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# Chemopreventive drugs: Mechanisms *via* inhibition of cancer stem cells in colorectal cancer

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**Core tip:** To develop optimal chemopreventive agents, we need to target cells and pathways that are essential and critical to carcinogenesis: early tumorigenic changes of stem cells and signals for dedifferentiation may be good targets for chemoprevention. Major chemopreventive drugs, such as non-steroidal anti-inflammatory drugs, statins, proliferator-activated receptor  $\gamma$  agonists and metformin, have cancer stem cell (CSC)-suppressing effects *via* regulation of stem cell-regulating pathways, stem cell niche in the tumor microenvironment, and altered tumor metabolism. These stem cell-related steps in tumorigenesis could be critical targets for chemoprevention and CSC-targeted adjunctive treatment of colorectal cancer.

## Abstract

Recent epidemiological studies, basic research and clinical trials on colorectal cancer (CRC) prevention have helped identify candidates for effective chemopreventive drugs. However, because of the conflicting results of clinical trials or side effects, the effective use of chemopreventive drugs has not been generalized, except for patients with a high-risk for developing hereditary CRC. Advances in genetic and molecular technologies have highlighted the greater complexity of carcinogenesis, especially the heterogeneity of tumors. We need to target cells and processes that are critical to carcinogenesis for chemoprevention and treatment of advanced cancer. Recent research has shown that intestinal stem cells may serve an important role in tumor initiation and formation of cancer stem cells. Moreover, studies have shown that the tumor microenvironment may play additional roles in dedifferentiation, to enable tumor cells to take on stem cell features and promote the formation of tumorigenic stem cells. Therefore, early tumorigenic changes of stem cells and signals for dedifferentiation may be good targets for chemoprevention. In this review, I focus on cancer stem cells in colorectal carcinogenesis and the effect of major chemopreventive drugs on stem cell-related pathways.

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## CHEMOPREVENTIVE DRUGS FOR COLORECTAL CANCER AND FUTURE DIRECTION

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers, and is a major cause of cancer morbidity and mortality worldwide<sup>[1]</sup>. Although there have been improvements in surgical and oncological therapies, the data have shown limited survival improvements in

advanced CRC<sup>[2]</sup>. Therefore, prevention strategies remain the most promising avenue for reducing both the incidence and mortality of CRC.

CRC development is a multi-step process that occurs over a span of about 10 years, thereby providing an opportunity for prevention and early detection<sup>[3]</sup>. CRC screening and polyp removal are effective interventions for CRC prevention<sup>[3,4]</sup>. However, along with screening efforts, we need a specific prevention strategy for patients at high-risk for developing CRC. Chemoprevention involves the use of a variety of agents that can prevent, delay, or even reverse the development of pre-malignant lesions by suppressing the multi-step carcinogenic process. Many studies have demonstrated that pre-malignant lesions can be reversed and prevented pharmacologically<sup>[5]</sup>. This effect is of particular importance to high-risk individuals with a hereditary predisposition for or susceptibility to the environmental causes of CRC. Chemoprevention shows great promise in this regard and the ideal chemopreventive agent, with an excellent safety profile, remains to be discovered.

Until now, there have been several major candidates for CRC chemopreventive drugs, including aspirin and non-steroidal anti-inflammatory drugs (NSAIDs), statins, peroxisome proliferator-activated receptor (PPAR)  $\gamma$  agonist, and metformin, which exhibit chemopreventive effects in epidemiologic studies, in *in vitro* and *in vivo* experiments, and in some clinical trials.

NSAIDs have received the most attention as chemoprevention agents in CRC, and experimental and clinical studies have consistently shown that NSAIDs may reduce the risk of colorectal adenoma or cancer<sup>[6,7]</sup>. In experimental models, either nonselective or cyclooxygenase-2 (COX2)-selective NSAIDs have been shown to suppress CRC growth through COX2-dependent and -independent mechanisms, such as activation of apoptotic and anti-inflammatory signals<sup>[8,9]</sup>.

Many clinical trials have addressed the cancer-preventive effect of aspirin, using colorectal adenomas as a surrogate primary end point for cancer, and the data support its benefits in reducing the risk of CRC. In patients with a history of a previous CRC<sup>[10]</sup> or a history of colorectal adenomas<sup>[6,11]</sup>, the recurrence of adenoma was reduced in patients who received aspirin *vs* those who did not. In addition, in patients with hereditary non-polyposis CRC, the long-term use of aspirin reduced the incidence of CRC, with an HR of 0.63 (95%CI: 0.35-1.13)<sup>[12]</sup>.

