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Aspirin in the prevention of colorectal cancer: mechanisms beyond COX inhibition and implications for chemoprevention

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Resumen

La aspirina es un fármaco antiinflamatorio no esteroideo ampliamente estudiado. Ha demostrado potencial en la prevención del cáncer colorrectal (CCR) mediante la inhibición de la ciclooxigenasa y la reducción de la síntesis de prostaglandinas. Sin embargo, la nuevas evidencias sugieren que los efectos y mecanismos de la aspirina contra el CCR podrían extenderse más allá de las vías de la ciclooxigenas (COX). Las estrategias antiinflamatorias centradas en la COX están siendo reemplazadas por enfoques multimodales de precisión que se dirigen a vías de señalización más allá de la síntesis de prostaglandinas, con el fin de mejorar los resultados en pacientes con cáncer colorrectal. Estos enfoques incluyen la modulación de las vías de señalización inflamatorias, la reprogramación del microambiente inmunitario tumoral, la terapia combinada, el aumento de la quimiosensibilidad en las células de CCR y las interacciones con alteraciones genéticas específicas del tumor. Esta revisión resume el conocimiento actual sobre los mecanismos de acción de la aspirina en el cáncer colorrectal, con énfasis en las vías independientes de la COX, destaca su relevancia potencial para la prevención del cáncer colorrectal y sus posibles implicaciones para los enfoques basados en la precisión.

Palabras clave
Aspirina, cáncer colorrectal, ciclooxigenasa, inhibición.

Conflicto de intereses
Este artículo no presenta conflicto de interés.

Summary

Aspirin is a well-researched nonsteroidal anti-inflammatory drug. It has shown potential in preventing colorectal cancer (CRC) by inhibiting cyclooxygenase and reducing prostaglandin synthesis. However, emerging evidence suggests that aspirin's effects and mechanisms against CRC may extend beyond cyclooxygenase pathways. Anti-inflammatory COX-centered strategies are being replaced by multi-pathway, precision-based approaches that target signaling pathways beyond prostaglandin synthesis to improve outcomes for patients with colorectal cancer. These include modulation of inflammatory signaling pathways, reprogramming of the tumor immune microenvironment, combination therapy, enhancement of chemosensitivity in CRC cells, and interactions with tumor-specific genetic alterations. This review summarizes the current understanding of aspirin's mechanisms of action in colorectal cancer with an emphasis on the COX-independent pathways, highlights their potential relevance for CRC prevention, and their possible implications for precision-based approaches.

Key words
Aspirin, Colorectal cancer, Cyclooxygenase, Inhibition.

Conflict of interests
This article does not present a conflict of interest.

Introduction

Aspirin (acetylsalicylic acid, $C_9H_8O_4$) (Ornelas *et al.,* 2017) is a widely used drug, recognized for its pharmacological activities as an anti-inflammatory, analgesic and antipyretic, as well as its role in cardiovascular prophylaxis. Over time, aspirin emerged as a promising agent for the chemoprevention of colorectal cancer (CRC) (Thorat and Cuzick, 2015), and is currently under investigation for its ability to inhibit tumor development, metastasis, and inflammation across many cancer types. Clinical data suggest a possible reduction in cancer incidence with aspirin's use, especially in colorectal, breast, and gastric cancers. Moreover, its capacity to augment the effectiveness of chemotherapeutic agents suggests its utility as an adjunct therapy in oncological treatment (Ornelas *et al.,* 2017).

According to Weitz et al. (2005), colorectal cancer is the fourth most common cause of cancer-related deaths globally and the third most common type of cancer. Genetic mutations, epigenetic changes, metabolic reprogramming, immunological regulation, and tumor-microenvironment crosstalk are some of the intricate molecular interactions that cause colorectal cancer (Sun *et al.,* 2025). Aspirin's role in chemoprevention may be associated with a lower risk of CRC metastases and reduced incidence of CRC (Rothwell *et al.,* 2012). The analyses of numerous randomized controlled trials comparing aspirin to a control group indicate a probable correlation between its daily usage and reduced incidence and mortality of CRC, with benefits after an interval of about 8 to 10 years or more of follow-up (Johansson *et al.*, 2012; Rothwell *et al.*, 2010).

Low-dose aspirin is associated with fewer T4 tumors and metastases in CRC patients, according to a 2013 study by Jonsson et al. It has been shown that aspirin can reduce the potential for metastasis by inhibiting platelet-tumor cell signaling and preventing the epithelial-mesenchymal transition in circulating tumor cells (Khorana, 2003). The potential of aspirin in the chemoprevention of colorectal cancer continues to be highlighted by recent studies. Inducing apoptosis, inhibiting the stemness of colorectal cancer cells, regulating oncogenic signaling cascades like Wnt/β-catenin, NF-kB, and PI3K–AKT–mTOR, and changing immune surveillance are just a few of the processes that aspirin is known to affect through COX-independent pathways (Din et al., 2012; Ying et al., 2023; Laila *et al.,* 2025).

