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REVIEW ARTICLE

A novel role for estrogen-induced signaling in the colorectal cancer gender bias

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Abstract

Colorectal cancer (CRC) is a malignancy whose incidence is increasing globally, and there is a gender difference in the increasing risk. Evidence from hormone replacement therapy studies points to a role for circulating estrogens in suppressing the development of CRC. Estrogen receptor-β has been identified as a tumor suppressor, but other actions of estrogen may also contribute to the difference in CRC incidence between men and women. The KCNQ1/KCNE3 potassium channel is regulated by estrogen in order to modulate chloride secretion during the menstrual cycle; the effect of estrogen on the colon is to promote fluid conservation during the implantation window. KCNQ1 is also a tumor suppressor in CRC, and its sustained expression has been linked to suppression of the Wnt/β-catenin signaling pathway that contributes to CRC tumor progression. KCNQ1 regulation may represent a link between the normal physiological actions of estrogen in the colon and the hormone's apparent tumor-suppressive effects in CRC development.

Keywords Beta-catenin · Colorectal cancer · Estrogen · KCNQ1 · Wnt

Introduction

Colorectal carcinoma (CRC) is becoming an increasingly significant cause of morbidity and mortality in an aging global population. With 1.4 million new cases diagnosed, CRC was the third most common cancer globally in 2012 and the incidence is expected to reach 2.2 million by 2030 [1]. CRC currently accounts for 8% of all cancer diagnoses worldwide and has overtaken infection-initiated malignancy to become the second most common cause of cancer in women [1]. The incidence and survival patterns for CRC vary markedly across the world. The highest incidence is seen in North America, Western Europe, Japan, and Australia while those countries with the worst overall survival are located in Eastern Europe and the former Soviet Union [1]. Multiple factors contribute to the development of CRC including predisposing genetic factors, environmental factors, and lifestyle. Interestingly, the identified risk factors do not make equivalent contributions to CRC development in men and women. For example, a high body mass index (BMI) increases the risk of CRC in men but this link is not consistent for women of different ages [14]. In a study to test the relationship between BMI and the risk of CRC in women, a strong association with CRC development was identified in premenopausal women but not in postmenopausal women [14].

A number of studies have suggested that CRC occurs at a higher frequency in men than in women. A total of 746,000 CRC cases were reported among men in 2012, while for women, a total of 614,000 cases were reported in the same year. Factors such as obesity and smoking undoubtedly contribute to this difference but a protective role for estrogen has also been proposed in the reduced CRC rates for women [22]. This raises the question of whether hormonal status has a direct impact upon incidence or whether hormone abundance influences the contribution of other established risk factors. A number of studies have investigated the gender difference in CRC incidence in the context of different lifestyles and different cultural backgrounds. Population-based data collected in the 1970s found that the age-standardized incidence rates (ASIR) for CRC were similar among men and women [26].

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However, within the past few decades, the incidence of CRC in men has increased and exceeded that seen for women, especially in high incidence populations such as in New Zealand, the USA, Canada, Australia, and the UK. A similar trend is now being observed in traditionally low-risk populations that are developing a rising incidence, such as Hong Kong, Japan, and Singapore. This trend is much less apparent in low incidence populations such as in India, Chile, and Thailand [26]. The importance of the environment and lifestyle has been confirmed in migrant studies, where it was revealed that the incidence of CRC in men rises more rapidly than that in women when they emigrate from low to high incidence areas [26]. Different studies have come up with divergent conclusions for variations in CRC survival, since multiple additional factors may contribute such as comorbidities and the quality of available medical care [26]. Gender does contribute to CRC risk, but this is modulated by lifestyle and environmental factors.

