Estrogen Receptors $\alpha$ and $\beta$ Are Inhibitory Modifiers of $Apc$-Dependent Tumorigenesis in the Proximal Colon of Min/+ Mice

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Abstract

Estrogen replacement therapy in postmenopausal women is associated with a reduction in colorectal cancer risk, potentially via interactions between 17$\beta$estradiol ($E_2$) and the estrogen receptors (ER) $\alpha$ and $\beta$. To study the role of $E_2$ in intestinal tumor inhibition, we separately crossed C57BL/6j-Min/+ (Min/+; $E_2$-sufficient) and C57BL/6j-Min/+ (Min/+; $E_2$-deficient) mice with $E_2$ to generate $ER$-deficient Min/+ progeny. We found an increased incidence of visible colon tumors and dysplastic microadenomas in $ER$-deficient Min/+ relative to $ER^{+/+}$ Min/+ controls. Small intestinal tumor numbers were unaffected. Invasive carcinomas were found only in $ER^{+/+}$ Min/+ mice, suggesting that ER$\alpha$ plays additional non–cell autonomous roles that limit tumor progression. Histologic analyses of $ER$-deficient Min/+ colons, as well as colons from ovariectomized Min/+ mice (OvxMin/+), and $E_2$-treated OvxMin/+ mice (OvxMin/+ + $E_2$), revealed significant differences in crypt architecture, enterocyte proliferation, and goblet cell differentiation relative to Min/+ and $ER^{+/+}$ (wild-type) controls. The expression of ER$\alpha$ and ER$\beta$ was regionally compartmentalized along the colonic crypt axis, suggesting functional antagonism. Our results indicate that ER$\alpha$ and ER$\beta$ are inhibitory modifiers of $Apc$-dependent colon tumorigenesis. As a result, loss of $E_2$ and ER signaling in postmenopausal women may contribute to colorectal cancer development.

Introduction

Signaling by 17$\beta$-estradiol ($E_2$) results from the binding of this steroid hormone to two nuclear receptors, estrogen receptor (ER) $\alpha$ or $\beta$ (1). Little is currently known about the role of $E_2$ signaling in the colon, but circumstantial evidence suggests a causal association between the loss of $E_2$ in women after menopause and colorectal cancer (2). Annual age-adjusted colorectal cancer incidence rates in the United States are higher for men than for women (3), and there are gender differences regarding cancer location and type within the colorectum. Women have an excess of right-sided cancers at all ages relative to men (4). Colon cancers in this location more commonly show high levels of methylation at CpG islands (CIMP+), a modification that silences tumor-suppressor genes (5). Methylation-associated silencing is responsible for defects in DNA repair capability due to loss of MLH-1, a condition that accelerates tumor progression. Promoter methylation also disrupts the function of the gene encoding ER$\alpha$ (5, 6). Finally, data from prospective randomized trials showed that hormone replacement therapy reduced the risk of colorectal cancer in postmenopausal women by 30% to 40% (7). Hormone replacement therapy also conferred protection against the incidence and size of adenomas, the colorectal cancer precursor lesions. Taken together, these data indicate that ER-mediated signaling plays a role in colorectal cancer prevention.

ER$\alpha$ and ER$\beta$ affect growth and differentiation by regulating gene transcription. These receptors share conserved DNA and ligand-binding domains, and both activate and repress target gene expression when homodimerized or heterodimerized and bound to the estrogen-response element, their cognate promoter site (1). Estrogen receptors are widely distributed throughout the body, and tissues that simultaneously express both ER$\alpha$ and ER$\beta$ exhibit cell type–specific patterns of expression (8). For instance, only ER$\alpha$ enhances the transactivation of other transcription factors that are associated with cell proliferation, such as activator protein-1 and Sp1 (9). ER$\beta$ negatively regulates the $E_2$-dependent activities of ER$\alpha$ that stimulate cell growth. When coexpressed with ER$\alpha$, ER$\beta$ caused a concentration-dependent reduction in ER$\alpha$-mediated transcription, suggesting that ER$\beta$ acts as a dominant regulator of ER$\alpha$ signaling (10, 11). ER$\beta$ may inhibit colorectal carcinogenesis because its expression suppressed the growth of colon cancer cells and was frequently lost in colon tumors (12, 13).