In addition to aspirin, other NSAIDs have also shown efficacy in CRC prevention trials. For example, in one clinical trial, in which patients with a history of resected adenomas were randomized to receive either sulindac plus difluoromethylornithine or matched placebos, promising results were seen, in that the risk ratio was 0.30 (95%CI: 0.18-0.49) for recurrent adenomas and 0.085 (95%CI: 0.011-0.650) for advanced adenomas in the intervention arm relative to the placebo arm<sup>[13]</sup>. In addition, recent long-term follow-up studies have reported that NSAIDs may also reduce the recurrence and mortality of CRC<sup>[14-16]</sup>.

Meanwhile, celecoxib, a selective COX2 inhibitor, showed promise in inhibiting adenoma occurrence in familial adenomatous polyposis patients<sup>[17]</sup> and in patients with a history of colorectal polyps<sup>[18,19]</sup>. However, COX2 selective inhibitors are no longer considered for prevention of CRC because of their cardiovascular toxicities<sup>[20-22]</sup>.

Statins, widely used cholesterol-lowering drugs, inhibit cholesterol synthesis *via* inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, the rate-limiting enzyme in the mevalonate and cholesterol-synthesis pathway. Many of the downstream products of this pathway are required for critical cellular functions, such as maintenance of membrane integrity, signaling, protein synthesis and cell cycle progression<sup>[23,24]</sup>. However, clinical studies examining the relationship between statins and CRC incidence have yielded mixed results. Although some case-control and cohort studies and a meta-analysis study<sup>[25-31]</sup> demonstrated a protective effect against CRC in statin users, other studies failed to do so<sup>[32-37]</sup>. Siddiqui *et al*<sup>[38]</sup> showed reduced recurrence of polyps (OR = 0.51, 95%CI: 0.43-0.60) and high-risk polyps (OR = 0.74, 95%CI: 0.52-0.96), and diminished polyp size and number in patients who used statins continuously for up to 5 years. Moreover, statins have an excellent safety profile. To overcome the possible discrepancies in the results from studies of statins in CRC chemoprevention, a thorough analysis of the underlying CRC risk, methodologies and exposure time are needed, along with data from well-designed large-scale clinical trials and epidemiological studies in the future. The exact role of statins in chemoprevention remains to be elucidated. In addition, recent data have suggested that statins may have beneficial effects on disease progression and survival, indicating that long-term use of statins may be associated with a less-advanced tumor stage and a better survival rate<sup>[34,39]</sup>.

PPAR $\gamma$  agonists, such as thiazolidinedione (TZD), an insulin-sensitizing diabetes drug, also have anti-cancer activities, involving inhibition of cell growth and induction of apoptosis and terminal cellular differentiation<sup>[40-43]</sup>. PPARs have central roles in the regulation of glucose and lipid homeostasis, and also regulate cell proliferation, differentiation, and inflammation<sup>[44]</sup>. Recently, several studies have reported that the use of TZDs may be associated with a decreased risk of CRC in patients with diabetes<sup>[45,46]</sup>, and in some cases, PPAR $\gamma$  agonists have also shown modest efficacy for chemoprevention in clinical trials<sup>[47,48]</sup>. In addition, PPAR $\gamma$  expression in CRC primary tumors correlates well with overall survival of CRC patients<sup>[49]</sup>, which is consistent with animal experiments showing that intestinal tumors are exacerbated in *APC* min/+ mice with genetic ablation of *Pparg*, compared with control *APC* min/+ mice<sup>[50]</sup>. However, controversy regarding the anti-tumor effect of PPAR $\gamma$  agonists persists because some studies indicated that activation of PPAR $\gamma$  promotes tumorigenesis<sup>[51-54]</sup>. Furthermore, clinical studies show that TZD may be associated with an increased risk of heart failure<sup>[55]</sup>, bone fractures<sup>[56-58]</sup> and

possibly bladder cancer<sup>[59]</sup>. Whether these are PPAR $\gamma$ -mediated side effects or off-target effects remain uncertain. Although PPAR $\gamma$  is currently considered a potential target for chemoprevention, previous results are based mainly on observational and preclinical studies, and rigorous clinical trials are needed to address the utility of PPAR $\gamma$  agonists in CRC chemoprevention.

