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Estrogen Receptor Beta as Target for Colorectal Cancer Prevention

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Abstract

Colorectal cancer (CRC) is a leading cause of death in the United States. Despite its slow development and the capacity for early diagnosis, current preventive approaches are not sufficient. However, a role for estrogen has been demonstrated in multiple epidemiologic studies, which may benefit CRC prevention. A large body of evidence from preclinical studies indicates that expression of the estrogen receptor beta (ERβ/ESR2) demonstrates an inverse relationship with the presence of colorectal polyps and stage of tumors, and can mediate a protective response. Natural compounds, including phytoestrogens, or synthetic ERβ selective agonists, can activate or upregulate ERβ in the colon and promote apoptosis in preclinical models and in clinical experience. Importantly, this activity has been associated with a reduction in polyp formation and, in rodent models of CRC, has been shown to lower incidence of colon adenocarcinoma. Collectively, these findings indicate that targeted activation of ERβ may represent a novel clinical approach for management of colorectal adenomatous polyps and prevention of colorectal carcinoma in patients at risk for this condition. In this review, we discuss the potential of new chemopreventive or dietary approaches based on estrogen signaling.

Keywords

colorectal cancer; estrogen; estrogen receptor beta; phytoestrogens; prevention; gene expression

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Conflict of Interest

The authors declare no conflict of interest.

1. Introduction

Colorectal cancer (CRC[1]) is the fourth most common cancer in the United States, and is the second leading cause of death, with 49,700 deaths projected to have occurred in 2015 [1]. CRC can be detected through physical exams, sigmoidoscopy colonoscopy, and/or a laboratory test that measures the level of carcinoembryonic antigen (CEA), a type of tumor marker in the blood. Current approved therapy includes surgery, radiofrequency ablation, cryosurgery, chemotherapy, radiation therapy, and targeted therapy. Choice of treatment is based on the stage (0–IV) and spread of the tumor, with stage 0 indicating a carcinoma *in situ* and stage IV representing a metastatic cancer. Stage 0 – I are treated through surgery alone, while chemotherapy using fluorouracil (5-FU), capecitabine (a prodrug that is enzymatically converted to 5-FU), or the platinum-based cytotoxic agent oxaliplatin, is added for stage III and IV CRCs. For metastatic and recurrent cancers, chemotherapy including the nucleoside analog trifluridine combined together with an inhibitor of its metabolism, tipiracil hydrochloride, or an inhibitior of topoisomerase 1 (irinotecan hydrochloride), can be added, along with targeted therapies. Approved targeted therapies include inhibitors towards kinases (regorafenib) and the vascular endothelial growth factor receptor VEGF (ziv-aflibercept), or antibodies directed towards the VEGF (bevacizumab, ramucirumab) and the epidermal growth factor receptor EGFR (cetuximab, panitumumab). Treatment targeting EGFR is used for patients with EGFR-expressing tumors and wild-type KRAS.

Most CRCs evolve slowly in a polyp-cancer sequence in which adenomatous polyps with malignant potential evolve into cancerous lesions over 10 to 15 years [2]. The importance of early identification is highlighted by a 90% 5-year survival rate after diagnosis of early-stage localized disease. This rate drops to 71% when the disease is spread beyond the colon site but is still within the region and to only 13% when the diagnosis of CRC is accompanied by distant metastases [1]. As a result, CRC prevention strategies have attracted a great deal of research interest.

1.1. Early Screening Efforts

Risk factors for adenomatous polyps include male gender, cigarette smoking, and family history [3]. The National Comprehensive Cancer Network guidelines recommend the use of screening colonoscopy or sigmoidoscopy beginning at age 50 for those with average risk for CRC [4]. In a meta-analysis of reports examining the diagnostic yield of screening colonoscopy in asymptomatic individuals with average risk of CRC, adenomatous polyps were found in 19%, advanced adenoma in 5%, and CRC in 0.78% of patients [5]. Thus, a significant part of the aging population would be candidates for a safe, preventive approach.

1.2. Current Preventive Measures

Inflammation of the colon is a known risk factor for CRC development. Cyclooxygenase-2 (COX-2) is an enzyme responsible for inflammation and pain. The nonsteroidal anti-

[1]Abbreviations: CRC: Colorectal cancer, ER: estrogen receptor, CIMP: CpG island methylator phenotype, FAP: familial adenomatous polyposis, IBD: inflammatory bowel disease, RR: relative risk, OR: odds ratios

inflammatory drugs aspirin and celecoxib, which both are COX-2 inhibitors, have demonstrated substantial efficacy in the prevention of adenomatous polyps and CRC. High doses of aspirin (>300 mg) reduces the incidence of CRC in populations without risk factors for CRC by approximately 26%, but the risk-benefit balance is not clear for aspirin and other nonsteroidal anti-inflammatory drugs owing to the risk of gastrointestinal toxicity, including peptic ulcers and dyspepsia [6]. Lower doses of aspirin (75–300 mg/day for 5 years) are equally effective, reducing the long-term incidence of CRC and associated mortality rates while diminishing gastrointestinal toxicity [7]. In patients with a history of adenoma or CRC, celecoxib (400 mg/day) has been shown to significantly reduce the risk of adenoma recurrence by 34% (relative risk [RR] 0.66; 95% confidence interval [CI] 0.60–0.72; $P<.00001$) and to provide a statistically significant 55% reduction in the RR of advanced adenoma incidence (RR 0.45; 95% CI 0.35–0.58; $P<.00001$). However, trials with celecoxib were terminated early due to an increased risk of cardiovascular events [6]. As current targeted preventive treatments do not outweigh the risks, new targets or treatments are needed. A number of other pharmaceutical agents and supplements with different mechanisms of action, including folic acid, calcium, vitamin D, and antioxidants, have been proposed in the prevention of adenomatous polyps and CRC [6, 8, 9]. This review examines the preclinical and clinical evidence of the estrogenic pathway in the development of adenomatous polyps and CRC, and the potential to modulate this process as a preventive measure based on current knowledge.