Aspirin induces G1 cell cycle arrest and modulates the p53–Cyclin Dependent Kinases (CDK1) pathway, causing upregulation of p53 and downregulation of CDK1 (Zhang *et al.*, 2024). It can facilitate the secretion of tumor growth factor beta 1 (TGF-β1), which can cause colorectal cancer cells to undergo apoptosis and stop proliferating (Wang et al., 2018). In a CRC mouse model of inflammatory carcinogenesis, aspirin reduced the number and size of CRC lesions. It suppressed COX-2, inducible nitric oxide synthase, the active form of Yes-associated protein 1, and cytosolic high mobility group box 1, all of which are highly expressed at tumor sites in colorectal cancer (Ohnishi *et al.*, 2021). Additionally, an experimental study in mice suggests that aspirin may modify histone H3 lysine 27 acetylation marks and inhibit histone deacetylases, causing a reduction in the expression of inducible nitric oxide synthase, tumor necrosis factor-α, and interleukin-6. These effects have been linked to a reduction in colorectal cancer development in mice models (Guo *et al.*, 2016).

A more comprehensive understanding of aspirin's intricate mechanisms of action may elucidate its potential role in colorectal cancer prevention and assist in identifying patient subgroups that potentially benefit, but this has yet to be

conclusively determined. Aspirin integration into customized therapy approaches based on molecular biomarkers, tumor genetics, and other factors is gaining popularity as precision oncology develops (Laila et al., 2025). In addition to highlighting aspirin's potential for CRC prevention outside traditional anti-inflammatory mechanisms, this review explores the emerging and growing evidence supporting aspirin's COX-independent effects in colorectal cancer.

2.0 ASPIRIN'S EMERGING MECHANISMS OF COLORECTAL CANCER PREVENTION

2.1 Antitumor-Effect of Aspirin: COX-Dependent Mechanism

Cyclooxygenases convert arachidonic acid into prostaglandins, prostacyclins, and thromboxane A2 (TXA2). Unlike COX-1, which is constitutively expressed in many tissues, COX-2 is generally inducible and is absent or present at very low levels in most cell types under basal conditions. However, during inflammatory and other pathological states, such as cancer, its expression increases (Patrignani and Patrono, 2016). It has been suggested that aspirin's anti-cancer properties might result from inhibition of COX-2, given its involvement in cancer (Eberhart et al., 1994). Additionally, studies using deletions of COX-1 and COX-2 have shown decreased intestinal tumorigenesis (Chulada et al., 2000). Uncontrolled platelet activation triggers local recruitment of immune cells, leading to inflammation. Aggregation of platelets at these sites releases platelet-derived growth factors (PDGF), cytokines (interleukin-1β), and lipid mediators (PGE2 and TXA2), promoting proliferation of adjacent nucleated cells in the colonic mucosa.

Furthermore, it is hypothesized that these agents may increase COX-2 levels (further enhancing PGE2) and drive the epithelial–mesenchymal transition (EMT) of intestinal epithelial cells, a characteristic early event in tumorigenesis. EMT activation generates tumor-initiating cells and triggers tumor cell invasion and metastasis to distant organs (Patrignani and Patrono, 2016; Dovizio *et al*., 2012). Notably, PGE2 binding to EP1-4 receptors (G-protein-coupled receptors) activates signal transduction pathways that promote adhesive, migratory, and invasive behavior of cells during the development and progression of cancer (Menter and Dubois, 2012). Therefore, in agreement with the platelet hypothesis, platelet aggregation and aberrant activation at the site of mucosal injury in the gastrointestinal (GI) tract are the driving factors for the genesis of adenomatous polyps and eventual progression to CRC. Aspirin's chemopreventive effect against CRC is thus attributed to its ability to inhibit COX-1 in platelets, prevent the release of lipid or protein mediators of inflammation, and subsequently decrease COX-2 expression in colorectal tissues.

Recent works by Yang et al. (2025) and Langley and Burn (2025) have demonstrated the potential of aspirin to reduce the risk of metastasis by limiting platelet TXA2-induced suppression of T cell immunity. This mechanism may contribute to aspirin's chemopreventive and anti-metastatic potential.

2.2 Limitations of the COX-Centric Model

John R Vane was awarded the Nobel Prize in 1982 for the discovery that aspirin irreversibly acetylates the cyclooxygenase enzymes, thereby inhibiting the conversion of arachidonic acid to prostaglandins (Vane, 1971). The upregulation of cyclo-oxygenase-2, the inducible form of the enzyme, in cancer, together with prostaglandin E2 (PGE_2), has been implicated in stem cell proliferation, migration, apoptosis resistance, invasion, and metastasis (Pang *et al*., 2016). Hence, inhibition of COX-2 activity was initially

proposed as the primary mechanism that can explain the anti-tumor effects of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) (Chan *et al*., 2007).