Effects of hormone replacement therapy on CRC

Some of the strongest evidence pointing to a role for steroid hormones, particularly estrogen, in inhibiting the development of CRC have come from retrospective studies of populations that have received hormone replacement therapy (HRT). HRT is usually prescribed to menopausal women to control postmenopausal symptoms or to prevent hormone deficiency-related diseases, such as osteoporosis [6]. Although HRT is associated with increasing the risk of cancers of the reproductive tissues such as the endometrium, breast, and ovary, evidence suggests that HRT may be beneficial in reducing the risk of CRC [9]. The influence of HRT on CRC development may be dependent on several factors such as the type of hormone, type of regimen prescribed, dose, duration, and route of administration. A retrospective meta-analysis study of 30 papers with data from more than one million women who were over 50 years of age found that estrogen-only HRT and estrogen combination HRT were associated with reduced risk of CRC, and that transdermal administration of estrogen was more effective than injection [18]. A second study found that HRT users had a lower risk of CRC than never users, but this study could not find a difference in risk of developing CRC based on current or past HRT prescriptions, the number of prescriptions, the duration of use, and type of HRT used [9]. The analyses showed that the risk of CRC was reduced for both estrogen and progestagen users compared to the never users, with little difference of risk reductions between these two types of HRT [9]. The inverse relationship between the risk of CRC and the use of HRT was also indicated by a population-based case-control study done on CRC patients diagnosed between 1998 and 2006, where there was a reduced relative risk of CRC and odds ratio of 0.67. But here, the combination of estrogen and progesterone in the form of pills showed a marked risk reduction compared to estrogen-only pills [6]. The preferred route of administration is orally, since estrogen would be converted and metabolized in the liver to its conjugated form, which would then enter the circulation and bile system. The conjugates are hydrolyzed in the intestine to active, reabsorbable forms, whereas the conjugated metabolites are excreted through the biliary system [6]. An alternative route of administration is via a transdermal patch, but estrogen administered transdermally is absorbed directly into the systemic circulation, avoiding the first pass effects, which makes it less effective in HRT but seems to render it more effective in CRC protection [18]. Studies such as these have added to the ongoing debate on whether HRT provides more benefits than harm, and so it is important to weigh up the potential risks. It is possible that hormone-based chemoprevention could make an impact on clinical practice in the future [12]. Treatment could be personalized for different individuals in terms of the type of HRT used, duration, and route of administration, but first it is important to understand the mechanisms that underpin the protective effects of estrogen in CRC.

Estrogen receptors

Estrogen is generally linked to carcinogenesis in reproductive tissues; however, estrogen action is also linked to malignancy in nonreproductive tissues such as the lung and the gastrointestinal system [3]. The effects of estrogen are mediated by estrogen receptors (ERs), ERα and ERβ [30]. ERs are nuclear receptors that mediate changes in ligand-dependent gene expression. ERs bound to estrogen translocate to the nucleus and to bind to specific DNA sequences in the promoter regions of target genes called estrogen response elements (EREs). ERs may recruit co-activators or coregulators to promoter sites, and so upregulate or downregulate the transcription of specific genes. ERs are also able to regulate genes that do not contain EREs, for example, by interacting with other transcription factors such as stimulating protein 1 (Sp1), activator protein-1 (AP1), and nuclear factor-κB (NFκB) [3]. ERs are also able to interact with receptor tyrosine kinases, scaffolding proteins, and other intracellular signaling proteins to activate a wide range of cytoplasmic signaling pathways [13]. Colonic epithelium expresses both ERα and ERβ in cells at the various stages of differentiation and the two receptors are linked to very different actions [22]. In the colonic crypts, ERα is expressed at the base and ERβ is expressed at the midsection and luminal surface [3]. ERα supports proliferation through differential expression of pro- and anti-apoptotic proteins and also cyclin D1 to promote cell cycle transition [3]. ERβ functions as a dominant regulator when both receptors are co-expressed and promotes apoptotic and antiproliferative effects

in more differentiated cells. ERβ is able to produce stand-alone apoptotic effects by activating pro-apoptotic signaling through the p38/MAPK (mitogen-activated protein kinase) pathway.