2. Estrogen and CRC incidence

2.1. Women Have a Lower Risk for CRC than Men

Colorectal polyps and tumors have been reported to occur more frequently in men than in women, with odds ratios (ORs) of 1.52 and 1.43, respectively [10]. The Women's Health Initiative showed that pre-menopausal women are 40% less likely to suffer from CRC compared to age-matched men [11]. Older women, on the other hand, have a worse overall survival prognosis than similarly aged men, presumably because of the loss of estrogen associated with menopause [12]. Furthermore, in patients with inflammatory bowel disease (IBD) followed for more than 10 years, the overall risk for CRC was 60% higher among men than women [13]. Various pieces of evidence suggest that female sex hormones, specifically estrogen, form the basis for this protective effect in women.

2.2. Estrogen Exposure Decreases CRC Risk

A meta-analysis of 20 English-language case-control and cohort studies reported in the medical literature through June 2000 demonstrated that women who had used oral contraceptives had a lower incidence of CRC than those who had never used it (RR 0.82; 95% CI 0.74–0.92) [14]. Hormone replacement therapy has also been reported to play a protective role in the prevention of CRC [11, 12, 15]. In The Women's Health Initiative, this finding was initially obscured because CRCs in the women who took estrogen plus progestin at the time of diagnosis presented with a more advanced stage than those in women who took placebo [11]. This indicates a complex role of estrogen, where it appears protective initially but may possibly have a mitogenic effect in later stages [16]. A longer-term follow-up over 7 to 8 years in the Women's Health Initiative observational study showed little if

any benefit of therapy with estrogen plus progestin or estrogen alone in protection against CRC in postmenopausal women [17]. However, a meta-analysis of 2,661 articles found consistent evidence supporting the association between estrogen plus progestin therapy and CRC risk reduction, and also that current use of estrogen alone was associated with a decreased CRC risk [18]. Interestingly, progestin-estrogen therapy proved to be more effective than estrogen alone, suggesting that progestins enhance the activity of estrogen [19]. However, therapy combination also increased the risk of coronary heart disease and breast cancer [20]. Also consumption of plant-derived compounds that are structurally similar to estrogens, phytoestrogens, has shown correlations with CRC. A meta-analysis showed that consumption of soy, which is rich in phytoestrogens, was associated with an approximately 21% reduction in CRC risk in women (combined risk estimate, 0.79; 95% CI, 0.65–0.97; P = 0.026), but not in men [21]. As the effect was not significant in men, it has been suggested that the effects of phytoestrogens are dependent on altering metabolism of endogenous estrogens [22].

3. Estrogen Receptor Beta (ERβ) Has a Role in CRC

The effects of estrogens are mediated by estrogen receptors (ERs) of which two with similar structures have been described; ERα (ESR1) in 1985 [23], and ERβ (ESR2) in 1996 [24]. Both are nuclear receptors, which dimerize and translocate to the nucleus after ligand binding and regulate transcription of target genes through binding to estrogen-response elements in the DNA [25–30]. The receptors can further interact with other transcription factor complexes, such as activating protein-1 (AP1), stimulating protein 1 (Sp1), and nuclear factor-κB (NFκB) in a process called transcription factor crosstalk [31–35]. Additionally, non-genomic effects via phosphorylation and regulation of enzymes that impact cell physiology, such as kinases and phosphatases, and activity through the membrane-bound G protein-coupled estrogen receptor 1 (GPR30/GPER1), have been described [36]. While ERα protein is not detected in the colonic epithelium or in CRC (http://www.proteinatlas.org/ENSG00000091831-ESR1/tissue/colon), ERβ has been confirmed as the predominant form of ER in a number of studies [37–45].

3.1 ERβ expression decreases during CRC and associates with survival

Substantial evidence has demonstrated an inverse relationship between ERβ expression in the colon and the presence and stage of colorectal polyps and tumors. Barone and colleagues evaluated ERβ expression in archival biopsy material from six patients with familial adenomatous polyposis (FAP) who underwent colectomy [46]. Results showed that ERβ expression was significantly reduced in both adenomatous tissue with high levels of dysplasia and in carcinomatous tissues compared with normal mucosa ($P<.001$). Further, in clinical samples from 25 patients with colonic polyps compared with 25 controls, expression of ERβ was demonstrated to be significantly lower in the polyps than in controls (10.1% ± 5.5% vs 44.2% ± 13.7%; $P<.03$) [47]. Konstantinopoulos and colleagues demonstrated similar results, with significantly reduced expression of ERβ in adenomatous tissue compared with neighboring healthy tissue ($P<.001$) [37]. The degree of loss of ERβ expression appears to correlate with worsening stage and grade of tumor [37, 39]. These investigators found that 41.7% of well-differentiated tumors from men and 45.5% from

women were moderately or strongly immunopositive for ERβ, compared with only 10% and 11% of poorly differentiated tumors, respectively [37]. Similarly, Nussler and colleagues found that ERβ levels were significantly reduced in CRC of both men ($P<.001$) and women ($P<.04$) compared with in normal colonic mucosa; this reduction in ERβ level was significantly greater in men vs women ($P<.04$) [48]. Further, lack of ERβ in the tumor has been independently associated with poor survival in patients [49–51], and for ERβ-positive tumors, CRC risk significantly decreased with duration of oral contraceptive use [52].