The COX-centric paradigm has provided a good framework for understanding the anticancer effect of aspirin. However, several findings and observations have challenged the completeness of this dominant model. Findings suggest that COX inhibition represents only one dimension of a larger, interconnected network of aspirin-mediated antitumor mechanisms. Understanding these alternative pathways optimizes the use of aspirin in colorectal cancer treatment and identifies patients most likely to benefit.

Some limitations of the COX-inhibition hypothesis have been described. The platelet hypothesis (COX-dependent pathway) proposes sequential inhibition of COX-1 activity in platelets and COX-2 expression in nucleated cells of the colonic mucosa (Thun *et al*., 2012, Patrignani, and Patrono, 2016). Although this is an interesting and attractive hypothesis, a critical issue is that the primary target (platelet COX-1) and the subsequent downstream effector (epithelial COX-2) are in different cell types. The platelet hypothesis lacks an explanation for the preferential protective effects of aspirin against CRC compared with other types of cancer. Aspirin influences multiple cellular compartments, including platelets, immune cells, stromal fibroblasts, and the intestinal microbiota, many of which act independently of tumor epithelial COX signaling (Sankaranarayanan *et al*., 2020). If platelet COX-1-dependent inhibition by aspirin is primarily responsible for cancer prevention following its absorption into the circulation, it is logical to expect aspirin to be equally effective against all cancers, which is not the case.

The hypothesis also suggests that the chemopreventive effects of aspirin apply only to CRC that develops after mucosal injury-induced platelet activation; it does not explain how low-dose aspirin works against cancers due to sporadic mutations, where platelets may not be involved. The hypothesis was derived to accommodate the inability of low-dose aspirin to directly inhibit COX-2, a COX isoform implicated in the development of many cancers (Sankaranarayanan *et al*., 2020). The only known effect of low-dose aspirin was COX-1 inhibition in platelets. The hypothesis argued that COX-1 inhibition in platelets may be central to the prevention of tumorigenesis (Dovizio *et al* 2012). However, the clinical efficacy of aspirin in the treatment of CRC remains under investigation. It should be noted that although COX-2 expression is implicated in the development of CRC, aspirin has been reported to inhibit cancer cell growth even in cells that do not overexpress COX-2 (Hanif *et al*., 1996). Further, some CRC cell lines do not produce COX-2 or express its inactive form (Hsi *et al*., 2000). The report by Rothwell *et al*. (2010) also showed that right-sided CRCs, which generally do not overexpress COX-2, were more responsive to aspirin use than those of the left-sided colon cancer. These observations raise the possibility that aspirin's chemopreventive effects in colon cancer treatment extend beyond COX inhibition, with other alternative pathways likely playing a significant role.

Another report showed that aspirin and other NSAIDs promote cell cycle arrest and apoptosis in colon cancer cell lines that do not express COX-1 or COX-2 enzymes and in mouse embryo fibroblasts that are COX-null (Rigas and Shiff, 2000). Additionally, their growth-inhibitory properties could not be reversed by the addition of prostaglandins (Drew *et al*., 2016). Moreover, NSAID derivatives that do not remarkably affect the catalytic activity of COXs maintain their anti-tumor properties in tissue culture (Piazza *et al.,* 1997) and animal models (Mahmoud *et al*., 1998). Hence, this provides further evidence that inhibition of COX is not the only mechanism by

which NSAIDs promote apoptosis and prevent the growth of neoplastic lesions (Tegeder *et al.,* 2001). Several COX-independent targets have been identified, including the WNT (Qiu *et al*., 2010), AMP-activated protein kinase (AMPK) (Hardie *et al.,* 2012) and MTOR (Din *et al*., 2012) signaling pathways, and the cross-talk between these pathways is shown in figure 1.

2.3 Rationale for Targeting Non-COX Pathways

Although COX-2 overexpression has been identified to play a role in CRC and the progression of tumorigenesis, the process involves many signaling pathways beyond prostaglandin synthesis. Other pathways that involve modulation of inflammatory signaling pathways, reprogramming of the tumor immune microenvironment, combination therapy, promotion of chemosensitivity of CRC cells, and interactions with tumor-specific genetic alterations are involved, as shown in Table 1. Targeting these non-COX pathways may overcome resistance to COX inhibitors, decrease toxicity, and enable more personalized therapeutic strategies. Furthermore, non-COX targets might improve health outcomes by working in concert with already available chemotherapeutic drugs.