Links between estrogen receptor expression and CRC

ERβ has been identified as a tumor suppressor in CRC and its expression is selectively lost by methylation-dependent gene silencing during tumor progression [22]. It is postulated that estrogen acts via ERβ-modulated transcription events to stimulate pro-apoptotic signaling, regulate mismatch repair proteins, and also modulate the antitumor immune response [3]. A study described experiments done by crossing ERβ−/− mice with ApcMin/+ C57BL/6J mice to obtain ERβ−/− ApcMin/+ mice that showed increase number and size of polyps and tumors that were significantly larger than those in control ApcMin/+ mice [8]. This suggested that the loss of ERβ alone may not be enough to promote carcinogenesis, but instead it would confer a selective advantage by eliminating protective mechanisms against oxidative stress. Another experiment done with SW480 CRC cells with ectopic expression of ERβ resulted in inhibition of proliferation and cell cycle arrest in G1 phase [10]. This further indicated that ERβ plays a role in CRC cell apoptosis. Male F344 rats treated with raloxifene had suppressed colon adenocarcinoma formation while female ApcMin/+ mice showed suppressed small intestinal tumor multiplicity and size, further confirming the role of estrogen in protection against CRC [8, 30]. Failure of mismatch repair protein to repair damaged DNA results in microsatellite instability (MSI) tumor. Women are more likely than men to have a MSI tumor at an older age [12]. This is in part due to the estrogen ability to induce expression of mismatch repair proteins [27]. ERβ modulates the immune system by downregulating interleukin-6 (IL-6) and activating dendritic cells. Analysis of SW480 cells by RT-PCR showed that IL-6 was downregulated by ERβ and confirms its role in cancer-induced inflammation [5]. A study was conducted to evaluate ERα and ERβ expression in long-lasting pancolitis through each grade of dysplasia to carcinoma [23]. The ERβ labelling index (LI) showed significant reduction in carcinoma-associated colitis (CAC) compared to both controls and ulcerative colitis (UC), while ERα showed a marked increase in high-grade dysplasia (UC-HGD) and CAC when compared to controls, UC, and low-grade dysplasia (UC-LGD) [22]. ERβ expression had an inverse relationship with tumor grade [30]. A clinical experiment done showed that 41.7% of well-differentiated tumors from men and 45.5% from women were moderately or strongly immune positive for ERβ compared with only 10% and 11% of poorly differentiated tumors respectively [8]. Again, this indicates that tumor-reduced ERβ expression is correlated with poorer grade and more advanced tumor stage [12].

Estrogen and the KCNQ1 potassium channel

The KCNQ1 potassium channel together with its KCNE3 regulatory subunit plays an important role in regulating secretion across the epithelium of the colon. KCNQ1/KCNE3 voltage-gated (Kv) channels are needed to maintain the electrochemical gradient by recycling potassium at the basolateral membrane, which is required for membrane repolarization. This then acts as a driving force for the conductance of chloride at the apical membrane via cystic fibrosis transmembrane conductance regulator (CFTR) channels [7]. Estrogen acting through ERs inhibits basolateral KCNQ1 channel activity and thus, rapidly inhibits secretagogue-stimulated cAMP-dependent Cl^- secretion [21].

The most active estrogen, 17β-estradiol (E2), is a regulator of the whole body fluid and electrolyte balance through its action on the distal colon, the principal site for water conservation in the body (20). The transport of ions especially Na^+ absorption and Cl^- secretion across the colonic epithelium determines the rate of water loss and absorption from the lumen of the colon. Cl^- is transported into the colonic epithelial cells at the basolateral membrane via the Na^+-K^+-$2Cl^-$ (NKCCl) cotransporter [20]. Cl^- is secreted across the apical membrane via the CFTR. Cl^- secretion provides the electrical driving force for trans-epithelial Na^+ secretion through paracellular pathways [7]. This generates the osmotic driving force for water flow that yields an isotonic secretory product. Na^+-K^+-ATPase located at the basolateral membrane produces the driving force required for Cl^- secretion [20]. Basolateral K^+ channels, for example, KCNQ1, facilitate the K^+ recycling required for the favorable membrane electrical potential that is needed for Cl^- secretion. The activity of ion transporters involved in Cl^- secretion is modulated by secretagogues acting through cAMP activity or the intracellular Ca^{2+} concentration. KCNQ1 is the critical K^+ channel involved in Cl^- secretion in the distal colon. KCNQ1 is regulated by cAMP activity and associates with a regulatory β-regulatory subunit, KCNE3 [24].

E2 inhibits Cl^- secretion across the colonic epithelium via protein kinase-dependent modulation of the KCNQ1 channel to promote fluid retention at the luteal stage of menstrual cycle [21]. The mechanism is nongenomic, and the signaling pathways stimulated potentiate and modulate the direct transcriptional effect of the nuclear ERs. The nongenomic signaling is initiated at membrane-associated estrogen receptors that are coupled to the rapid activation of protein kinase signaling cascades (Fig. 1). The membrane-localized ERs may be splice variants of the nuclear receptors or truncated forms of ERα associated with cell membrane through acylation [3, 13]. E2 inhibited KCNQ1 channel currents in isolated female rat distal