3.2. ERβ is Associated with Risk of CRC

Further, assessing the association between SNPs and distal colorectal adenoma risk in a case-control study of 1–351 subjects, Levine and colleagues found that distal adenoma risk was significantly decreased for an *ESR2* SNP (per minor allele OR = 0.78; 95 % CI = 0.66, 0.91; P (act) = 0.041) [53]. Using data obtained from the Fukuoka Colorectal Cancer Study (a large case-control study of Japanese residents of Fukuoka City and adjacent areas), Honma and colleagues investigated the association between ERβ gene cytosine-adenine (*ESR2* CA) repeat polymorphism and colon cancer risk [54]. Cases included a series of patients with histologically confirmed incident colorectal adenocarcinomas consecutively admitted to 1 of 8 hospitals between September 2000 and December 2003 for surgical treatment; controls met the same inclusion criteria as cases except for not having a diagnosis of colorectal cancer. Classifying the CA repeat alleles into either short (S) alleles (<22 repeats) or long (L) alleles (>22 repeats), the authors found that the risk of colon cancer, but not rectal cancer, increased with the increasing number of L alleles present among postmenopausal women below 75 years of age. In postmenopausal women, age-adjusted OR and 95% CI were 1.83 (1.11–3.00) for the SL genotype and 2.53 (1.41–4.52) for the LL genotype ($P=0.002$). The authors suggested that the increased risk associated with the L allele reflected impaired ERβ signaling caused by lower wild-type ERβ expression [54]. Passarelli and colleagues found three SNPs in the promoter of *ESR2* (rs2987983, rs3020443, and rs2978381) that were statistically significant predictors of CRC-specific and overall survival [55]. No associations were noted for SNPs of the hormone receptor genes *AR*, *ESR1*, or *PGR*, leading the authors to suggest that SNPs in the promoter of *ESR2* may be important to pathways related to the association between ERβ and tumor progression and metastasis.

4. Experimental Evidence Supports Activation of ERβ in the Prevention of CRC

4.1. ERβ Deletion Affects Tumor Formation in CRC and Intestinal Cancer Models

Estrogen has been shown to mediate protective effects in wild-type mice but not in ERβ-knockout mice [56], supporting the role of ERβ as the mediator of these effects. Cho and colleagues further demonstrated that ERβ was an inhibitory modifier of APC-dependent tumorigenesis in the proximal colon of $Apc^{Min/+}$ mice [57]. Using the $Apc^{Min/+}$ model, together with ERα or ERβ knockout animals, they showed that 17β-estradiol deficiency in ovariectomized $Apc^{Min/+}$ females increased tumor incidence and that $ERβ^{+/-}$ $Apc^{Min/+}$ and $ERβ^{-/-}$ $Apc^{Min/+}$, as well as $ERα^{+/-}$ $Apc^{Min/+}$ mice, all developed more tumors in the proximal colon when compared with $ER^{+/+}$ $Apc^{Min/+}$ littermates [57, 58]. Similarly,

Apc$^{Min/+}$ mice deficient in ERβ have shown enhanced intestinal tumorigenesis in independent experiments [59]. Cleveland and colleagues showed similar results, again in *Apc*$^{Min/+}$ mice, demonstrating an association between both ERα and ERβ deficiency and increased incidence of colon tumors [60].

In recent years, a mouse model of the colitis-associated neoplasia dextran sodium sulfate (DSS)-azoxymethane (AOM) has been used. AOM is a colon carcinogen that leads to development of adenocarcinoma in the colon of mice and rats. These tumors can be further promoted by application of DSS, an inflammatory agent that enhances colitis-induced colon cancer, similar to in patients with long-lasting ulcerative colitis (UC). Deletion of ERβ leads to an increase in size and number of colon adenomas in this model, indicating a possible anti-inflammatory and anti-neoplastic mechanism of action for ERβ in the colon [61]. However, one group using this model found that ovariectomy protected female mice against colitis-associated tumor development, and that estradiol as well as medroxyprogesterone acetate (MPA) or a combination of both promoted tumorigenesis [62]. Using both ERα and ERβ knockout animals, they found that the protumorigenic effect of estrogen depended on both ERα and ERβ [62]. The apparent contradiction reported by this group could indicate that estrogen under certain circumstances may play a mitogenic role, possibly once a tumor is formed, as was also indicated in The Women's Health Initiative (see Section 2.2). Interestingly, Armstrong and colleagues found an upregulation of ERα in a minority of lesions following inflammation-associated colon tumor formation in mice, along with increased proliferation following estrogen treatment [63].

4.2. ERβ Activation Reduces Intestinal Tumor Formation in Animal Models

Consistent with the experiments indicating promoted cancer incidence in the absence of ERβ, activation using ERβ-selective agonist diarylpropionitrile (DPN) reduced intestinal tumorigenesis in male and female *Apc*$^{Min/+}$ mice by 39% and 36%, respectively [64]. Weyant and colleagues suggested that endogenous estrogens protected against APC-mediated tumorigenesis through upregulation and activation of ERβ and downregulation of ERα [58]. These authors found that ovariectomy increased tumor production in *Apc*$^{Min/+}$ mice, with the tumor number reduced in mice supplemented with 17β-estradiol [58]. At the same time, ERα expression was reduced and ERβ expression was increased in ovariectomized mice given 17β-estradiol [58].