The C57BL/6j-Min/+ mouse carries a germ line mutation in the $Apc$ tumor-suppressor gene and is a model for studying early events in intestinal tumorigenesis and chemoprevention. Min/+ mice develop dozens of adenomas in the small intestine and have an average life span of 16 to 24 weeks. Unlike humans carrying $Apc$ mutations, macroscopic tumors in the colon of Min/+ mice are rare. In previous studies, we showed that $E_2$ suppresses intestinal tumor formation in the Min/+ model. We found that $E_2$ loss produced by ovariectomy of 5-week-old Min/+ mice caused a 77% increase in the small intestinal tumor burden by 3 months of age and that $E_2$ replacement inhibited tumors to the basal number found in age-matched intact Min/+ females (14). We also reported that dietary treatment of ovariectomized Min/+ (OvxMin/+; $E_2$-treated) mice with coumestrol, a phytoestrogen and selective ER$\beta$ ligand, inhibited tumorigenesis and increased ER$\beta$ expression in the intestinal mucosa (15).

Based on these human and animal data, we hypothesized that $E_2$-activated signaling via either ER$\alpha$ or ER$\beta$ or both modifies intestinal tumor formation. To test this, we did separate crosses of heterozygous $Erx$ and $Er\beta$ mice in a homogeneous genetic background (BL/6) with the Min/+ mouse to produce $ER$-deficient Min/+ strains. We compared the characteristics of these animals with $Er^{+/+}$Apc$^{+/+}$ (wild-type [WT]), Min/+; OvxMin/+; and $E_2$-treated OvxMin/+ (OvxMin/+ + $E_2$) mice. Our results show that $E_2$ and its receptors are inhibitory modifiers that cooperate to regulate colonic tissue homeostasis.
Materials and Methods

Materials. Erα−/−C57BL/6-Min/+ mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Antibodies directed against β-catenin (clone 14) were from BD Bioscience (San Diego, CA); ERα (PA1-310B) was from Affinity Bioreagents (Golden, CO); ERα (MC-20) was from Santa Cruz Biotechnology (Santa Cruz, CA); and Ki-67 (clone TEC-3) was from DAKO (Carpinteria, CA). The DNeasy Tissue Kit was from Qiagen (Valencia, CA). The AmpliTaq Gold kit for PCR was from Applied Biosystems (Branchburg, NJ).

ER-deficient Min/+ crosses, tissue collection, and genotyping. C57BL/6 Erα−/− male and female and Erβ−/− male and Erβ−/− female mice pairs were generously provided by Dr. Pierre Chamoun (Ilkirch-Cedex, France) and were bred to produce heterozygous progeny. Both male and female Erα−/− mice are infertile, and Erβ−/− females have significantly impaired fertility (16). A standard maintenance diet was provided ad libitum, and mice were examined daily. Genotyping was done using DNA extracted from tail biopsies of 1- to 2-week-old pups, and new breeding hares of 4- to 5-week-old mice were established to expand the ER-deficient population before initiating separate crosses of Erα−/− and Erβ−/− mice with Min/+ mice. Offspring from these crosses yielded Erα−/−Min/+ and Erβ−/−Min/+ and, final breeding hares were separately created to produce Erα−/−Min+/+, Erβ−/−Min+/+, and Erα−/−Min−/− mice. All mice were sacrificed at ages 3 to 4 months. The entire small intestine and colon of each animal were excised and opened longitudinally. An investigator blinded to the genotype of each animal counted all visible tumors. Primers of each animal were excised and opened longitudinally. An investigator prepared specimens and did immunohistochemistry by standard procedures.

Statistical analyses. An unpaired t test evaluated tumor multiplicity in both small intestine and colon of ER-deficient mice relative to Min/+ controls. A Fisher’s exact test was used to distinguish colon tumor incidence in ER-deficient mice relative to Min/+ controls, and also to compare the incidence of dysplastic aberrant crypt foci in OvxMin/+ and OvxMin/+ + E2 relative to Min/+ controls. For the proliferative index, a nuclear Ki-67 staining was recorded as the total number of positive cells in 10 to 15 distinct, well-oriented crypts per strain or treatment (three separate animals of each strain). The proliferative index was expressed as the ratio of positively staining nuclei to the total number of cells present per crypt. An unpaired t test compared results relative to Min/+ controls. A similar approach was used to analyze the number of differentiated goblet cells in specimens.