Metformin is a classic biguanide drug that has been used as first-line therapy for type 2 diabetes mellitus (DM). Metformin inhibits hepatic gluconeogenesis and reduces insulin resistance. It is a safe and economical drug that has been used for more than 50 years. Most CRC-specific observational studies and meta-analyses reported that patients with type 2 DM who were taking metformin have a lower risk of CRC and better outcomes compared with patients not taking the drug<sup>[60-64]</sup>. Moreover, metformin showed a protective effect for colorectal adenoma recurrence in colonoscopic surveillance of CRC patients with diabetes<sup>[65]</sup>. Preclinical studies in animal models support these findings, showing that metformin induced AMP-activated protein kinase (AMPK) activation and inhibited tumor development and growth, including colon tumorigenesis<sup>[66,67]</sup>. In terms of its molecular mechanism, metformin regulates insulin/insulin-like growth factor-related pathways, inflammatory activity, and the AMPK/mammalian target of the rapamycin (mTOR) pathway<sup>[68]</sup>. Activated AMPK inhibits the mTOR-mediated synthesis of key proteins responsible for malignancy and growth of cancer cells<sup>[69]</sup>, and is thought to be a main mediator of the potential anti-cancer mechanism of metformin. Despite the promising results in these studies, data pertaining to metformin from well-designed clinical trials for CRC and its precancerous lesions are lacking. Furthermore, the anti-tumor effect of metformin on non-diabetic patients should also be assessed, because the safety of metformin is well known and it has no glucose-lowering effects in non-diabetic patients. One clinical trial demonstrated an inhibitory effect of metformin in aberrant crypt foci formation of the rectum in patients who did not have diabetes<sup>[70]</sup>. However, this study showed a short-term effect of metformin in a small number of subjects. Therefore, large-scale randomized controlled trials are required to confirm the chemopreventive and therapeutic effects of metformin, especially for non-diabetic patients<sup>[71]</sup>.

Among the most-promising chemopreventive drugs currently being studied, NSAIDs have consistently shown a protective effect against CRC; however, they are generally not recommended for widespread chemoprevention because of the increased risk of bleeding<sup>[72]</sup>. In addition, COX2-selective inhibitors showed increased cardiovascular morbidity<sup>[73]</sup>. However, statins, PPAR $\gamma$  agonists and metformin have a relatively good safety profile, and these drugs show similarities in their abilities to improve metabolic disorders that are known to be associated with increased cancer risk, such as diabetes, obesity, dyslipidemias, and chronic inflammation. For widespread acceptance of these chemopreventive drugs, more defi-

nite chemopreventive effects in large-scale well-designed clinical trials need to be demonstrated, along with a more acceptable safety profile for PPAR $\gamma$  agonists.

Future directions in CRC chemoprevention will include genetic and molecular approaches for identifying pathways that are associated with cancer initiation and development, and personalized approaches to predict risk, drug susceptibility and toxicity. In addition, mechanism-based combinations of agents will also maximize effectiveness, while limiting drug toxicity. From the perspective of identifying new targets for chemoprevention, besides the traditional targeting of the multi-step process in colorectal carcinogenesis, new evidence has demonstrated that targeting essential cell types and critical signaling pathways, such as tumor-initiating stem cells and stem cell-related pathways, could be another effective strategy for preventing colorectal tumorigenesis.

## STEM CELLS IN CRC AND EARLY CARCINOGENESIS: OPPORTUNITIES FOR CHEMOPREVENTION

Much evidence has shown that genomic instability, including chromosomal and microsatellite instability, and epigenetic changes are important mechanisms for multi-step tumorigenesis of CRC<sup>[74]</sup>. However, we have come to understand that tumors show inter- and intra-tumoral heterogeneity, even in the same patient, and more complex intercellular interactions. Therefore, we need to identify the cells and cellular interactions that are critical in tumors to eradicate cancer cells and prevent cancer development. Recent evidence revealed that stem cells, the tumor microenvironment and metabolic alterations are closely related and critical steps in colorectal carcinogenesis. With recent advances in our understanding of the homeostatic control of intestinal crypts and microenvironments (niches), we are able to delineate the steps in early carcinogenesis of CRC, which could lead to development of new targets for chemoprevention of CRC.

Within the crypts of the intestinal mucosa, the intestinal epithelium is a permanently renewing tissue, the architecture of which is maintained by the ability of the intestinal stem cells to self-renew and generate a hierarchy of proliferative and differentiated cells<sup>[75]</sup>. The balance between proliferation and cell death is important for homeostasis of the intestinal epithelium. Using genetic lineage-tracing methods for stem cell markers, several markers of intestinal stem cells, including B-lymphoma Mo-MLV insertion region 1 (BMI1), telomerase reverse transcriptase, leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5), leucine-rich repeats and immunoglobulin-like domains 1 (Lrig1), olfactomedin 4 and achaete-scute complex homolog 2 (ASCL2), have been identified<sup>[76-80]</sup>.