Another group studied the chemopreventive efficacy of the selective estrogen receptor modulator raloxifene in male F344 rats where colon tumors were induced with AOM [65]. Raloxifene has estrogenic actions on bone but anti-estrogenic actions on the uterus and breast. It is not known whether it is an agonist or antagonist to either ER in the colon, but one report indicates it may be an agonist to ERβ in colon cancer cell lines [66]. Raloxifene has also been reported to have ER-independent effects, such as inhibiting the voltage-gated ion channel Kv4.3 (KCND3) [67]. At early adenoma stage, groups of rats (36 or 45 per group) were fed diets containing raloxifene (1.5 or 3 ppm), for 40 weeks. Raloxifene significantly suppressed colon adenocarcinoma formation in terms of multiplicities (mean ± SE): control, 3.59 ± 0.25; 1.5 ppm raloxifene, 2.51 ± 0.29 ($P < 0.004$); 3 ppm raloxifene, 2.14 ± 0.28 ($P < 0.0001$) [65]. The same group also showed that raloxifene suppressed small

intestinal tumor multiplicity and size in female *Apc*$^{Min/+}$ mice, and suggested the treatments modulated immune signaling and decreased stem-like cells [68]. Phytoestrogens such as the coumestan coumestrol have been shown to modulate ERβ expression and reduce small intestinal mucosa tumor number in *Apc*$^{Min/+}$ mice [69]. Also, soy protein rich in phytoestrogens has been shown to protect mice from colon cancer, and estrone to further reduce colon tumorigenesis, independently of ERα [70]. In conclusion, as summarized in Table 1, the majority of animal studies clearly indicate a protective role for ERβ. The varying roles for ERα, as reported in some studies, may possibly be affected by indirect effects, as its deletion also modifies the levels of estradiol [60].

5. The Cellular Function of ER*β* in Colon Epithelial Cells Has Therapeutic Potential

5.1. ERβ Is Related to Apoptosis in Patient Samples

Barone and colleagues evaluated cell proliferation (using Ki-67/MKI67 as marker), and apoptosis (measured using terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling [TUNEL]) in relation to ERβ expression in patients with FAP [46]. Results showed that high ERβ expression correlated directly with increased apoptosis (r=0.76; $P<.001$) and inversely with cell proliferation (r=−0.54; $P<.05$). The effect of ERβ expression has also been assessed in patients with UC, a disease that increases the risk of developing colonic carcinogenesis. In this small study of 16 subjects (normal, n=4; UC without dysplasia, n=8; UC with dysplasia, n=4), the modifications of ERα and ERβ expression were assessed [71]. The authors found no significant variations in either ERβ or ERα expression, or in the ERβ:ERα ratio, when assessed in subjects with UC with or without dysplasia. This result might be explained by the inclusion of patients with only low-grade dysplasia in the UC with dysplasia group. Nevertheless, the results suggest that ERβ functions as an overseer of apoptosis/proliferation. Indeed, there was a significant increase ($P=0.002$) in cell proliferation in UC and UC with dysplasia subjects that was inversely proportional to ERβ. This was accompanied by a rise in apoptosis, albeit ineffective in completely countering Ki-67 overexpression [71]. The reduction of apoptotic rate in parallel with reduced ERβ expression was confirmed in a larger study, in which also a dramatic decrease of ERβ expression in high-grade dysplasia and cancer was found, when compared to low-grade dysplasia, [72].

5.2. ERβ$^{-/-}$ Knockout Mice Exhibit Changes in Colonic Epithelia

In an ERβ$^{-/-}$ knockout mouse model, silencing of ERβ led to hyperproliferation, loss of differentiation, and decreased apoptosis of the colonic mucosa [73]. Another group found that *Apc*$^{Min/+}$ wild-type for ERβ mice and treated with estrogen exhibited significantly fewer aberrant crypt foci and increased epithelial apoptosis than ERβ-knockout mice [56]. In addition, Hasson and colleagues found that loss of either ER promoted crypt expansion and impaired TGFbeta (Tgfb1) and HNF3beta (Foxa2) signaling in *Apc*$^{Min/+}$ mice [74]. However, in a different study, there was no association between ERβ deficiency and enhanced Wnt/β-catenin (Ctnnb1) pathway activation, which is known to play a critical role in intestinal cancers. Conversely, results showed that ERα deficiency was associated with activation of the colonic Wnt/β-catenin pathway, suggesting that ERα but not ERβ acts via

this pathway [60]. Furthermore, tumors exposed to raloxifene, showed increased apoptotic cells (3-fold) and an increase in the CDK inhibitor p21 (Cdkn1a) expression (3.8-fold) as compared to tumors of rats fed control diet [65].

In addition to cellular effects, also gut microbiota are known to influence the development of colorectal cancer [75]. Presence of estrogen in e.g. waste water has been shown to negatively correlate with species diversity in the microbiota [76], and it is possible that ERβ may affect the composition of intestinal microbiota. Menon and colleagues set out to explore this relationship using terminal restriction fragment length polymorphism (TRFLP) analysis to compare the 16S rRNA genes from fecal DNA from wild-type mice with ERβ knockout mice, fed a complex isoflavone-containing diet or a simple semisynthetic diet [77]. They showed that the ERβ status did affect the composition of the microbiota, with a lower diversity while on the complex diet in the presence of ERβ, and different microbiota responses to changes in diet depending on ERβ expression [77].

