER-subtype selective inhibitors or stimulators.

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Introduction

Estrogens play key roles in maintaining the structure and function of various reproductive and non-reproductive organs. In general, the biological actions of estrogens are mediated through binding to estrogen receptor α (ERα) and ERβ via genomic and non-genomic mechanisms [1]. ERα and ERβ are encoded by different genes known as ESR1 and ESR2, respectively, that are localized on different chromosomes and several splice variants are described for both receptor types. Structural analysis revealed that whilst the DNA-binding domain has a high degree of homology (95%) the ligand-binding domain has only 58% homology (Fig. 1) [2]. The activation of genomic estrogen signaling pathway is mediated through binding to its receptors which then bind to estrogen responsive element (ERE), which is located in the promoter region of various estrogen target genes [3, 4]. Activation of the genomic mechanism takes several hours, whereas the non-genomic mechanism can be activated much faster within seconds or minutes. The non-genomic mechanism mediated through either ERs located in or near the plasma membrane, or other non-ER proteins associated with the plasma membrane, results in the activation of kinases [5]. Although both receptors are involved in activation, a very recent report has indicated that ERα is more important for the genomic mechanism and that ERβ is more important for the non-genomic mechanism [6].

ERα and ERβ are expressed in various tissues but ERβ is the predominant ER type in the intestinal tract [7, 8]. Structural and functional differences as well as distinct ER expression patterns are revealing the potential for the use of receptor

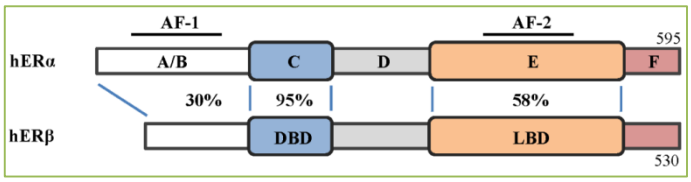

Figure 1. Domain structure representation of human ERα and ERβ isoforms. ERs consists with two domains (DNA binding domain and ligand binding domain) as well as two transactivation functions (AF1 and AF2). The degree of homology between ERα and ERβ in the A/B, C and D domains is indicated.

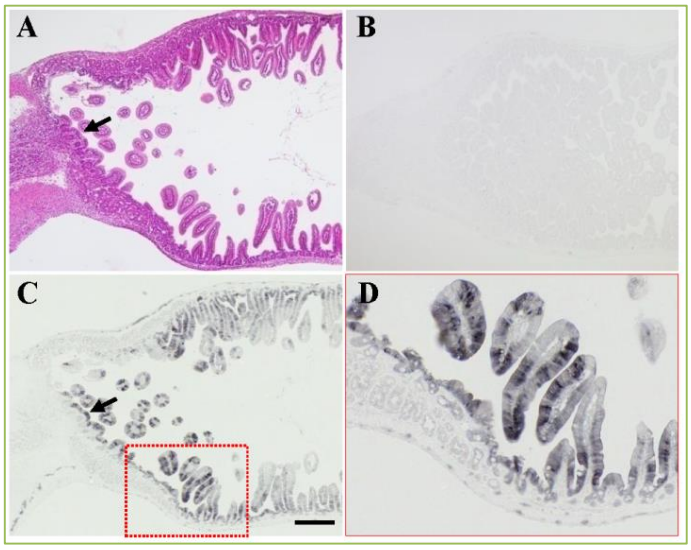

Figure 2. ERβ is the predominant estrogen receptor in mouse duodenum (Choijookhuu *et al*, 2015). A. H&E staining revealed the gastroduodenal junction and proximal duodenum in PND 10 mice. Arrow indicates the proximal part of the duodenal epithelium at the gastroduodenal junction. Immunohistochemistry for ERα and ERβ in duodenum is shown in **B** and **C**, respectively. Black arrow indicates ERβ-positive epithelial cells at the gastroduodenal junction. A magnified micrograph is shown in **D**. Epithelial cells were stained in nuclei and cytoplasm. Magnification, 100 × (A-C); Scale bar 500 µm.

type-specific treatments in the clinical setting. Selective estrogen receptor modulators (SERMs) such as tamoxifen are successfully being used against breast cancer based on an antagonistic effect in the breast, although it also has an agonistic effect in the uterus and bone [9]. Currently, estrogen targeted treatment strategies include inhibitors for both receptors, such as faslodex and SERMs, and aromatase inhibitors which inhibit the conversion of androgen to estrogen. In this review we have focused on estrogen regulated intestinal diseases and the potential use of estrogen targeted treatments in clinical practice.