Results

ER-deficient Min/+ mice have increased colon tumors and abnormal mucosal histology. ER-deficient (Erβ+/−, Erβ−/−, and Erα−/−) Min/+ mice were generated. None of the viable pups resulting from Erα−/− × Erα−/− Min/+ pairings were of Erα−/−Apc−/− genotype. Animals were sacrificed at ages 3 to 4 months, and the entire intestinal tract of each was harvested and examined. At this age, Min/+ mice typically contain 20 to 60 visible tumors in the small intestine, and approximately 1 animal in 20 has a visible tumor in the colon. Tumor numbers in ER-deficient Min/+ mice were not affected by gender (data not shown). The mean number of small intestinal tumors in the ER-deficient Min/+ crosses was similar to Er−/− Min/+ littermate controls (Fig. 1A, top). However, both ERα and ERβ deficiency resulted in increased tumor formation in the cecum and proximal one third of the colon. Because colon tumors were still relatively few in all animals, this effect was most clearly seen when the number of animals with colonic tumors were compared with those without tumors for each genotype (Fig. 1A, bottom). Colonic adenomas were of both sessile and pedunculated forms. This increase in colon tumors was not previously noted in either ERα- or ERβ-deficient Apc−/− mice, and E2 loss minimally affected the number of colon tumors in OvxMin/+ mice (16, 18).

Next, we examined differences in colonic mucosa resulting from either ER deficiency or E2 loss by ovariectomy. First, the histology
of proximal colon sections from WT, Min/+, OvxMin/+ , OvxMin/+ +E2, Erα−/− Min/+ , Erβ−/− Min/+ , and Erβ−/− Min/+ mice was evaluated by H&E staining (Fig. 1B). Normal crypt morphology was found in the proximal colon of WT, Min/+ , and OvxMin/+ +E2, and Erβ−/− deficient Min/+ mice. In the colon of OvxMin/+ mice, however, we observed significant changes in crypt morphology, with reduced crypt length and distortion of the circular shape of surface epithelial cuffs. Architectural changes were also present in the submucosa, including thickening of the muscularis mucosae and enrichment of stromal components, suggesting an increased inflammatory cell infiltrate. Similar morphologic changes were observed in Erα−/− Min/+ mice, but were less consistent. The presence of mononuclear cell infiltrates was also a common feature in the colons of Erβ−/− Min/+ mice.

Compartmentalized E2 signaling by ERα and ERβ modulates enterocyte growth and differentiation within colon crypts. To learn whether changes in the crypt dimensions reflected the relative proliferative index, we did Ki-67 immunohistochemistry using sections from the proximal colons of study animals (Fig. 2A, right). The number of proliferating cells in the colon of Min/+ mice was similar to that of WT (Fig. 2A, left). Consistent with the observed loss of crypt length, ovariectomy of female Min/+ mice resulted in a 20% reduction in the number of positively stained nuclei relative to colon from intact female and male Min/+ mice. E2 replacement of OvxMin/+ mice reversed this effect, increasing colonocyte proliferation 2-fold. Taken together, these results indicate that signaling via ERα produces trophic effects on the proximal colonic mucosa. We also examined the effect of ER deficiency on proliferative index. Consistent with the tumor phenotype, ER deficiency was also associated with increased enterocyte proliferation (44–87%) in the colon relative to that of Erα−/− Min/+ mice.

The majority of differentiated cells in the colon are mucin-producing goblet cells, and expression of acidic mucins is detected by Alcian blue staining. We used this technique to assess differentiation in the proximal colons of the study mice and found relatively fewer mature goblet cells in OvxMin/+ +E2, Erα−/− Min/+ , and Erβ−/− deficient Min/+ mice than in the normal colons of WT, Min/+ , and OvxMin/+ mice (Fig. 2B, right). Morphologically different, pre-goblet cells were especially prevalent in OvxMin/+ +E2 and Erβ−/− deficient Min/+ tissues. The change in the ratio for all ER-deficient Min/+ strains and OvxMin/+ +E2 was significantly reduced (24–42%) relative to Min/+ controls (Fig. 2B, left). In contrast, the goblet cell ratio for OvxMin/+ mice was increased relative to Min/+ mice. Thus, goblet cell maturation was inversely correlated with colonocyte proliferative index.