5.3. Therapeutic Effects of ERβ in Human CRC Cell Lines

In cell lines, re-introduction of ERβ into colon cancer cells has been found to reduce proliferation in cell lines SW480, HT29, HCT8 but not HCT116 [66, 78, 79]. We have also shown that the expression of ERβ in SW480 xenografts implanted in immunodeficient mice reduced tumor weight by 65% compared with SW480 xenografts from controls that did not express ERβ, demonstrating that ERβ attenuates tumor growth *in vivo* [78]. A recent study by Tu and colleagues sought to determine whether the adenoviral vector–delivered human ERβ gene (Ad-ERβ), raloxifene, or the combination thereof, could be used to treat HCT116 colon cancer cells [66]. The proliferation of HCT116 cells was no different in the ERβ-transfected group compared with controls. In the presence of ERβ and raloxifene, however, there was a strong concentration-dependent antiproliferative effect that was greater than that produced by raloxifene alone. Likewise, ERβ produced no significant difference in apoptosis rate compared with controls, whereas both raloxifene alone and raloxifene + Ad-ERβ significantly increased the rate of apoptosis in HCT116 colon cancer cells ($P< 0.01$ and $P<0.001$, respectively, vs controls). ERβ overexpression was strongly associated with reduced malignant cell transformation as exemplified by a significant reduction of the HCT116 cell colony–forming efficiency compared with controls ($P<0.01$). Lastly, in a colon cancer xenograft model, Ad-ERβ reduced average tumor volume by 18% vs controls (P=NS) and raloxifene reduced average tumor volume significantly by 53% vs controls ($P<0.01$). Interestingly, the combination of Ad-ERβ + raloxifene brought about an even higher tumor volume reduction (89% vs controls, $P<0.001$) [66]. Possibly, the ER-independent effects of raloxifene [67] explain the raloxifene effect in the essentially ER-negative HCT116 cells, and the enhanced effect of raloxifene together with ERβ may be a consequence of its binding to and potential activation of ERβ. A follow-up study tested the therapeutic effect of combined Ad-ERβ and thermotherapy in a HCT116 colon cancer xenograft model. Thermotherapy is based on tumor cells' higher sensitivity to heat, and uses the wavelength of near-infrared region in combination with photothermal agents. The authors concluded that Ad-ERβ enhanced the therapeutic effect of thermotherapy *in vivo* [80].

6. The Molecular Mechanism of Action of ERβ in Colon Epithelial Cells

6.1. Molecular Pathways behind CRC

In order for prevention to be effective, it needs to modify tumorigenesis in the colon of patients identified as high-risk in screening. Three major pathways have been identified. One is the chromosomal instability pathway, exemplified by mutations in the *adenomatous polyposis coli (APC)* tumor suppressor gene and the *ras* (*KRAS*) gene, as well as deletions found on chromosomes 5, 17 and 18 [81]. This pathway is associated with up to 60% of sporadic adenomatous polyps and CRCs [82–84]. A second, more recently described pathway, is the serrated pathway leading to the CpG island methylator phenotype (CIMP+) [84]. This pathway is responsible for ~35% of colon cancers, the most common of which involve an activating mutation of the *BRAF* gene, inhibiting normal apoptosis of colonic epithelial cells [84]. The remainder (~5%) are thought to arise from the microsatellite instability (mutator) pathway, which involves the silencing of mismatch repair genes, including *MLH1* (*mutL homolog 1*), *MSH2* (*MutS homolog 2*), *MSH6* (*MutS homolog 6*), *PMS1*, and *PMS2* [82, 84, 85]. These major pathways are, however, difficult to modify efficiently for prevention purposes.

6.2 ERβ Regulates DNA Repair and Apoptosis through p53 Signaling

Researchers have proposed several ways in which ERβ may reduce tumorigenesis in human cells. Jin and colleagues noted an upregulation of DNA mismatch repair gene expression in human colonic epithelial cells that correlated with serum estrogen concentrations of >45 pg/mL [86]. ERβ has been shown to induce apoptosis through various mechanisms, including DNA fragmentation in COLO205 colon cancer cells [41] and increased p53 (TP53) signaling in LoVo colon cancer cells [87]. The study reporting the latter finding is important, as it demonstrated that overexpression of ERβ led to increased p53 signaling, which increased apoptosis and reduced cell proliferation [87]. p53 has also been shown to increase estradiol-induced DNA repair in nonmalignant colonocytes [88]. Martineti and colleagues demonstrated that overexpression of ERβ reduced cell proliferation in HCT8 human colon cancer cells through modulation of cell cycle regulators, an effect that included a decrease in Cyclin E (CCNE1) and an increase in p53-target gene p21 [79]. We have also found that ERβ reduces cell proliferation in HT29 and SW480 colon cancer cells through regulation of G1-phase cell cycle genes [78].