Estrogen receptors and duodenal ulcers

Epidemiological studies have indicated that the male to female sex ratio in the prevalence of duodenal ulcers was 4:1 in Asia, 2.2:1 in Europe and 1.7:1 in the United States [10-12]. Furthermore, gender specific protection by estrogen against duodenal ulcers was reported in women, and a protective role was demonstrated in animal models where a gastric acid induced mucosal injury was attenuated after estrogen and isoflavonoid treatment [13, 14]. The incidence of duodenal ulcers is reduced in pregnant women and in women taking oral contraceptives, suggesting the direct involvement of estrogen in the pathogenesis of duodenal ulcers [15]. In the proximal duodenum, mucosal bicarbonate (HCO_3^-) neutralizes acidic contents derived from the stomach and protects against peptic ulcer formation. In premenopausal women, basal and acid-stimulated duodenal bicarbonate secretions are significantly higher than in postmenopausal women [16]. In contrast, decreased bicarbonate secretion after treatment with ICI 182,780 (an ER antagonist) suggests that the duodenum expresses functional ERs [13].

We recently reported that ERβ is the predominant ER type in mouse duodenal epithelium, and the highly similar co-localization of ERE-binding proteins demonstrates that ERβ is functionally active in mouse duodenum [8]. Immunohistochemistry revealed only ERβ, but not ERα expression in mouse duodenal epithelium (Fig. 2). Tuo *et al.* reported the mechanism of protective effect of genistein, an isoflavone phytoestrogen that stimulates duodenal bicarbonate secretion through the phosphatidylinositol 3-kinase pathway in mice [17]. The physiological concentration of estrogen potentiates prostaglandin E_2-stimulated duodenal mucosal bicarbonate secretion [18]. Based on these facts, ERα may have a minor role in the pathogenesis of duodenal ulcers. In summary, recent reports and our data suggest that ERβ has a major role in mediating the protective effects of estrogen against duodenal ulcer development through increased duodenal mucosal bicarbonate secretion. The reduced incidence of duodenal ulcers in postmenopausal women using hormone replacement therapy and in women taking contraceptive pills emphasizes the importance of an estrogen targeted treatment approach for prevention and treatment of duodenal ulcers not only in women, but also in men.

Estrogen receptors and inflammatory bowel disease

Inflammatory bowel disease (IBD) is an idiopathic disease caused by the disrupted regulation of the immune responses to the host intestinal microbiota [19]. Two major clinically defined forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC). There are relatively few and conflicting reports of gender differences in IBD incidence. Females have a higher incidence in Europe and North America but male predominance in Asian populations has also been reported [20-23]. Gender differences in IBD incidence may be explained by gender specific cultural

habits, socioeconomic status and different exposures to environmental risk factors for IBD [24].

Intestinal microbiota is a major environmental driving factor for IBD and increased epithelial permeability leads to intestinal inflammation [19]. Interestingly, increased epithelial permeability was associated with decreased ERβ expression in an animal model of colitis as well as in patients with IBD [25]. This finding was also demonstrated in ERβKO mouse where a significant decrease in α-catenin and plectin was found in colonic epithelium. These are important proteins for adherens junctions and hemidesmosomes, respectively [26]. Therefore, the lack of ERβ leads to increased epithelial permeability causing direct exposure of intestinal microbiota to colonic immune cells and further progression of inflammation.