Because proliferation takes place predominantly in the cells at the base of crypts and mature goblet cells are mostly found near the surface mucosa, we asked whether the expression of ERα and ERβ differed by location within the colonic crypt unit. First, we did immunohistochemistry to examine the cell of origin and location of ERα in the colons of the study animals (Fig. 3, right). In all of the specimens, strong nuclear ERα expression was present in enterocytes extending from the base of crypts to approximately three fourths of their lengths. Confirming our localization of ERα in intestinal crypts, expression of this receptor was reported in progenitor cells microdissected from the mouse intestine (19). By immunohistochemistry, ERα expression was not visibly diminished in the colon of Erα−/− Min/+ mice. Next, we examined the cell of origin and location of ERβ in the colons of the study animals (Fig. 3, left). Positive staining

![Figure 2](https://example.com/figure2.png)
for ERβ was found in the cytoplasm and nucleus of enterocytes located in the upper regions of colonic crypts and at the surface mucosa. This staining pattern is consistent with the results of others using the same antibody (PA1-310B) on human colon sections (13). ERβ staining was scant and cytoplasmic in enterocytes at the base of colonic crypts from Erα+/- Min/+ mice and absent, as expected, in the Erβ-/- Min/+ animals (negative control). The observed difference in receptor location indicates that ERα and ERβ activities are regionally compartmentalized, a result compatible with their functional antagonism in enterocytes undergoing growth arrest. Taken together, these data suggest that the proper balancing of E2-dependent effects on epithelial cell proliferation and differentiation in the colon is achieved by separate and nonredundant activities of ERα and ERβ.

Characterization of colon tumors from Erα+/- Min/+ and Erβ+/- Min/+ mice. Invasive tumors are rarely found in Min/+ mice, and when observed are virtually always found in animals older than 3 to 4 months. Microscopic examination of intestinal tumors from Erα+/- Min/+ and Erβ+/- Min/+ mice revealed a significant difference in tumor aggressiveness in the former. Erα+/- Min/+ mice developed from one to three invasive carcinomas each that were located in both the small intestine and colon (Fig. 4A). Invasive carcinomas were detected in seven of seven (100%) Erα+/- Min/+ mice, but were not found in any of the other animals examined. To verify the Erα dependence of this phenotype, we reviewed H&E-stained sections of small intestinal tumors (three to five per mouse) from our archival collection of 19 OvxMin/+ mice (14, 15). No invasive tumors were found in these animals, suggesting that Erα deficiency accelerated tumor progression independent of E2.

Noninvasive adenomas from Erα+/- Min/+ and Erβ+/- Min/+ mice were histologically similar (Fig. 4B). Typical of Min/+ adenomas, immunostaining showed up-regulated nuclear and cytoplasmic β-catenin. The number of positively stained nuclei by Ki-67 immunohistochemistry in these tumors was not significantly different (data not shown). Nuclear expression of ERα was evident in both Erα+/- and Erβ+/- colon tumors. Maintenance of nuclear ERα expression in ApC-null tumors of the ER-deficient Min/+ mice supports the view that Erα is a modifier gene and not a tumor suppressor. Finally, immunohistochemistry for ERβ in Erα+/- Min/+ colon tumors revealed low, cytoplasmic expression of this protein. Again, only background staining was apparent in Erβ-/- Min/+ tumor cells.

Deficiency of either ERα or ERβ in Min/+ mice stimulates tumor progression without affecting tumor initiation. Colon tumor growth in Min/+ mice requires several genetic and
The similar incidence of dysplastic single crypts in the proximal colon of ER-deficient and E2-replete Min/+ mice indicates that ER loss enhances precursor lesion progression to microadenomas and visible tumors. In the table (left top), the incidence of dysplastic single crypts in the proximal colon of the E2-replete (Min+ and OvxMin+/++E2) mice was compared with OvxMin/. Immunohistochemistry (IHC) for β-catenin was used for aberrant crypt foci detection as described (21). One-centimeter proximal colonic specimens (three serial 4-μm sections) from Min+/+ mice of the different treatment groups were also used. No adenomas were present in any of the specimens tested. Fisher’s exact test was used to compare results from each treatment group. As previously reported (14, 15), ovariectomy of Min/+ mice increased the number of small intestinal tumors but had no effect on the typically low number of colon tumors in these animals; E2 replacement of OvxMin/+ mice reduced small intestinal tumors to the baseline number of Min+. Examples of dysplastic aberrant crypt foci (ACF) in colons of Erα−/− Min/+ mice revealed by immunohistochemistry for β-catenin (right top). Similar lesions were found in Erβ-deficient Min/+ (data not shown). A two-step model for dysplastic microadenoma formation in Min/+ mice depicts the proposed role of E2 signaling (bottom).