6.3. ERβ Represses Oncogenes

Using transcriptome profiling to explore the effects of ERβ in an unbiased manner, we found that the antitumorigenic effect of ERβ across different cell lines may be a combined result of cell cycle regulation; anti-inflammatory response regulation; increased DNA repair capacity and downregulation of oncogenes (PROX1, MYC, and MYB) and of the microRNAs cluster miR-17-92 [45, 89]. Using target prediction and anticorrelation between microRNA and mRNA expression, followed by focused mechanistic studies, we demonstrated that repression of miR-17 was a secondary event following ERβ's downregulatory effect on MYC and we showed that re-introduction of miR-17 can reverse the antiproliferative effects of ERβ [45]. Further, of special interest in CRC is PROX1. PROX1 is upregulated in colon cancer and is associated with a poor grade of tumor differentiation and worse outcome,

especially in women [90]. Overexpression of PROX1 has been shown to enhance colon cancer progression *in vivo*; indeed, it has been associated with transition from benign adenoma to carcinoma, and silencing of this transcription factor inhibits the growth of human colorectal tumor xenografts [91]. We have demonstrated that PROX1 is repressed by ERβ, through the regulation of microRNA miR-205, and results in a repression of metastatic potential of CRC cells [92]. This pathway was confirmed in clinical specimens and could be recapitulated in intestine-specific ERβ knockout mice [92]. In summary, a number of studies support that the molecular mechanism whereby ERβ protects against tumorigenesis by enhancing DNA repair and apoptosis while repressing oncogene expression, proliferation and metastasis (Figure 1).

7. Modulation of ERβ Expression of Activity as Therapeutic Preventive Approach

7.1. Modulation of ERβ through Naturally Occurring Phytoestrogens

The administration of dietary phytoestrogens, of which many have a higher affinity for ERβ than for ERα, has emerged as a potential source of management of adenomatous polyps [26, 93]. For example, the major active constituent of milk thistle seeds extract silymarin, silibinin, binds selectively to ERβ [94]. Silymarin upregulated ERβ mRNA and protein in the *Apc*$^{Min/+}$ mouse model, and was associated with fewer and smaller tumors [95]. In a study of Fischer 344 (F344) rats, those fed silibinin showed reduced AOM-induced aberrant crypt foci formation [96]. Similarly, F344 rats fed silibinin also had a significant reduction in aberrant crypt foci, including a notable decrease in aberrant multicrypt foci [97] and *Apc*$^{Min/+}$ mice fed silibinin also had reduced formation of polyps [98, 99]. Barone and colleagues also fed four groups of male *Apc*$^{Min/+}$ mice silymarin, the nonstarch, insoluble dietary fiber lignin, both silymarin and lignin, or a high-fat, low-fiber diet (control) [95]. All of the groups of mice which received silymarin, lignin, or both, showed reductions in the total number of polyps, the total number of polyps in the distal small intestine, and the number of mice with more than one colonic polyp. Silymarin in combination with lignin achieved the greatest reductions of polyps ($P<.001$) and also increased apoptosis in adenomatous tissue to levels similar to those found in nonadenomatous tissue (Table 2) [95]. Lignin may enhance the activity of silymarin by delaying its absorption from its preferred uptake in the duodenum to the more distal ileal segments. The potential of a blend of silymarin plus lignin given in the dietary management of colorectal adenomatous polyps was demonstrated in a randomized, double-blind, placebo-controlled clinical study [100]. Sixty subjects received dietary supplementation or placebo for 60 days. After 60 days, 63% of subjects given the combination mixture had ERβ protein levels above the median (0.82 OD), compared with only 35% of subjects given placebo ($P<.05$ for difference). Notably, increased ERβ expression showed a trend to correlate with markers of apoptosis, TUNEL and caspase 3 (CASP3), for the mixture group but not for the placebo group [100].

7.2. ERβ as therapeutic approach

The hypothesis that estrogens are protective against inflammation-associated cancers, suggests a role for estrogenic compounds in the dietary management of high-risk patients, including patients with IBD and FAP. Calabrese and coworkers [101] have demonstrated

that a mixture of phytoestrogen and fibers is able to reduce the size and the number of duodenal polyps in such a population. Similarly, Bringiotti *et al.* [102] reported a case in which the same mixture achieved reduction in size and number of small intestinal polyps in a patient affected by Lynch syndrome. In addition, using human CRC HT29 xenografts to athymic nude mice, Singh and colleagues found that silibinin suppressed tumor growth without safety concerns, decreased the proliferation index (reduced expression of PCNA and cyclin D1), slightly increased apoptosis, and strongly inhibited tumor angiogenesis [103]. These findings suggest that silibinin may have potential as an antimetastatic agent for patients with existing cancer [104].

8. Concluding remarks

Numerous experiments and studies supports that activation of ERβ reduces colorectal adenomatous polyps and modulates important pathways in CRC. Management of colorectal adenomatous polyps through diet, dietary supplements, and medical foods that are generally recognized as safe represents one potential adjunctive option. Clinical trials are required to determine the full potential clinical utility of ERβ activation in the prevention of CRC. It will also be necessary to characterize the impact of systemic effects.