As for the stem cell niche or microenvironment, subepithelial mesenchymal cells, their secreted growth factors, and basement membrane factors that regulate

epithelial cell function support the intestinal crypts. Pericryptal myofibroblasts (PCMFs), one of the micro-environment or niche components, have crucial functions and roles in intestinal organogenesis, regulation of epithelial cell proliferation and differentiation, mucosal protection, wound healing and extracellular matrix (ECM) metabolism that affects the growth of the basement membrane<sup>[81,82]</sup>. In addition, many inflammatory cells, such as macrophages and lymphocytes, are also important components of the microenvironment in both normal and pathological states. PCMFs and inflammatory cells are located immediately beneath the basement membrane, just under the epithelial cells, and function through the secretion of growth factors, cytokines and basement membrane/ECM proteins; they become activated through direct and indirect interactions<sup>[82]</sup>. Several studies showed that interstitial myofibroblasts and inflammatory cells increase in neoplastic lesions, suggesting that myofibroblasts and inflammatory cells play critical roles in colorectal neoplasia, as well as under normal conditions<sup>[83-85]</sup>.

The stem/progenitor cells, transient amplifying cells, and, finally, the terminally differentiated cells in intestinal crypts are under homeostatic control through important signals, such as the Wnt, bone morphogenic protein (BMP), NOTCH, Hh and phosphoinositol 3-kinase (PI3K)/mTOR pathways<sup>[86]</sup>. The regulation of these signals occurs *via* the tight control of interactions between stem/progenitor cells and the niche microenvironment, such as the PCMFs, smooth muscle cells and inflammatory cells<sup>[86]</sup>. The dysregulation of cryptal homeostasis can induce tumorigenesis and the micro environmental factors secreted by inflammatory cells and myofibroblasts in tumors, as well as accumulation of epithelial changes, have important roles in early tumor progression<sup>[86]</sup>.

Recent evidence suggests that CRC may arise from mutated colorectal stem or progenitor cells that have been termed colorectal cancer stem cells (CSCs) or initiating cells because of their exclusive ability to sustain tumor formation<sup>[87,88]</sup>. Colorectal CSCs have been identified based on the expression of specific cell surface markers, such as cluster of differentiation (CD)133, CD44, CD166, aldehyde dehydrogenase, doublecortin-like kinase 1 (Dclk1), Lgr5 and Eph receptor B2, and these cells demonstrated stem/progenitor cell properties, in terms of their ability to self-renew, differentiate, and proliferate indefinitely to drive continuous expansion of the malignant cell population<sup>[89-93]</sup>. These data emphasize the importance of better characterization of CSCs, because the limited numbers of CSCs within the bulk of the tumor may account for their capability to escape conventional therapies, leading to relapse and metastasis. CSCs are now recognized as a specific target for the complete elimination of CRC (Figure 1). In addition, because these CSCs appear in the very early stages of colorectal carcinogenesis, the early changes that occur in normal and cancer stem cells during carcinogenesis might be an effective target for chemoprevention, as well as treatment

of advanced CRC.

### **Effect and mechanisms of chemopreventive drugs on CSCs**

Recent evidence suggests interesting similarities in the effects of the above-mentioned major chemopreventive drugs, such as aspirin, NSAIDs, statins, PPAR $\gamma$  agonists, and metformin; these include their CSC-suppressing effect, anti-inflammatory action, and regulation of altered tumor metabolism. In addition, both anti-inflammatory effects and regulation of altered tumor metabolism are also associated with the CSC-suppressing effects of these drugs.