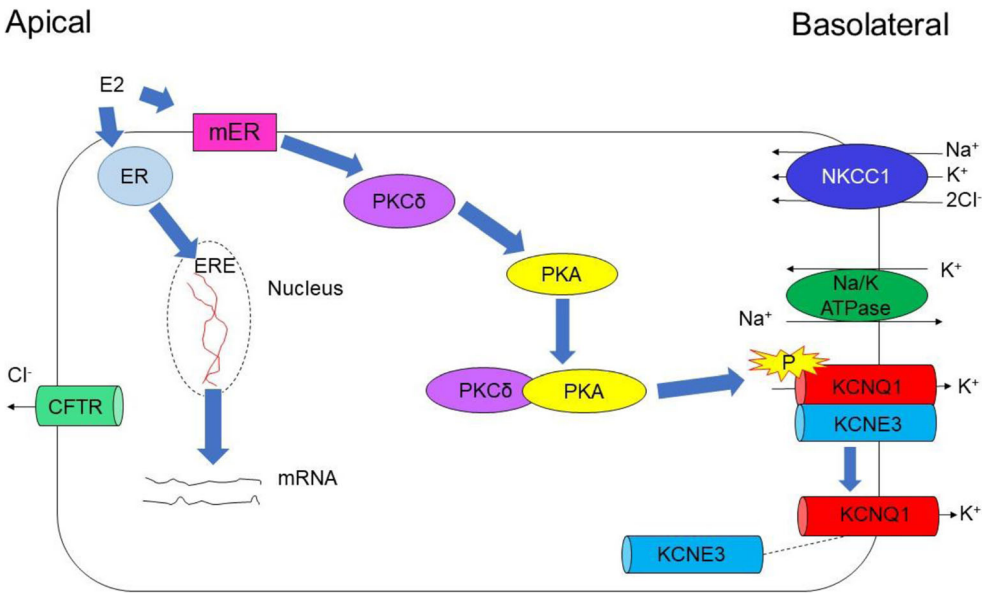

Fig. 1 The most active estrogen, 17β-estradiol (E2), may promote the nuclear localization of estrogen receptors (ERs) to modulate gene expression or initiate protein kinase cascades via membrane-associated receptors (mERs). The activation of a PKCδ/PKA cascade in colonic epithelial cells promotes the dissociation of the KCNQ1 potassium channel from its KCNE3 regulatory subunit. This blocks the basolateral recycling of potassium which is required for apical chloride secretion in the colon, and so inhibits fluid secretion at the estrogen peak in the menstrual cycle

colonic crypts, and no effect was found in distal colonic crypts isolated from male rats in whole cell patch-clamp analysis [20]. This suggested that the estrogen antisecretory effect is gender-specific, even though the KCNQ1 channel is expressed by males and females. Another study tested if the estrogen antisecretory effect can be reproduced in a human colonic adenocarcinoma cell line [24]. Ussing chambers were used to mount confluent HT29cl.19A monolayers that were subjected to E2 or chromanol 293B treatment. After 15 min of treatment, the cells were stimulated by the addition of the secretagogue forskolin. Chromanol 293B (a KCNQ1 blocker) reduced the forskolin-stimulated channel current by 65%, while E2 reduced the effect of forskolin on channel current by 30%. E2 inhibits KCNQ1 via activation of protein kinase Cδ (PKCδ) and protein kinase A (PKA) activation. These protein kinases promote the phosphorylation of KCNQ1 subunit resulting in the dissociation of the regulatory subunit KCNE3, and a collapse in the channel conductance. This is supported by the observation from Ussing chamber studies, which found that both the antisecretory response to E2 and inhibition of the KCNQ1 conductance were diminished by pretreatment with rottlerin, a PKCδ-specific inhibitor [20]. The cells of the female distal colonic crypts expressed PKCδ at a greater abundance compared to the male tissue, rendering the female tissue a more response to signaling actions stimulated by E2.

The role of KCNQ1 in CRC

Potassium channels play a critical role in cell growth and proliferation. Progress through the G1/S checkpoint of the cell cycle requires membrane hyperpolarization and is achieved by the efflux of K^+ [4]. A potential role of KCNQ1 in gastrointestinal tract cancers was indicated by its identification as a high-frequency common insertion site (CIS) locus in Sleeping Beauty (SB) DNA transposon-based forward mutagenesis genetic screens for intestinal cancers [17]. KCNQ1 acts as a tumor suppressor gene that regulates inflammation, growth regulatory signaling pathways, innate immune response, intestinal stem cells growth, cellular detoxification, and mucin secretion. The loss or inactivation of KCNQ1 contributes to CRC development, defining KCNQ1 as a tumor suppressor (18). In a recent study, CRC development was evaluated in C57BL/6J mice, C57BL/6J-*Apc*^{Min} mice and C57BL/6-*Kncq1* knockout mice were used, and results showed that *Kcnq1* mutant mice developed significantly more intestinal tumors which some progressed to become aggressive adenocarcinomas [29]. Heterozygous or homozygous activation of KCNQ1 expression resulted in increased adenoma frequency as well as increased progression to adenocarcinoma and poor overall survival [29].