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Highlights

- Estrogen exposure decreases the risk of colorectal cancer
- Experimental evidence supports activation of ERβ in prevention
- ERβ regulates DNA repair and apoptosis through p53 signaling
- ERβ repressed oncogenes and metastasis
- Phytoestrogens activate ERβ and show therapeutic efficacy

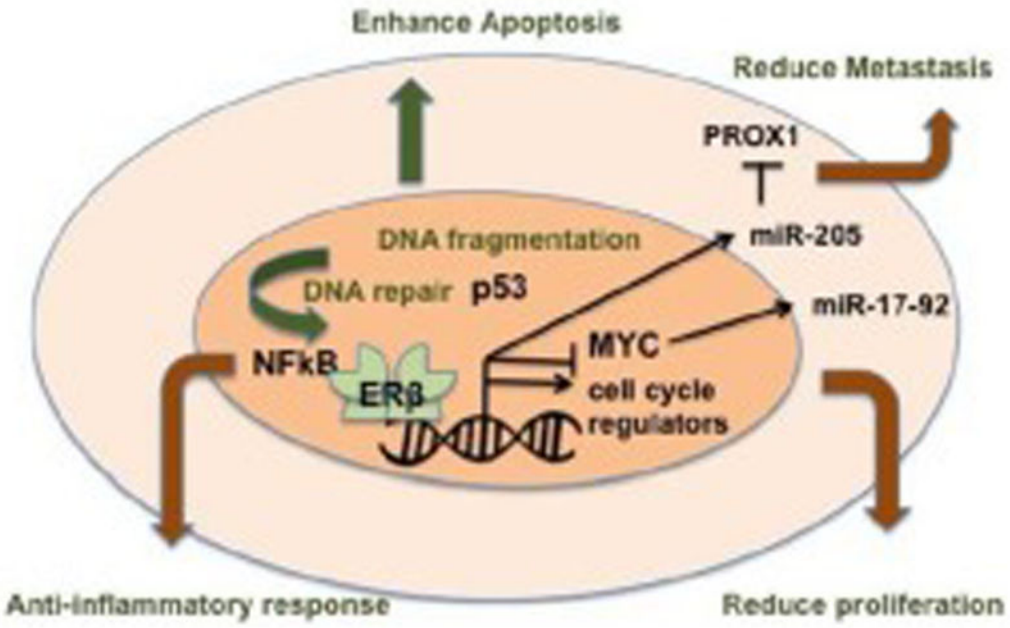

Figure 1.
Proposed molecular mechanism for ERβ-mediated anti-tumorigenic activity.

Table 1

Summary of studies investigating the impact of estrogen signaling on the intestinal epithelium and its cancers *in vivo*.

Strains	Experiment	Findings	Ref #/year
BERKO (Gustafsson lab) mice.	Colon of mice with homozygous deletion of ERβ compared to WT.	Higher proliferation, decreased apoptosis and abnormalities in tight junctions in BERKO colon epithelia.	[73]/2006
BERKO (B6.129P2-Esr2tm1Unc/J) and C57BL/6J (both Jackson Laboratory) mice.	WT and homozygous deletion of ERβ exposed to different diets followed by analysis of fecal microbiota 16S rRNA gene.	ERβ affects the microbiota environment response to changes in diet complexity	[77]/2013
	Apc tumorigenesis model		
C57B1/6J *Apc*$^{Min/+}$ mice.	Ovariectomized *Apc*$^{Min/+}$ mice treated with E2.	Estrogen depletion increased tumor formation in small intestines and colon of *Apc*$^{Min/+}$ mice, which was rescued by E2 treatment.	[58]/2001
C57B1/6J *Apc*$^{Min/+}$ (the Jackson Laboratory) mice.	*Apc*$^{min/+}$ mice treated with ERβ agonist DPN for 12 weeks.	DPN decreased the number of small intestinal polyps in male and female mice. ERβ transcript expression in colonocytes was not different between vehicle- and DPN-treated mice. DPN upregulatated TGFβ1 and TGFβ3.	[64]/2011
C57B1/6J *Apc*$^{Min/+}$ (the Jackson Laboratory) mice.	Ovariectomized *Apc*$^{Min/+}$ and WT mice treated with genistein, coumestrol and E2 for 10 weeks.	Ovariectomy increased tumor formation in *Apc*-associated small intestinal tumors. E2 and coumestrol reduced tumor number in both small intestine and in colon. Genistein had no significant effect.	[69]/2005
C57B1/6J *Apc*$^{Min/+}$ (Charles River, Italy) mice.	*Apc*$^{Min/+}$ male mice on control diet, high-fiber, and silymarin supplemented diet compared to WT.	Silymarin and insoluble lignin individually or in combination acted as selective ERβ inducers in *Apc*$^{Min/+}$ mice and reduced neoplasia and polyps through the entire male intesine. Silibinin exert chemopreventive activities against CRC through modulation of β-catenin pathway.	[95]/2010
C57B1/6J *Apc*$^{Min/+}$ (Jackson Laboratory) mice.	*Apc*$^{Min/+}$ and WT mice treated with silibinin for 6 weeks.	Silibinin at three dose levels significantly reduced the total number of polyps in small intestine of *Apc*$^{Min/+}$ mice.	[98]/2009
C57B1/6J *Apc*$^{Min/+}$ (Jackson Laboratory) mice.	*Apc*$^{Min/+}$ and WT mice treated with silibinin for 13 weeks.	Silibinin significantly reduced the total number of polyps in both small intestine and colon of *Apc*$^{Min/+}$ mice.	[99]/2010
BERKO C57B1/6J (Chambon lab) crossed with C57BL/6J–*Apc*$^{Min/+}$ mice.	Wt type and ERβ$^{-/-}$ *Apc*$^{Min/+}$ male and female mice, females ovariectomized and E2 supplemented.	ERβ decreased tumor formation in small intestines of *Apc*$^{Min/+}$ mice. TGF pathway implied.	[59]/2008
BERKO and ERKO C57BL/6 (Chambon lab) crossed with C57BL/6 *Apc*$^{Min/+}$ (The Jackson Laboratory) mice.	Wt, heterozygous deletion of ERα, homozygous and heterozygous deletion of ERβ *Apc*$^{Min/+}$ mice.	Both estrogen receptors inhibited tumorgenesis in proximal colon of *Apc*$^{Min/+}$ mice in both genders, whereas small intestinal tumors were unaffected. Homozygous ERα deletion in Apc $^{Min/+}$ genotype not viable. ER deficiency stimulated intestinal crypt expansion, disrupted intestinal stem cell microenvironment and reduced TGFB signaling.	[57]/2007 [74]/2014
ERKO (B6.129-Esr1tm1Ksk, D. Lubahn lab), and BERKO (B6.129-Esr2tm1Ksk, Taconic) crossed with C57B1/6J *Apc*$^{Min/+}$ (The Jackson Laboratory) mice.	Homozygous/heterozygous deletion of ERβ and ERα *Apc*$^{Min/+}$ mice.	Both ERα and ERβ deficiency increase tumorgenesis in colon of *Apc*$^{Min/+}$ mice. ERα deficiency increases E2 levels. Wnt signaling pathway implied, particularly through ERα.	[60]/2009