2.4 COX-Independent Mechanisms

2.4.1 Immune Modulation

The importance of aspirin in boosting immune responses against inflammation-associated colorectal cancer has been highlighted (De Matteis *et al*., 2022). Aspirin suppresses PD-1 expression in macrophages and CD8+ T cells by controlling specific pro-resolving mediators (SPMs) in colonic tissues. Experimental investigations suggest that aspirin may alter important transcription factors (Sp1, Sp3, Sp4), which could contribute to CRC prevention, though clinical validation is lacking (Marimuthu *et al*., 2011). Additionally, frequent aspirin use has been linked to a rise in tumor-infiltrating lymphocytes (TILs), which may improve the results of conventional chemotherapy or immunotherapy (Cao *et al.,* 2016). It enhances the activity of effector T cells (CD8+ T cells, Th17 cells, and B cells) by suppressing TIGIT expression on T cells and enhancing immune responses against CRC progression (Liu *et al*., 2024). This modulates gut microbiota composition by reducing Enterococcus cecorum. Immunogenic cell death has been identified in colon cancer cells treated with aspirin. This is characterized by the expression of calreticulin (CRT) and HSP70, together with activation of endoplasmic reticulum stress and glycolytic changes, which could be leveraged in combination with immune checkpoint inhibitors like anti-PD-1 and anti-CTLA-4 (Lei *et al*., 2023).

2.4.2 Combination Therapy-Dependent Approach

Many studies have explored the combination therapies involving aspirin in the treatment of colorectal cancer. Its combination with oleanolic acid inhibits CRC cell proliferation and invasion, by inducing S-phase arrest, and promoting apoptosis through the Akt/NFκB/IκBα/COX2 pathway (Zhou *et al*., 2024). Aspirin and zinc suppress tumor progression in colitis-associated colorectal cancer in mice, improving markers such as PCNA and STAT3, while also enhancing antioxidant defenses such as Nrf-2, catalase, and superoxide dismutase (Babu *et al*., 2023). Studies in FAP model Min mice suggested that aspirin obstructs intestinal tumorigenesis by regulating β-catenin signaling, oxidative stress, and inflammation pathways (Hamova *et al*., 2023). Additionally, aspirin combined with genistein exhibited cytotoxicity in HCT-116 colorectal cancer cells by inhibiting cell migration and altering cell morphology.

Table 1: COX-Independent Aspirin's Mechanisms of Action in Colorectal Cancer

COX-Independent Mechanisms	Molecular Targets	Biological Effects	Type of Study	Role	Dosage	References
Nuclear Factor (NF-Kappa B) Inhibition	RelA (P65), IκBα	Increased chemosensitivity, induction of apoptosis	Preclinical	Anticancer effect	High	Stark *et al.*, (2001)
Immune Modulation	Effector T cells (CD8+ T cells, Th17 cells, and B cells)	Improved Immune Response	Preclinical	Chemoprevention and anticancer-effect	Low	Marimuthu *et al.*, (2011)
Inhibition of Wnt Signaling and β-Catenin Phosphorylation	β-catenin	Ubiquitination and proteolysis of the oncoprotein. inhibition of protein phosphatase 2A	Preclinical	Anticancer effect	High	Bos *et al.*, 2006
Activation of AMP-Kinase and Inhibition of mTOR Signaling	AMPK, mTOR, effectors S6K1 and 4E-BP1	Cell survival, regulation of intracellular energy homeostasis and metabolism	Preclinical	Anticancer effect	High	Din *et al.*, 2012
Downregulation of c-myc, cyclin A2 and CDK2	c-myc mRNA, proteasomal pathways	Cell cycle arrest and inhibition of cell proliferation	Preclinical	Anticancer effect	High	Ai *et al.,* 2016; Dachineni *et al.,* 2016
Induction of Polyamine Catabolism	Spermidine/spermine N1-acetyltransferase (SSAT)	Reduction of tumorigenesis	Preclinical and clinical	Anticancer effect and Chemoprevention	Moderate	Babbar *et al.*, 2006
Induction of DNA Mismatch Repair Proteins	DNA mismatch repair proteins	G0/G1 cell cycle arrest, apoptosis	Preclinical	Anticancer effect	High	Goel *et al.,* 2003
Microbiota Modulation	Gut bacteria	Increase in chemosensitivity of colorectal cancer cells	Preclinical and clinical	Chemoprevention	Moderate to high	Ying *et al.*, 2023

A promising therapeutic approach for this genetic subtype of colorectal cancer is the combination of metformin and aspirin, which has demonstrated a substantial antiproliferative, cytotoxic, and antimigratory effect on PIK3CA-mutant HT-29 CRC cells (Goncalves *et al.,* 2024). Additionally, aspirin and cisplatin have shown synergistic effects. The effect inhibited cell invasion, proliferation, and migration, while also triggering apoptosis by targeting the PI3K/Akt, NF-κB/COX-2, and Bcl-2 signaling pathways (Jiang *et al.*, 2020).

In a colitis-associated cancer model, aspirin and γ-tocopherol reduce tumor size and multiplicity while reducing aspirin-induced gastric lesions with changes in the gut flora. Also, this combined therapy inhibited HCT-116 growth, indicating a new chemopreventive approach (Liu *et al.*, 2023). Delta-tocotrienol (DT3) and aspirin together prevented colon cancer stem cells from proliferating and induced apoptosis (Husain *et al.*, 2024). This is a result of more research on the effects of aspirin on Wnt signaling in colon cancer stem and adenoma development in APCmin/+ mice.