In addition to membrane hyperpolarization, KCNQ1 is also involved in other processes linked to oncogenesis including inflammation, detoxification, stem cell homeostasis, growth regulatory signaling pathways, and ion channel activity. Knockout of KCNQ1 had significant effects on lipid metabolism resulting in the downregulation of lipid oxidation [29], and the switch to lipogenesis due to the inhibition of fatty acid oxidation was associated strongly with oncogenesis. KCNQ1 KO mice had dysregulated inflammatory genes and increased the risk of CRC due to the occurrence of inflammatory bowel diseases. Several genes involved in detoxification including cytochrome oxidase P450 enzymes and several glutathione S-transferases were also dysregulated in KCNQ1 KO and MUC2 KO mice [29].

The KCNQ1 opposite strand/antisense transcript 1 (KCNQ1OT1, also known as LIT1) has been identified as

an antisense long noncoding RNA which can epigenetically regulate the expression of neighboring genes in cis or of distant genes in trans. Alteration of the LIT1 sequence may lead to dysregulation of its target genes, leading to the manifestation of various diseases including cancers [28]. Loss of imprinting (LOI) at LIT1 was observed at high frequency in CRC and in CRC cell lines, suggesting that LIT1 expression was controlled by epigenetic status at the KvDMR1 imprinting control region. KvDMR1 was primarily responsible for altered growth characteristics and transformed phenotype of cells with LOI. A study conducted with tissue samples from 69 CRC patients showed LOI of LIT in 53% of cases. This suggested that LIT1 LOI and loss of methylation (LOM) at KvDMR1 may be associated with CRC tumorigenesis [19]. CRC tumors and cancer cell lines have aberrations in KCNQ1OT1 transcription, and epigenetic changes including histone modifications and DNA methylation at the KCNQ1 cluster [28]. This evidence points to KCNQ1OT1 transcription being closely related to the initiation and progression of CRC.

β-Catenin and KCNQ1

In the normal progression of the cell cycle, β-catenin regulates normal cell proliferation and embryonic development [15]. In the absence of Wnt signals, cytoplasmic β-catenin degradation is promoted by an axin complex consisting of adenomatous polyposis coil (APC), a tumor suppressor gene, casein kinase 1α (CK1α), and glycogen synthase kinase 3β (GSK3-β) [11]. This complex promotes the phosphorylation of the amino terminal region of β-catenin so that it can be recognized by β-TRCP, an E3 ubiquitin ligase subunit for the ubiquitination and proteosomal degradation of β-catenin (Fig. 2). This consequently prevents the nuclear accumulation of β-catenin which ensures the repression of the Wnt target genes by DNA-bound T cell factor/lymphoid enhancer factor (TCF/LEF) family of proteins [16]. CRC cell lines which possess an active Wnt signaling pathway contain an increased amount of β-catenin in the cytoplasm which eventually translocates and accumulates in the nucleus. Two families of receptors are involved in the activation of the Wnt signaling pathway, namely the Frizzled receptor and the LDL receptor-related proteins 5 and 6 (LRP5 and LRP6) [16]. When a Wnt ligand binds to one of these receptors, the pathway is activated, leading to the recruitment of the axin complex to the receptor. The phosphorylation of β-catenin is thus inhibited leading to its accumulation in the nucleus and consequently activating Wnt target gene expression. Mutation of Wnt target genes such as APC or Axin, and also the loss of APC expression are strongly associated with CRC development due to the stabilisation of free β-catenin [2].