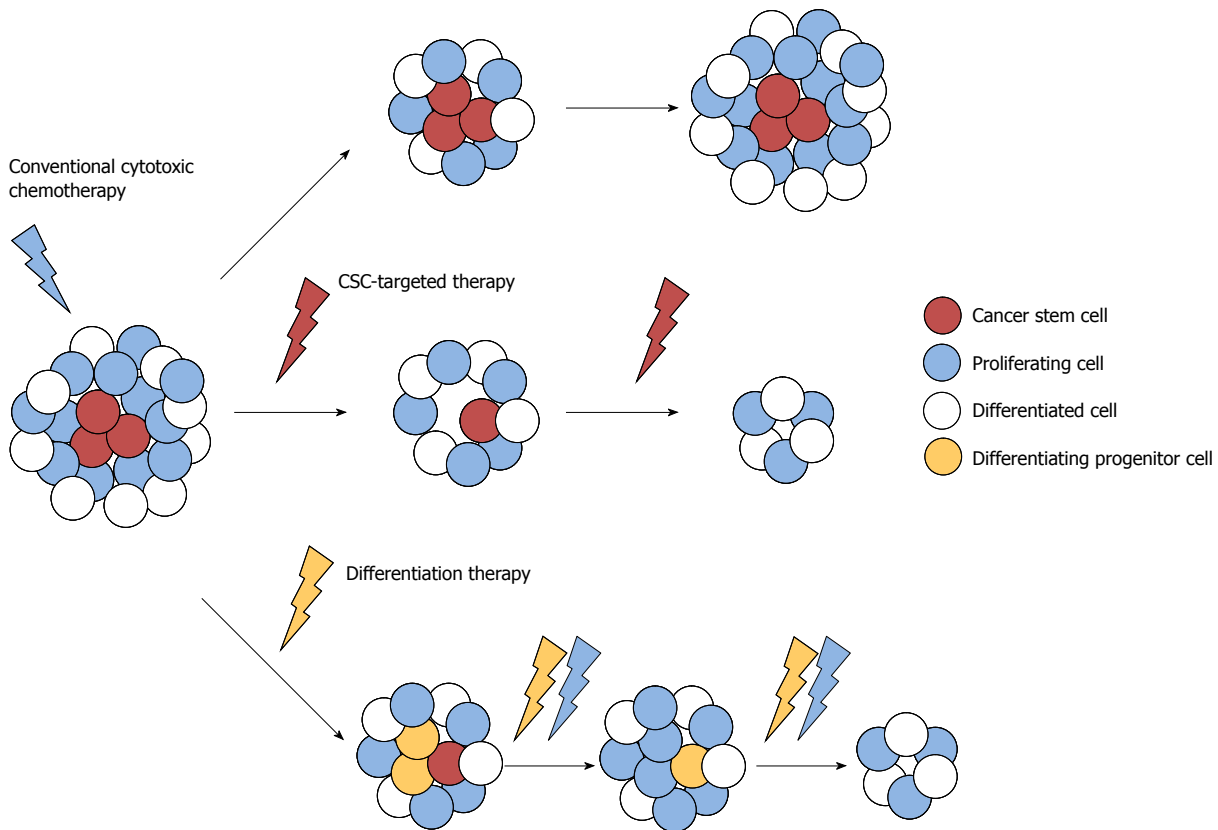
CSCs are involved in tumor initiation, growth, recurrence, and metastasis; therefore, these data suggest that the preventive and survival-improving effects of chemopreventive drugs on CRC might be related to their CSC-suppressing ability. Therefore, in this section, I focus on the relationship between normal/cancer stem cell-related pathways and the mechanism of the chemopreventive drugs.

### **Direct effect on stem cell and cancer stem cells via regulation of Wnt, NOTCH and BMP pathways**

The anticarcinogenic activity of NSAIDs in CRC may depend mostly on the inhibition of COX2 activity, because prostaglandins (PGs) play an important role in tumorigenesis in CRC<sup>[94-97]</sup>. COX2 is reported to be overexpressed in 85% of human CRC cases and in about 50% of colorectal adenomas<sup>[94]</sup>, and was also identified in an animal model<sup>[97]</sup>, in which a COX2 null mutation significantly reduced the number and size of polyps in *Apc* <sup>$\Delta$ 716</sup> mice<sup>[98]</sup>. An earlier study reported that COX2 over-expression leads to the production of PGE2, which ultimately stimulates  $\beta$ -catenin-mediated transcription in colon cancer<sup>[99]</sup>. The WNT/ $\beta$ -catenin pathway is thought to be involved in the regulation of CSCs, and is one of the most interesting therapeutic targets in CSCs<sup>[100]</sup>. In terms of the anti-CSC effect of NSAIDs, Moon *et al*<sup>[101]</sup> showed that the anti-CSC effects of NSAIDs were related to both COX2-dependent and -independent pathways.