2.4.3 Gut Microbiota-Dependent Approach

The amount of 4-Hydroxybenzoic acid (4-HBA) generated in the intestine through cytochrome P450 (CYP450)-mediated metabolism within cells or via microbial degradation in the lumen has been implicated in aspirin's colorectal cancer prevention. Following aspirin therapy, patients with a CYP2C9 variant allele had a lower risk of colorectal adenomas. According to Bigler *et al.* (2001), this might be because CYP2C9 cannot generate 2,5-DHBA, highlighting the significance of aspirin metabolites in cancer prevention. However, the capacity of microbes to biotransform aspirin to produce HBAs may vary. The observed heterogeneity in epidemiological research can be explained by the interaction between intestinal microbiota and nutrition, which suggests that both factors may contribute to the production of HBA. Numerous studies have shown how nutrition affects the composition of the microbiota (Singh *et al.,* 2017; Leeming *et al.*, 2019).

The association between aspirin's cancer-preventive qualities and HBAs may provide novel approaches for achieving the intended chemopreventive benefits in all populations. This could involve taking aspirin and a healthy, sustainable diet that includes more fruits and vegetables, or consuming appropriate probiotics with aspirin in areas where dietary change is challenging. According to a randomized clinical study, aspirin decreased the levels of Bacteroides, Parabacteroides, and Dorea species, which have been linked to a lower risk of colorectal cancer while increasing the abundance of Prevotella, Akkermansia, and Ruminococcaceae species (Prizment *et al.*, 2020).

An in vivo experiment with germ-free and conventionalized mice also demonstrated that aspirin was able to increase the probiotic bacteria Bifidobacterium and Lactobacillus, indicating its role in CRC prevention. Furthermore, the study identified aspirin's ability to prevent tumor formation in germ-free mice, reinforcing the possibility that aspirin may prevent CRC through a microbiome-independent mechanism, such as inhibiting COX-2 and PGE2 synthesis (Zhao et al., 2020). This anticancer effect is independent of metabolite formation. Additionally, Mignozzi et al. (2025) provided insights into the potential benefits of aspirin on intestinal adenoma and CRC, which may be connected to changes in the gut microbiota. The translational potential of microbiome-based interventions in the development of future therapeutic paradigms for colorectal cancer has been described by Neagu et al. (2025). Future research should explore how aspirin may increase beneficial bacteria that contribute to human gut health and cancer prevention. During oral absorption, some aspirin may remain unabsorbed and could act directly on colorectal tissues. However, aspirin may also be metabolized, producing 2,3-DHBA and 2,5-DHBA, which may also influence colorectal tissues to prevent cancer. Since 2,5-DHBA and 2,3-DHBA can inhibit cancer cell growth (Sankaranarayanan et al., 2020), it would be valuable to investigate whether these metabolites enhance the cancer-preventive effects of aspirin when given to germ-free mice. The study should assess the combined roles of intact aspirin and its metabolites in preventing CRC.

Studies have demonstrated that metabolites of aspirin, 2,3-DHBA/pyrocatechuic acid and 2,5-DHBA/gentisic acid, are notable contributors to its anti-cancer effects against CRC (Sankaranarayanan *et al.,* 2020). 2,5-DHBA has been proven to be effective in arresting cancer cell growth in colon cancer cell lines (HCT-116 and HT-29) and breast cancer cell lines (MDA-MB-231), whereas 2,3-DHBA was more selective against the MDA-MB-231 cell line. Cyclin-

dependent kinases are likely cellular targets of the metabolites, as they were both found to inhibit them, particularly CDK1 and CDK6 (Dachineni *et al*., 2017).

It is speculated that these metabolites are generated from the biotransformation of aspirin and salicylic acid, which are left unabsorbed in the intestine. Only 40–50% of the orally consumed aspirin is bioavailable (Dovizio *et al*., 2012), because it undergoes rapid hydrolysis in the intestine and liver to generate salicylic acid (Rowland *et al*., 1972). The *in vitro* studies by Sankaranarayanan *et al*. (2020) demonstrated the inhibitory effect of 2,3-DHBA and 2,5-DHBA on colon cancer cell growth at effective concentrations ranging from 100–500 µM. These concentrations are believed to be pharmacologically achievable in the human intestine. Interestingly, another cellular target of 2,5-DHBA besides CDKs has been discovered. For example, 2,5-DHBA can inhibit fibroblast growth factor (FGF) receptor function, suggesting that this pathway may also contribute to aspirin's anti-cancer effects (Fernandez *et al*., 2010). In addition, its inhibitory effect on COX-2-mediated PGE2 synthesis in murine macrophages (Hinz *et al*., 2020) through its antioxidant properties has been reported (Borges and Castle). Recently, a study also showed that conjugation of 2,5-DHBA to gelatin induces heparin-like properties and hinders the invasion of cancer cells (Snigireva *et al.,* 2020). The alteration of the structure of matrix proteins surrounding cancer cells is also part of the potential of 2,5-DHBA reported to inhibit the growth of cancer cells (Altinoz *et al*., 2018). Taken together, these suggest that 2,5-DHBA may be an important metabolite responsible for aspirin's chemopreventive actions. Interestingly, Hydroxybenzoic acids (HBAs) are also generated from flavonoid degradation and are present in fruits and vegetables (Russell *et al.,* 2009). The metabolite hypothesis thus proposes that HBAs produced from the degradation of aspirin/salicylic acid, flavonoids and the presence of HBAs in the diet may contribute to their chemopreventive properties (Sankaranarayanan *et al*., 2020).