NF-κB is one of the most important regulators of pro-inflammatory gene expression in the pathogenesis of IBD. It has been reported that NF-κB transcriptional activity is inhibited by estrogen ligand and receptor complexes [27]. Both ERα and ERβ can suppress the transcriptional activity of the RelA subunit of NF-κB in response to 17β-estradiol [28]. Surprisingly, in the rat model of IBD, treatment with estrogen receptor ligand inhibited the transcriptional activity of NF-κB. The rats' chronic diarrhea rapidly resolved and histological features indicated significant improvements such as decreased ulceration, fibrosis and inflammatory infiltration. All of these effects were blocked by co-administration of ER antagonist ICI 182,780, suggesting that the anti-inflammatory action was mediated through ERs [29]. Edvardsson *et al.* reported that re-expression of ERβ induced the anti-inflammatory and anti-tumorigenic networks of colorectal cancer cells [30]. In addition, the increased expression of ERβ was strongly correlated with down regulation of IL-6 and its downstream molecules, which are critically important in IBD pathogenesis. Armstrong *et al.* also demonstrated that E_2 treatment suppresses the inflammation associated with colon cancer through an ERβ-mediated mechanism [31]. An azoxymethane/dextran sodium sulfate induced colitis mouse model revealed that ERβKO mice showed more severe inflammation and earlier clinical manifestation than control mice, highlighting the protective role of ERβ against IBD [32]. Although accumulated evidence in basic and clinical settings suggests that estrogen protects from IBD through an ERβ-mediated mechanism, there are conflicting reports that oral contraceptives can increase the risk of IBD and particularly CD. Moreover, the risk was reduced when oral contraceptive pill use ceased [33]. In colorectal mucosal biopsy, ERα promoter methylation was higher in UC patients compared with controls and it is the only a report regarding with ERα and IBD correlation [34]. Therefore, ERα may have a minor role in the pathogenesis of IBD. Based on animal models and clinical data, we concluded that estrogen has a protective role against IBD through ERβ. Importantly, the strong correlation between increased ERβ and down regulation of inflammatory cytokines suggests that ERβ-mediated treatment options may be helpful for IBD treatment.

Estrogen receptors and constipation

The causes of constipation appears to be multi-factorial, such as hormonal effects on intestinal transit time and motility, increased water absorption, reduced physical activity, and dietary and mechanical factors [35, 36]. Among these factors, intestinal transit time and motility, salt and water absorption are already known to be affected by estrogen [37, 38]. Epidemiological studies have reported that up to 30% of the general population suffers from constipation at least once and the incidence was 2-3 times higher in reproductive aged women than in age-matched men, suggesting that women are more susceptible to constipation [39-41]. In childhood, constipation is more common in boys, but it is reversed during the pubertal period and continues until postmenopausal age [42]. In people aged over 70 years the incidence of chronic constipation is similar for men and women, demonstrating that sex steroid hormones affect intestinal function [40]. In fact, oral contraceptive pills have also been reported as a causative factor for constipation [43]. Indeed, estrogen effect on constipation during the menstrual cycle has been reported controversially. Several studies have suggested that the effect of estrogen on constipation is minimal in normal healthy women during the menstrual cycle, whereas other studies reported that constipation occurred more often during the menstrual period and / or luteal phase [38, 44-46]. Although the effect of estrogen on constipation during the menstrual cycle is not obvious, the dramatic increase in estrogen correlates with constipation during pregnancy with up to 40% of pregnant women experiencing it [35, 47]. In our recent study, during late pregnancy, the ERβ expression was increased in the mouse proximal colon, following the increase in circulating estrogen concentration. Immunohistochemistry revealed ERβ expression in the proximal colon, but not in the middle or distal colon. NHE3 expression was also increased on the colonic surface together with increased ERβ expression [7]. Surprisingly, in ovariectomized (OVX) mice, the E_2-treatment induced ERβ expression as well as increased expression of NHE3 in the proximal colon. OVX mice treated with E_2 + ICI 182,780 were negative for ERβ, and the NHE3 signal was at a basal level, similar to that of the control mice. This indicates the direct regulation of estrogen in the expression of NHE3 in colon epithelium, through ERβ, suggesting that increased colonic salt and water absorption

may lead to constipation during pregnancy [7]. Another study in the mouse model also confirmed our findings that constipation is caused by estrogen rather than progesterone in both male and female mice [48].