As traditional NSAIDs exert anticancer effects *via* COX2-independent mechanisms<sup>[102]</sup>, the COX2-independent pathways of NSAIDs could be involved in their anti-CSC activity. In several previous reports, NSAIDs were shown to inhibit NOTCH/hairy and enhancer of split 1 (HES1) signaling pathway as a  $\gamma$ -secretase inhibitor<sup>[103,104]</sup> and activate the PPAR $\gamma$  expression as a PPAR $\gamma$  agonist<sup>[105,106]</sup>. NOTCH/HES1 signaling has been shown to be oncogenic in CRCs, inhibiting the terminal differentiation of epithelial cells<sup>[107]</sup>, and the dysregulation of the NOTCH/HES1 signaling was implicated in the self-renewal and maintenance of CSCs in CRC<sup>[108]</sup>. Meanwhile, PPAR $\gamma$  activation resulted in growth arrest and induced differentiation of colon cancer cells<sup>[45]</sup>. In addition, the CSC-inhibitory effect of PPAR $\gamma$  agonists through the inhibition of the Janus kinase-signal transducer and activator of transcription (STAT) pathway was demonstrated





**Figure 1 Role of cancer stem cell and anti-cancer stem cell therapy in colorectal cancer.** The limited numbers of cancer stem cell (CSC) within the bulk of the tumor may account for their capability to escape conventional therapies, leading to relapse and metastasis; therefore, CSCs are now recognized as a specific target for the complete elimination of colorectal cancer. CSCs are resistant to conventional chemotherapy: the tumor is reduced in size in the short term, but eventually relapses, driven by CSCs. When CSC-targeted therapy or CSC-differentiating therapy is combined with conventional therapy, a tumor will progressively exhaust its growth potential.

in brain CSCs<sup>[109]</sup>. In this context, Moon *et al*<sup>[101]</sup> showed that the NOTCH pathway and PPAR $\gamma$  could be related to CSCs in CRC and be down- and up-regulated by NSAIDs, respectively, suggesting that NSAIDs suppress colon CSCs *via* COX2-independent pathways.

In addition, Qiu *et al*<sup>[110]</sup> demonstrated that sulindac induces apoptosis to remove the intestinal stem cells with aberrant Wnt signaling, and that diablo IAP-binding mitochondrial protein (also referred to as SMAC), a mitochondrial apoptogenic protein, has a central role in this tumor-suppressive effect of sulindac. These results suggested that the chemopreventive effect of NSAIDs is mediated through the elimination of stem cells that are inappropriately activated by oncogenic events.

Some natural chemopreventive dietary compounds, such as curcumin, sulforaphane and piperine, also have been shown to suppress CSCs through inhibition of WNT/ $\beta$ -catenin signaling<sup>[111-113]</sup>.

Statins also have anti-CSC activity. Kodach *et al*<sup>[114]</sup> showed that tumor-suppressive BMP signaling was silenced by promoter hypermethylation of BMP2 in CRC, and downregulation of DNA methyltransferase activity by statin led to BMP2 promoter demethylation and up-regulation of BMP2 expression, culminating in the differentiation of CRC cells and reduction of "stemness".

Therefore, the suppression of Wnt and NOTCH signaling, and activation of BMP and PPAR $\gamma$  signaling

that is induced by NSAIDs, statins, PPAR $\gamma$  agonists, and some natural chemopreventive dietary compounds might have potential effects on the fate of stem cells, inducing cell differentiation, cell cycle arrest, and apoptosis.

### **Regulation of stem cell niche and the inflammatory nuclear factor- $\kappa$ B pathway**

Recent studies show that bidirectional conversion between CSCs and non-CSCs can be triggered by stromal factors secreted by inflammatory cells or myofibroblasts in the tumor microenvironment<sup>[115,116]</sup>. These factors enhance Wnt activation, induce dedifferentiation of non-stem cells and expand stem cell properties during tumorigenesis. In this regard, the anti-inflammatory effect of chemopreventive drugs can retard this de-differentiating effect.

As an important component of the tumor microenvironment, chronic inflammation is also a key factor in the progression of many cancers. Nuclear factor (NF)- $\kappa$ B represents a key transcription factor within the inflammatory tumor microenvironment. Schwitalla *et al*<sup>[116]</sup> demonstrated NF- $\kappa$ B's function in CSCs, showing that elevated NF- $\kappa$ B signaling enhances Wnt activation and induces de-differentiation of non-stem cells that have acquired a tumor-initiating capacity. Subsequently, epithelial cell-specific ablation of the RelA/p65 subunit of NF- $\kappa$ B retards crypt stem cell expansion; these data support the

concept of bidirectional conversion and the importance of inflammatory signaling for de-differentiation and generation of CSCs<sup>[116]</sup>.