2.4.4 Chemo Sensitivity-Dependent Approach

The development of resistance to chemotherapy during CRC treatment and the side effects on normal cells have seriously limited the application and therapeutic efficacy. Study by Ying *et al*., (2023) identified an unexpected function of aspirin in sensitizing colon cancer cells to chemotherapeutic treatment of 5-Fu and cisplatin. The two agents displayed remarkably improved anti-tumor efficacy *in vitro* upon co-treatment with aspirin.

Cancer stem cells are a minor subpopulation within a tumor that exhibits regenerative capacity, induces chemotherapy resistance, and initiates metastasis, thereby advancing tumor progression and recurrence. Therefore, suppression of colon cancer stemness may be an important strategy to improve the resistance of colon cancer to chemotherapy. Ying *et al*. (2023) demonstrated that aspirin could efficiently inhibit the stemness of colorectal cancer cells. Lipopolysaccharide (LPS) plays an important role in tumor occurrence and metastasis (Wang *et al.,* 2003), and toll-like receptor 4 (TLR4) on CRC cells serves as a receptor for LPS (Watts *et al*., 2013). Also, TLR4 is a key player in connecting inflammation and cancer invasion and progression (Ren *et al*., 2015). One mechanism by which aspirin inhibited the invasive potential of CRC cells is by down-regulating LPS/TLR4/NF-κB signaling pathway (Ying *et al*., 2023). Ying *et al* demonstrated that TLR4-positive colorectal cancer cells demonstrated high chemotherapy resistance, which indicates that TLR4 may also serve as a marker of colon cancer stem cells in colon cancer. Further research revealed that aspirin could increase the chemosensitivity of colorectal cancer cells by reducing stemness

via TLR4 downregulation. Thus, TLR4 could be used as an intervention target to increase the chemosensitivity of colon cancer cells during aspirin treatment.

2.4.5 Inhibition of Wnt Signaling and β-Catenin Phosphorylation

Aspirin reduces the expression of critical epithelial-mesenchymal transition (EMT) regulators in SW480 tumor cells via suppressing Wnt signaling (Jin and Wu, 2019). This suppression occurs via blocking cyclooxygenase metabolism, which reduces pro-inflammatory responses, specifically by lowering WNT6 via the NR4A2 transcription factor (Feng et al., 2021). These data imply that aspirin may have a role in the treatment of inflammation-related colon cancer. A study on the liquid formulation of aspirin, IP1867B, and its effects on TXB2 production and cyclooxygenase isozymes has been published (Hofling et al., 2022). Furthermore, NO-ASA, a derivative of aspirin that releases nitric oxide, has been demonstrated to dramatically suppress colon cancer cell proliferation. Its mechanism includes glutathione depletion, activation of oxidative stress, activation of apoptosis, as well as disruption of adherens. junctions and inhibition of Wnt signaling, which strengthens its chemopreventive characteristics and provides a more effective strategy to colon cancer treatment (Gao et al., 2005).

Wnt-signaling is constitutively activated in colorectal epithelial progenitor cells. However, in mature cells, adenomatous polyposis coli (APC) expression inhibits the pathway. APC works by attaching to excess β-catenin in the cytoplasm, inducing ubiquitination and proteolysis of the oncoprotein. Colorectal cancer is caused by abnormal activation of the Wnt-signaling system, which is defined by a mutation of the APC gene, rendering it inactive (Fodde, 2002). The mutation favors unregulated proliferation and differentiation of the colonic epithelia. An experimental study by Bos *et al*. (2006) suggested that aspirin may induce phosphorylation at doses ranging from 0.05 to 5 mM (through inhibition of protein phosphatase 2A) and subsequent ubiquitination of β-catenin, leading to the inhibition of the Wnt/β–catenin pathway. It should be noted that these effects were observed at concentrations that may exceed those achievable with low-dose aspirin in clinical settings.