Intestinal motility is one of the major driving factors in constipation and is controlled by the enteric nervous system. There is evidence to suggest that estrogen influences intestinal motility and transit time. Interestingly, in humans, colonic contractility was inhibited by estrogen through a non-genomic signaling pathway. This inhibitory effect was abolished by ICI 182,780, indicating that ERs were involved to mediate estrogen signaling for colonic motility [37]. Increased circulating estrogen as well as progesterone was shown to correlate with reduced colonic transit in rodent animal models [49]. In our study, expression of both ERα and ERβ was detected in intestinal neurons of Auerbach and Meissner plexuses (Fig. 3A, B). Therefore, ERα and ERβ-positive intestinal neurons could be a target of estrogen signaling. In summary, estrogen caused decreased colonic motility and increased sodium and water absorption which are two major factors in constipation.

Estrogen receptors and colon cancer

A meta-analysis survey of colon cancer incidence revealed a substantially lower rate in women than in men, with an overall sex ratio of 1:1.4 [51]. Hormone replacement therapy, the usage of oral contraceptive pills, high parity and early age at the time of first birth were all correlated with a reduced risk of colon cancer in women [52-54]. All of this evidence suggests that estrogen has a protective role against colon carcinogenesis. In clinical and laboratory settings, the ERβ is known to predominate in the colon and demonstrated that it is a key factor in protection against colon cancer. *In vitro* experimental results with SW480 and HCT116 colon cancer cell lines indicate that ERβ has suppressive and preventative roles against colon carcinogenesis [55, 56]. The protective role of ERβ has been explained by 17β-estradiol induced ERβ-mediated oxidative stress leading to apoptosis of colon cancer cells [57] and the anti-proliferative effect in colonic epithelial cells [26, 56]. Jassam *et al.* [58] revealed the correlation between loss of ERβ and increased Duke's stage of colon cancer, while another study demonstrated that the lack of ERβ expression was strongly associated with higher tumor grade and greater tumor extent [59]. Moreover, the loss of ERβ expression was three times higher in descending colon cancers compared to ascending colon cancers [58]. This difference may be explained by the subsite-specific expression, such as higher ERβ expression in the superficial epithelium of the ascending colon than in the descending colon [60]. In addition, our experimental results confirmed the hypothesis that ERβ was specifically localized in mouse

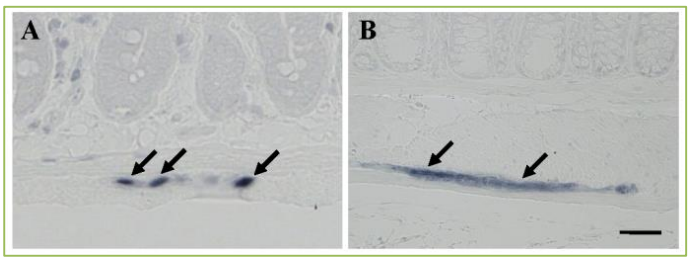

Figure 3. ERα and ERβ expression in mouse small and large intestine. The localization of ERα and ERβ in mouse intestinal neurons is shown in **A** and **B**, respectively. Black arrows indicate positive cells. Magnification 200 ×, scale bar 20 μm.

proximal colon but not in the distal colon [7]. A lower incidence of proximal colon cancer was found in young women compared to men. Interestingly, this difference was abolished in postmenopausal women, emphasizing that the protective role of ERβ in the colon depends upon circulating estrogen levels [61]. A colitis-associated colorectal cancer model in ERβKO mice revealed direct evidence of a protective role for ERβ against colorectal cancer. A more severe colitis developed in ERβKO mice with a significantly higher disease activity index, inflammation score and dysplasia [32].

The highest incidence of colorectal cancer was reported in North America, Oceania and European countries, while the lowest rate was observed in Asia [51, 62]. Low rates of colorectal cancer in Asian countries may be explained by high intake of dietary phytoestrogen from soyfoods, such as genistein and daidzein [63]. Genistein is an ERβ-specific agonist that is mainly derived from soyfoods which induces anti-proliferative and pro-apoptotic effects in the intestinal tract of ovariectomized rats [64]. Genistein binds to ERβ with higher affinity than 17β-estradiol, thus potentiating an ERβ-mediated protective role against colorectal cancer [65]. Moreover, 6,7,4′-trihydroxyisoflavone, a metabolite of daidzein, inhibits HCT-116 human colon cancer cell proliferation through cyclin-dependent kinase 1 (CDK1) and CDK2 [66].