**Figure 5.** The similar incidence of dysplastic single crypts in the proximal colon of ER-deficient and E2-replete Min/+ mice indicates that ER loss enhances precursor lesion progression to microadenomas and visible tumors. In the table (left top), the incidence of dysplastic single crypt in the proximal colon of the E2-replete (Min+ and OvxMin+/++E2) mice was compared with OvxMin/+.

Dysplastic crypt formation is the first step in this process. This lesion occurs spontaneously in the colons of Min/+ mice, arises clonally from a single stem cell that has sustained Apc+ loss of heterozygosity, and each exhibits β-catenin overexpression (21). Dysplastic crypts are potential but not obligate tumor precursors; in the colon of Min/+ mice, they typically do not progress to become adenomas. We anticipated that dysplastic crypts would be more prevalent in the proximal colon of Erα and Erβ mutant Min/+ strains if these receptors played a role in adenoma initiation.

To test this, we stained grossly normal-appearing colon sections using an antibody for β-catenin to identify microscopic changes associated with adenoma formation. Immunohistochemistry of proximal colonic sections from Erα−/− Min/+ mice revealed dysplastic aberrant crypt foci in the form of single crypts and microadenomas (Fig. 5, top right). The dysplastic crypt shown on the left is an example of a lesion undergoing crypt fission. The dysplastic microadenoma on the right has already undergone several crypt divisions, and the newly budded crypts remain tethered together beneath the surface mucosa. The incidence and appearance of these lesions were similar in Erβ−/− and Erβ−/− Min/+ colons (Fig. 5). Next, we used β-catenin immunohistochemistry to identify dysplastic aberrant crypt foci in our archival proximal colon specimens from 3-month-old female Min−/−, OvxMin−/−, and OvxMin−/− +E2 mice (Fig. 5, left). In Min−/− colons, 14 dysplastic crypts were found in 10 out of a total of 37 mice. All were single crypts that had not undergone stem cell duplication and further crypt fission. Similar results were obtained using OvxMin−/− +E2 colons in which eight single dysplastic crypts and one microadenoma were found in 5 of 20 mice. Surprisingly, no dysplastic lesions were discovered in any of the 28 OvxMin+/+ colon specimens. The incidence of single dysplastic crypts in the ER-deficient Min/+ mice was not significantly different from that of Min/+ mice, but that of microadenomas and visible tumors was significantly greater in the compound mutant animals. As illustrated (Fig. 5, bottom), these results suggest that dysplastic crypts may be products of normal or increased colonocyte proliferation in the Min/+ mouse and that E2 is an important stimulus of colonocyte growth. In addition, our results suggest that Erα and Erβ deficiencies in a heterozygous Apc mutant background promote aberrant crypt fission and microadenoma progression in the proximal colon.

**Discussion**

Human colorectal cancer is a heterogeneous disease, and multiple genetic and environmental factors contribute to its development. The Min/+ mouse is a useful colorectal cancer model, as Apc mutation plays an initiating role in the great majority of colorectal cancers. For reasons not understood, intestinal tumors in Min/+ mice occur primarily in the small intestine. The combination of Apc and Er loss, however, produced a phenotype more consistent with that of human colorectal cancer, with development of adenomas and invasive carcinomas in the colon. This condition also occurs in human colorectal cancer, as loss of ER expression is one of the tumor-associated consequences of methylation-associated gene silencing (6). This mouse model, therefore, provides important insights into the role of ER signaling in normal intestinal function and in colorectal carcinogenesis.

Colonic epithelium is a dynamic microenvironment. Progenitor cells in the crypts undergo cell division and give rise to epithelial cells that differentiate into various lineages as they migrate to the crypt lumen. The mucosal architecture is maintained by adhesive and repulsive interactions among enterocytes, signaling to and from stromal elements, and properly timed shedding of senescent or apoptotic cells at the crypt lumen. The studies reported here show that signaling via ERα and ERβ plays important roles in these processes. ERβ is thought to functionally antagonize the growth-promoting activities of ERα (9–11), and our results are consistent with this view. The compartmentalization of ERα and ERβ expression within the crypt axis (Fig. 3) suggests that a negative regulatory function of ERβ is directed at ERα as colonic enterocytes differentiate and undergo growth arrest. The increasing expression of ERβ in enterocytes ascending crypts can be expected to cause competition for ligand, as well as coactivators. Also, the repertoire of genes subject to the regulatory control of these receptors may change due to ERα-ERβ heterodimerization, in contrast to the situation in proliferating cells where ERα abundance may differentially favor its homodimerization. We did not measure serum E2 levels in the animals, as others have shown that serum E2 levels were normal in the Erα−/−, Erβ−/−, and Erβ−/− BL/6 mice used in this study (22).