Metformin inhibits initial cellular transformation and selectively suppresses CSCs by inhibition of NF- $\kappa$ B and STAT3<sup>[117]</sup>. In addition, metformin reduces inflammatory responses *via* inhibition of tumor necrosis factor (TNF)-production in human monocytes<sup>[118]</sup>, and metformin-induced AMPK signaling inhibits the NF- $\kappa$ B-mediated inflammatory responses<sup>[119]</sup>. Thus, metformin may target the inflammatory processes in the microenvironment of most neoplastic tissues and cancer cells<sup>[117,119]</sup>.

Similarly, PPAR $\gamma$  agonists can attenuate NF- $\kappa$ B-dependent signaling and induce downregulation of pro-inflammatory target genes, such as TNF and interleukin-6<sup>[120]</sup>, and statins also reduce colon tumorigenesis *via* their potential anti-inflammatory and immunomodulatory properties<sup>[121,122]</sup>. In addition, the anti-inflammatory action of NSAIDs is already well established. All the major chemopreventive drugs discussed in this review have anti-inflammatory properties, which suggest their association with anti-CSC activity through an anti-inflammatory action on the tumor microenvironment, along with their direct effects on CSCs.

Vermeulen *et al*<sup>[115]</sup> demonstrated that myofibroblast-secreted factors, specifically hepatocyte growth factor, activate  $\beta$ -catenin-dependent transcription and subsequently CSC clonogenicity, indicating that Wnt activity and cancer stemness are regulated by microenvironmental factors. They also showed that myofibroblast-secreted factors restore the CSC phenotype in more differentiated cells, suggesting the dynamic bidirectional conversion of stemness of colon cancer cells<sup>[115]</sup>.

Currently, the regulation of microenvironmental factors has become one of the major targets for development of anti-CSC drugs, and they could be important targets for chemoprevention as well, because the stem cell niche is involved in the very early stages of tumorigenesis.

### Regulation of altered tumor metabolism and the AMPK/mTOR pathway

In terms of cancer metabolism, recent evidence reveals that metabolic alterations and reprogramming of cancer cells are not indirect responses to cell proliferation, but altered metabolism itself can be tumorigenic by changing cell signaling and blocking cellular differentiation<sup>[123]</sup>.

The AMPK/mTOR pathway is a central cellular energy sensor<sup>[124]</sup>. Liver kinase B1 (LKB1), the upstream activator of AMPK, is a tumor suppressor, and the major pathway controlled by LKB1-AMPK activation is the mTOR signaling pathway, which regulates cell growth and proliferation<sup>[124,125]</sup>. Activation of AMPK led to inhibition of the mTOR through phosphorylation and subsequent activation of the tumor suppressor tuberous sclerosis complex 2. mTOR is a key regulator of growth factor and nutrient signals, as well as a critical mediator of the PI3K/protein kinase B/Akt pathway, one of the

most frequently dysregulated signaling pathways in human cancer<sup>[124,126]</sup>.

In preclinical studies, metformin induced tumor suppression through mTOR inhibition by AMPK activation<sup>[127]</sup>. In addition, activation of the Akt/mTOR pathway has been associated with malignant progression, resistance to many types of cancer therapy and poor prognosis<sup>[126]</sup>. The PI3K/Akt/mTOR pathway has, therefore, recently been identified as a target for novel cancer therapy, and the inhibition of mTOR signaling is thought to be one of the major mechanisms involving the anti-cancer effect of metformin. Activation of AMPK also induces cell cycle arrest by inhibiting the expression of cyclin D1 and activating p21/p27, resulting in cellular senescence, quiescence, and apoptosis<sup>[128,129]</sup>.

Recent studies have demonstrated that metformin could selectively suppress cancer stem cells using *in vitro* experiments and *in vivo* xenograft models<sup>[117,130,131]</sup>. Metformin has also been shown to improve tumor response to chemotherapy, by activating a cytotoxic effect on CSCs that exhibit chemoresistant features<sup>[117,130,132]</sup>. The PI3K/Akt/mTOR pathway is activated for maintenance and proliferation of CSCs<sup>[133-135]</sup>; therefore, the mechanism of action of metformin-induced CSC suppression involves the activation of AMPK and the consequent inactivation of mTOR. CSCs are known to be resistant to conventional chemotherapy, and are a cause of cancer recurrence and metastasis; therefore, metformin's effect of eliminating cancer stem cells suggest the possibility of metformin as an adjunctive agent combined with conventional chemotherapy, as well as a chemopreventive drug.