Strains	Experiment	Findings	Ref #/year
	AOM tumorigenesis model		
ERKO and WT mice	Ovariectomized ERα deficient and WT, six i.p. injections AOM +/− diets with soy, genistein, and estrone.	Soy protein protected mice from colon cancer, and estrone (E1) further reduced colon tumorigenesis independently of ERα.	[70]/2004
BERKO and WT c57BL6/J (The Jackson Laboratory) mice.	WT and ER β deficient, ovariectomized female mice treated with E2 and neoplastic lesions induced by AOM.	Estrogen treatment showed protective effects against pre-neoplastic lesions/ aberrant crypt foci (ACF) in distal colon of wt mice but not mice lacking ERβ.	[88]/2009
BERKO (B6.129P2-Esr2tm1Unc/J, Jackson laboratory) and background-WT (C57BL/6J) mice.	Homozygous deletion of ERβ and WT female mice and AOM/DSS treatment.	ERβ has anti-inflammatory and anti-tumor activity in colitis-associated colorectal cancer. ERβ deficient females produce normal serum levels of E2.	[61]/2012
BERKO and ERKO (Jackson laboratory) and background WT C57BL/6J mice	Ovariectomized WT, ERα or ERβ deficient mice treated with AOM/DSS and E2.	Estrogens promote cancer development via both ERα and ERβ.	[62]/2014
Fisher 344 rats (Harlan Breeding Laboratories)	Male rats treated with two AOM injections, and raloxifene for 40 weeks.	Raloxifene alone significantly suppressed colon adenocarcinoma formation in males.	[65]/2013
Fisher 344 rats (The Jackson Laboratory)	Induction of aberrant crypt foci through two s.c. AOM injections +/− treatment with silibinin, in male rats.	Silibinin reduced the number of aberrant crypt foci (ACF) in the colon in a dose-dependent manner.	[96]/2008
Fisher 344 rats (Charles River Laboratories)	Male rats treated with two s.c. AOM injections +/− treatment with quercetin, curcumin, rutin, silymarin and ginseng.	Silymarin, quercetin, curcumin, ginseng and rutin decreased the number of ACF in colon. All except silymarin induced apoptosis.	[97]/2005
	Xenograft models		
SCID mice (Taconic)	S.c. xenografts with SW480 cell line +/− ERβ.	ERβ reduces tumor size and regulates G1-phase cell cycle genes.	[78]/2009
Athymic BALB/c nu/nu mice (National Cancer Institute)	S.c xenograft with HT29 cell line +/− silibinin treatment.	Silibin has an antiproliferative effect in colorectal cancer xenografts. Downregulates ERk1/2 and AKT signaling pathways.	[103]/2008

Table 2

The effect of diet supplemented with silymarin and lignin on number of polyps and high-grade dysplasia in the intestinal tract of male *Apc*$^{Min/+}$ mice.

Group	Mice, n	Polyps in entire intestinal tract, n[a]	Polyps in DSI, n[b]	Mice with >1 colonic polyp, n (%)	*p*[c]	Mice with high-grade dysplasia, n (%)	*p*[d]
Control	15	56.5 ± 21.0	23.5 ± 10.5	13 (87)[e]	—	11 (73)	
SIL	15	40.9 ± 16.3[f]	17.6 ± 7.9	8 (53)	<0.002	7 (47)	<0.025
LIG	9	46.8 ± 14.0	16.4 ± 6.9	6 (60)	<0.025	3 (30)	<0.001
SIL + LIG	6	34.0 ± 10.7[f]	11.5 ± 5.1[f]	1 (17)	<0.001	2 (33)	<0.01

The number of polyps represents the mean ± SD obtained using all mice in each group.

[a] One-way analysis of variance demonstrated a significant difference among groups (*P*=0.029).

[b] One-way analysis of variance demonstrated a significant difference among groups (*P*=0.028).

[c] Significantly reduced vs value for control group (13 [87%]) by multiple comparisons for independent proportions.

[d] Significantly reduced vs control group by multiple comparisons for independent proportions.

[e] Among these 13 animals, only 3 showed high-dysplastic colonic polyps, whereas no dysplastic colonic polyp was observed in dietary-managed groups.

[f] Significantly reduced vs control group by Holm-Sidak method.

DSI= distal small intestine LIG=lignin; SIL=silymarin.