2.4.6 Inhibition of Nuclear Factor (NF)-κB Signaling

In healthy cells, the activation of NF-κB pathway is usually temporary (Hoesel and Schmid, 2013). In chronic inflammatory conditions and cancer, NF-κB becomes abnormally active, transforming from a defense mechanism into a contributor to disease progression by increasing inflammation, blocking differentiation, driving cell proliferation, and inhibiting apoptosis (Vlahopoulos *et al*., 2015). Research indicates that dysregulated NF-κB activity plays a significant role in intestinal carcinogenesis, which is highly responsive to aspirin treatment. A meta-analysis of expression studies found that increased expression of NF-κB is substantially associated with late-stage colorectal cancer with 3- to 5-year survival (Wu *et al*., 2015). Studies showed that transgenic mice with active IKK in intestinal epithelial cells develop intestinal tumors and display accelerated adenoma development when crossed to *Min*/+ mice (Shaked *et al*., 2012). However, inactivation of IKK in intestinal epithelial or myeloid cells attenuates the inflammation associated with tumor development (Greten *et al*., 2004). Furthermore, deletion of *RelA* in intestinal epithelial cells suppresses the formation of adenomas in the *Min*/+ model (Myant *et al.,* 2013). These data have highlighted the inhibition of NF-κB activity as a promising therapeutic target for the treatment of this disease.

Effect of aspirin with pharmacologically relevant doses (0.5–5 mM) on NF-κB signaling revealed that prolonged treatment of colorectal cancer cells stimulates the NF-κB pathway, as evidenced by phosphorylation of IκB and nuclear translocation of RelA (Stark *et al*., 2001). Furthermore, Stark *et al* reported that cells expressing degradation-resistant IκB demonstrated that this stimulation is absolutely required for the pro-apoptotic effects of aspirin. Additionally, an *in vivo* study demonstrated the effects of aspirin on NF-κB signaling in colorectal neoplasia using the HT-29 xenograft and Min/+ mouse models. Aspirin, at doses relevant to humans (0.5–1.5 mM) was found to induce phosphorylation and degradation of IκBα, nuclear translocation of RelA, and the induction of apoptosis in xenografted HT-29 tumours and in adenomas from *Min*/+ mice (Stark *et al*., 2007). Furthermore, exposure to low doses of aspirin at 100 μM *ex vivo* was recently shown to stimulate the NF-κB pathway, as indicated by increased phosphorylation of RelA at serine 536, in 5 of 6 freshly resected human colorectal tumors. Collectively, these findings from experimental studies suggest that aspirin and other non-steroidal anti-inflammatory drugs may stimulate the NF-κB pathway in neoplastic epithelial cells.

2.4.7 Activation of AMP-Kinase and Inhibition of mTOR Signaling

Adenosine monophosphate-activated protein kinase (AMPK) is a crucial cellular energy sensor that is activated in response to stress. It is involved in processes that generate ATP to meet the body's energy demands. AMPK regulates several pathways, including those involving p53, fatty acid synthase, and mechanistic target of rapamycin (mTOR). AMPK has also been implicated in cancer progression, including CRC, because these pathways are vital for cell survival and metabolism (Steinberg *et al*., 2013). Aspirin, at a concentration of 5 mM, was shown to decrease mTOR signaling by inhibiting the effectors S6K1 and 4E-BP1, which are involved in translation and protein synthesis, leading to cell death. Din *et al*. (2012) revealed that aspirin could modify the AMP: ATP ratios in cells, leading to the activation of AMPK and subsequent inactivation of mTOR signaling. Also, it was noted that aspirin inhibited mTOR signaling in CRC patients treated with analgesic doses (600 mg/day for one week). The decrease in phosphorylation of S6K1 and S6 effectors in these patients suggests that their inhibition facilitated the regulation of intracellular energy homeostasis and metabolism, which may contribute to the protective effect of aspirin against CRC (Din *et al*., 2012)

2.4.8 Downregulation of c-Myc, Cyclin A2 and CDK2

The cellular myelocytomatosis oncogene (c-myc) is a nuclear transcription factor that controls 15% of all genes. It influences many cellular functions, including cell proliferation, metabolism, apoptosis, growth, and differentiation. Its frequent overexpression in about 20% of all cancers is linked to poor clinical outcomes and a malignant metastatic phenotype. Aspirin and salicylic acid have been shown to reduce levels of c-myc mRNA and protein. This effect was observed at concentrations between 0.25 and 2.5 mM (Law et al., 2000; Ai et al., 2016), which might be higher than what can be achieved with low-dose clinical use. Another mechanism involves the downregulation of cell cycle regulatory proteins cyclin A2 and CDK2 through proteasomal pathways at concentrations from 0.5 to 2.5 mM (Dachineni et al., 2016), potentially leading to cell cycle arrest and reduced cell proliferation.

2.4.9 Induction of Polyamine Catabolism

The transcriptional regulator c-myc activates the transcription of ornithine decarboxylase (ODC), the first enzyme involved in polyamine

synthesis. This results in elevated levels of mucosal polyamines that have been implicated in tumorigenesis. The use of aspirin between 20 and 100 µM, in Caco-2 cells, was associated with the transcriptional activation of spermidine/spermine N1-acetyltransferase (SSAT), a driver for polyamine catabolism. The resulting reduced levels of polyamines may reduce tumorigenesis (Babbar *et al.*, 2006).