The overexpression of nuclear β-catenin induces the dysregulation of the KCNQ1OT1 transcription in CRC cells. Such a phenomenon has also been observed in other malignancies including melanoma, ovarian carcinoma, and gastric cancer [28]. KCNQ1/KCNE3 expression and activity is also linked to β-catenin expression. In cardiac cells, the abundance of KCNE1/KCNQ1 in the cell membrane was increased with the presence of β-catenin, and the induced depolarization current in these Kv channels was increased by 30% in the presence of β-catenin. A recent study found that the interaction between KCNQ1 and β-catenin was bidirectional in CRC cells [25]. The expression of KCNQ1 was lost during epithelial mesenchymal transition as a consequence of repression by β-catenin. When KCNQ1 was overexpressed, β-catenin was

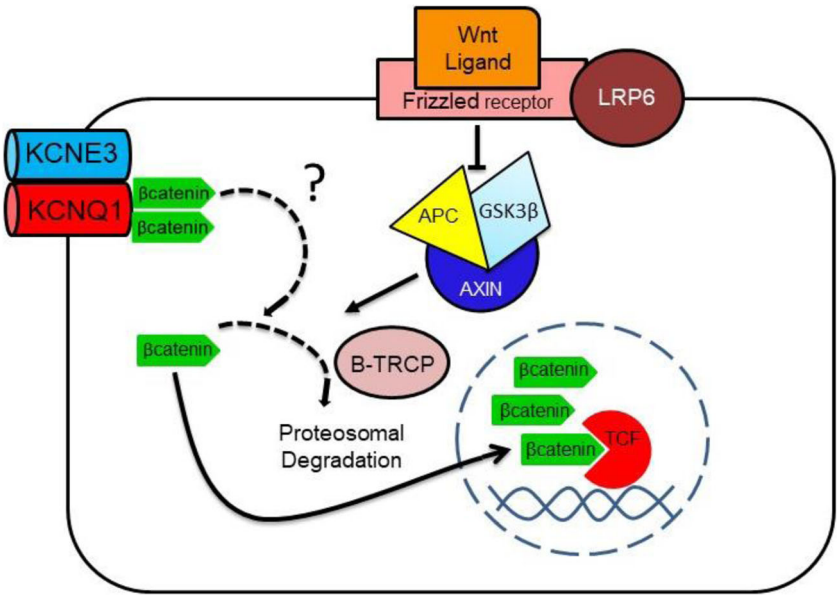

Fig. 2 The ubiquitination of β-catenin by the β-TRCP ubiquitin ligase is promoted by phosphorylation of β-catenin by the axin complex. Activation of Wnt signaling blocks axin-mediated degradation of β-catenin, resulting in its accumulation in the cytoplasm and nucleus. The nuclear accumulation of β-catenin results in the activation or derepression of Wnt-regulated genes by TCF family transcription factors. Overexpression of KCNQ1 recruits β-catenin to the membrane and may facilitate its subsequent ubiquitination

trapped at the membrane and the cells displayed a more differentiated phenotype, but when KCNQ1/KCNE3 activity was blocked with chromanol 293B, β-catenin correlated with better disease-free survival in a cohort of 286 CRC patients [25].

Conclusion

Multiple factors influence the development of CRC, but evidence points to steroid hormones, particularly circulating estrogens acting in a protective role. One of the key actions of estrogen in normal physiology is the regulation of secretion by the colon over the course of the menstrual cycle. The target for estrogen in this process is the KCNQ1/KCNE3 Kv channel. Estrogen promotes the phosphorylation of KCNQ1 in colon epithelial cells and also promotes the sequestration of the channel from the membrane to endocytic vesicles [20, 24]. This redistributive process is female gender-specific and does not affect the total abundance of KCNQ1. KCNQ1 is now emerging as an important tumor suppressor in CRC. Sustained KCNQ1 expression is associated with better CRC survival, and KCNQ1 overexpression suppresses nuclear accumulation of β-catenin [25]. In view of the action of estrogen in promoting the redistribution of KCNQ1 and its association with Nedd4-2 ubiquitin ligase [24], it remains to be shown whether the internalization of KCNQ1 also contributes to the ubiquitination of β-catenin and the attenuation of its tumor promoting action in the nucleus. Further studies are needed to establish whether the action of estrogen on the KCNQ1 channel subunit contributes to the suppression of CRC tumor promotion via the Wnt/β-catenin pathway.

Compliance with ethical standards

Cooperation between the Perdana University and the Royal College of Surgeons in Ireland is covered by an Institutional Memorandum of Understanding. The research is a focused review and did not involve human participants or the use of animals; consequently, ethical approval and participant consent were not required.

Conflict of interest The authors declare that they have no conflicts of interest.

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