A recent report revealed decreased ERβ expression concurrent with increased ERα expression in both mRNA and protein levels during cancer progression [31]. Another study reported that ERα deficiency in $Apc^{min/+}$ mouse correlated with increased expression of Wnt-β-catenin target genes, which are critically important in intestinal carcinogenesis [67]. A controversial role for ERα in the colon was reported where dietary soy isoflavone and estrone protected ERαKO mice from colon carcinogenesis, suggesting that ERα may not be required to mediate the protective effects of estrogen against colon cancer [68]. Although there are several reports of the presence of functional ERα in the colon, estrogen signaling is known to

Table 1. Role of Estrogen and its receptors in diseases.

Intestinal disease	Function	Receptor	Reference
Duodenal ulcer	In mouse duodenal epithelium, the ERβ is the predominant ER subtype, but not ERα	ERβ	8
	Genistein stimulates duodenal bicarbonate secretion through the PI3K pathway in mice, and the secretion was decreased after treatment of ICI 182,780	ERβ	13, 17
	Estrogen promotes prostaglandin E_2-stimulated duodenal mucosal bicarbonate secretion	ERα	18
Inflammatory bowel disease	The expression of α-catenin and plectin were significantly decreased in ERβKO mouse. Epithelial permeability was increased in animal model of colitis as well as IBD patients	ERβ	25, 26
	The re-expression of ERβ was induced the anti-inflammatory and anti-tumorigenic gene expression in colorectal cancer cells	ERβ	30
	Transcriptional activity of NF-κB suppressed by ERα and ERβ	ERα, ERβ	28, 29
	In colorectal mucosal biopsy, the ERα promotor methylation was higher in UC patients comparing with healthy control	ERα	34
Constipation	NHE3 expression was regulated by estrogen through ERβ in proximal colon of pregnant mouse	ERβ	7
	Oral contraceptive pills are causative factor for constipation	-	43
Colon cancer	E_2 induced oxidative stress leads to apoptosis of colon cancer cell. Genistein, has pro-apoptotic effects in mouse intestine	ERβ	57, 64
	Estrogen have an anti-proliferative effect in SW480 and HCT-116 colon cancer cell lines through CDK1 and CDK2	ERβ	26, 55, 56, 66
	Higher expression of ERβ in ascending colon correlates with lower incidence of colon cancer	ERβ	58, 60
	The deficiency of ERα in $Apc^{min/+}$ mouse correlated with increased expression of Wnt-β-catenin target genes, which is critical important for intestinal carcinogenesis	ERα	67
	Hormone replacement therapy, oral contraceptive use, high parity and early age at first birth are all correlated with reduced risk of colon cancer in women	-	52-54

Phosphatidylinositol 3-kinase (PI3K), Ulcerative colitis (UC), Cyclin-dependent kinase (CDK)

be mediated predominantly through ERβ by a combination of genomic and non-genomic mechanisms. Briefly, clinical studies and experimental models have suggested that ERβ has a protective action against colon cancer and that estrogen replacement therapy and phytoestrogens could be used for prevention and or treatment of colon cancer.

Conclusions

In this review, we focus on new insights into estrogen and the role of its receptors in intestinal diseases. Anti-inflammatory and anti-tumorigenic effects of estrogen through ERβ suggest that it may be possible to prevent or treat duodenal ulcers, inflammatory bowel disease and its progression to colitis associated neoplasia. Estrogen and its receptors have not only protective roles in intestinal diseases, but also contribute to the progression of constipation, suggesting that treatment should be specific for the individual patient (Table 1). We hope that future studies focusing on estrogen and the role of its receptors in pathogenesis of intestinal diseases will support further development of effective estrogen targeted treatments.

Conflicts of interest

The authors have declared that no conflicts of interest exist.

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