Previously, we reported that E2 loss by ovariectomy increased small intestinal tumor formation in Min/+ mice (14). Here, we show that the effect of ovariectomy on the colon is also significant, with a 20% reduction in proliferative index and architectural changes, suggesting loss of a mucosal trophic factor. Ovariectomy did not increase tumor formation in the colons of Min/+ mice and in fact
had the opposite effect, with a reduction in microscopically dysplastic aberrant crypt foci compared with intact or E2-replaced Min/+ mice (Fig. 5). Interestingly, loss of ERα function in the Erα+/-/Min/+ mouse produced histologic changes similar to that of ovariectomy; however, in this case, the architectural disorder was accompanied by increased proliferative index and loss of goblet cell differentiation. Consistent with these changes, Erα+/-/Min/+ mice developed a spectrum of neoplastic colonic lesions, including dysplastic aberrant crypt foci, visible adenomas, and invasive adenocarcinomas. Taken together, these observations suggest that loss of signaling via ERα is a major contributor to colorectal cancer development.

We observed stimulation of colonic crypt fission in ERα-deficient Min/+ strains, suggesting that ER activities inhibit interactions between stromal and epithelial elements that are necessary for adenoma formation. One possibility is that E2-activated ERα regulates signaling by bone morphogenetic proteins (BMP2 and BMP4) in the colon. BMP4 is secreted by the stromal mesenchyme and negatively regulates stem cell duplication and intestinal crypt fission (23). Binding of BMP4 to receptors on intestinal stem cells initiates signaling via transforming growth factor β, leading to phosphorylation of Smad1 and Smad5 and inhibition of Wnt signaling. This mechanism may involve E2 signaling because nuclear interactions between the BMP4 effector, Smad1, and ERα were inducible by BMP4, and ERα-dependent induction of BMP4 transcription occurred in response to the selective estrogen response modulator drug, raloxifene (24, 25). The net effect of these ERα-mediated activities would be intestinal tumor suppression.

BMP2-dependent mechanisms may also mediate tumor suppression in the colon. This factor enforces the growth arrest of mature enterocytes when secreted by intercrypt fibroblasts or enterocytes via an autocrine route (26, 27). BMP2 expression is strongly dependent on E2-activated ER, and induction of BMP2 transcription was 3-fold greater for ERα relative to ERβ (28). We showed that ERβ deficiency in Min/+ mice increased proliferation and decreased differentiation (Figs. 2 and 3). Thus, defective growth arrest may have occurred in our compound mutant mice due to reduced BMP2 expression at the surface mucosa. Similarly, BMP6 is expressed in the colon and its gene promoter is E2 dependent (29).

ER deficiency may also promote intestinal tumor formation due to loss of Smad4 activity. Loss of Smad4 in Apc mutant mice is sufficient for carcinoma formation (30). Regulatory cross-talk between ER and Smad4 occurs at the transcriptional level (31), and Smad4 transmits BMP-elicited signals in the nucleus. Finally, E2-activated, ERα-dependent colorectal cancer may also promote the inhibition of Snail transcription factors. These transcriptional repressors are regulators of the epithelial-mesenchymal transition and are overexpressed in human colorectal cancer (32). Both MTA3 and LIV1 are ERα-dependent Snail inhibitors and implicated in human cancer (33, 34).

In conclusion, we found that the functions of both ERα and ERβ inhibited Apc-dependent tumor formation in the proximal colon of Min/+ mice. These functions involved coordination of stromal-epithelial interactions to promote maintenance of normal colonic crypt architecture. This work is consistent with results of human clinical trials and cancer epidemiology, indicating that hormone replacement therapy is beneficial for preventing postmenopausal colorectal cancer. In addition, the model presented here provides a framework for further investigating the downstream signaling mechanisms responsible for these antitumor effects.

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