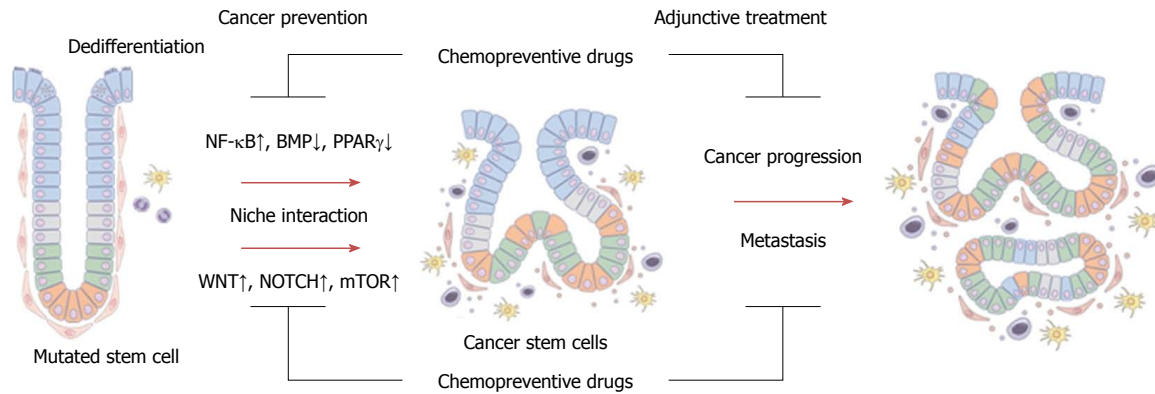
Moreover, aspirin, statin, and PPAR $\gamma$  agonists also induce the activation of AMPK, targeting regulators of intracellular energy homeostasis and metabolism<sup>[136-140]</sup>. These could contribute to their protective effects against development of CRC.

## CONCLUSION

Candidates for effective CRC chemopreventive drugs have been identified through epidemiological studies, basic research and clinical trials. However, to develop more effective chemopreventive drugs with good safety profiles, we need to target cells and pathways that are essential and critical to carcinogenesis, along with targeting the traditional multi-step process of CRC tumorigenesis.

With recent advances in our understanding of intestinal crypt homeostasis and its dysregulation, mutated stem cells and CSCs in early carcinogenesis are likely to be promising targets for chemoprevention of CRC. However, to target the CSCs and stem cell-specific signaling pathways in early carcinogenesis, a detailed understanding of the mechanisms of stem cell maintenance and differentiation, and their relationship with the carcinogenic pathways is needed.

Several recent reports indicate that major chemopreventive drugs like aspirin, NSAIDs, statins, PPAR $\gamma$  agonists, and metformin have CSC-suppressing effects



**Figure 2** Effects of chemopreventive drugs acting on cancer stem cells and their related signaling pathways in colorectal carcinogenesis. Changes and crosstalk in the stem cells, microenvironment, and metabolism are closely related to essential steps in early carcinogenesis and tumor progression. Mutated stem cells and dedifferentiated stem-like cells can progress to cancer stem cells through dysregulation of stem cell-regulating pathways (Wnt, NOTCH, and BMP), interaction with stem cell niche or tumor microenvironment (inflammatory NF- $\kappa$ B and stromal factor-induced Wnt pathways), and alteration of tumor metabolism [AMP-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) pathway]. The major chemopreventive drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs), statins, PPAR $\gamma$  agonists, and metformin, have cancer stem cell (CSC)-suppressing effects via regulation of these pathways. The development of CSC-suppressing chemopreventive drugs could be useful for adjunctive treatment to avoid relapse or metastasis, as well as more potent chemoprevention in colorectal cancer. PPAR: Peroxisome proliferator-activated receptor; NF: Nuclear factor; BMP: Bone morphogenic protein.

via regulation of stem cell-regulating pathways (Wnt, NOTCH, and BMP), stem cell niche or tumor microenvironment (inflammatory NF- $\kappa$ B and stromal factor-induced Wnt pathways) and altered tumor metabolism (AMPK/mTOR pathway) (Figure 2).

Changes in the stem cells, microenvironment, and metabolism are closely related, underlying essential steps in early carcinogenesis and tumor progression, and could be critical targets for chemoprevention and treatment of CRC (Figure 2). In addition to being more effective anti-neoplastic and chemopreventive drugs, either alone or in combination with other agents, these chemopreventive drugs could also be the basis for development of chemically modified drugs with better chemopreventive activity and a more desirable safety profile.

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