2.4.10 Induction of DNA Mismatch Repair Proteins

DNA mismatch repair is a highly conserved mechanism that corrects mutations arising during DNA replication or damage. Epigenetic changes to mismatch repair genes have been associated with CRC development (Li and Martin, 2016). Evidence showed that in colon cancer cells, exposure to aspirin at concentrations of 1 to 10 mM induced key DNA mismatch repair proteins by 2–7-fold, depending on the cell line studied. This was accompanied by G0/G1 cell cycle arrest and apoptosis, which may contribute to aspirin's anticancer effects (Goel *et al.,* 2003).

2.4.11 Acetylation of p53 and Glucose-6-Phosphate Dehydrogenase

p53 is a tumor suppressor protein that regulates cell proliferation. In normal cells, its activation is through acetylation and phosphorylation, after which it translocates to the nucleus and acts as a transcription factor. Approximately 50% of all tumors contain a mutated p53. Such mutations inactivate the functions of its tumor suppressor, leading to enhanced cell proliferation. Aspirin has been linked to direct acetylation of both wild-type and mutant p53, at concentrations between 0.05 and 2.5 mM, and this was associated with induction of p21 and Bax, suggestive of a role in restoration of p53 function and tumor suppression (Alfonso *et al.*, 2014; Ai *et al.*, 2016). Aspirin was also shown to acetylate glucose-6-phosphate dehydrogenase (G6PD; at ≥100 µM), with decreased enzyme activity. Studies by Marimuthu *et al. (*2011) and Ai *et al.* (2016) suggested that selective inhibition of G6PD may be a crucial mechanism by which aspirin exerts its anticancer effects by inhibiting ribonucleotide synthesis. According to Dore *et al.* (2016), aspirin has been reported to reduce G6PD, a mechanism that could potentially prevent cancer cell proliferation.

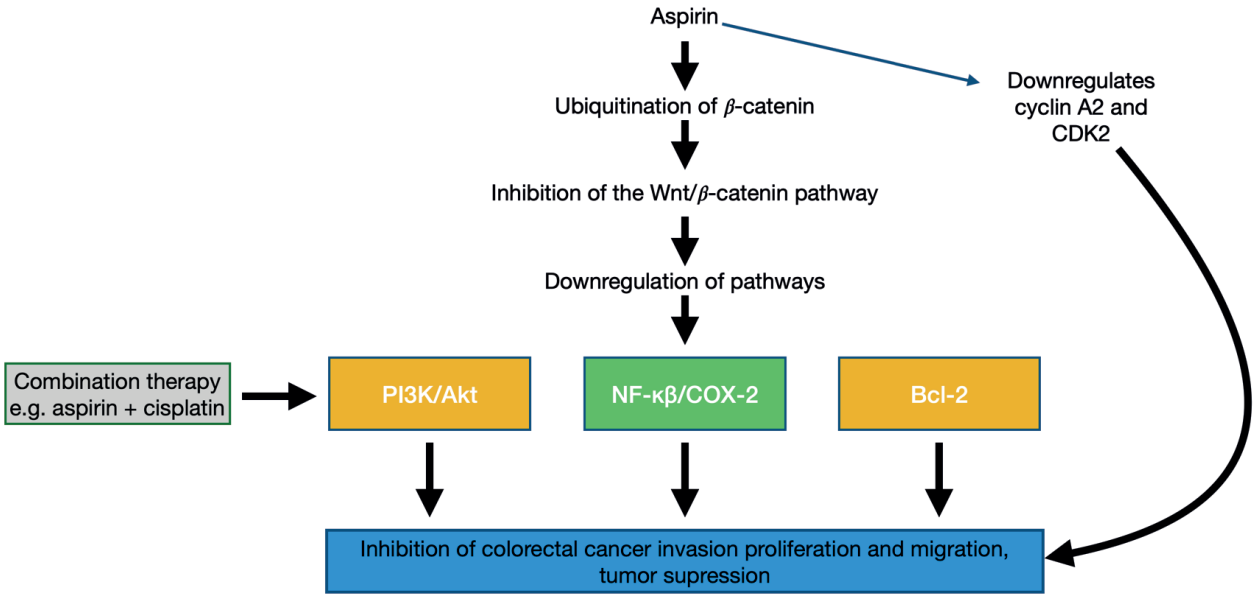

Figure 1: COX-Independent Pathways Crosstalk

Conclusions

Clinical Outcome of Non-COX-targets for CRC Patients: Implications for Precision Cancer Prevention and Therapy and Future Directions

Targeting non-COX pathways represents a shift from anti-inflammatory COX-centered treatments to multi-pathway, precision-based approaches to improving CRC patient outcomes. The method has the potential to overcome resistance to COX inhibitors while also reducing toxicity. Non-COX routes might be able to overcome resistance to COX inhibitors, minimize toxicity, and enable more tailored therapy approaches. Furthermore, non-COX targets may interact synergistically with existing chemotherapeutic, targeted, or immunotherapeutic treatments to improve clinical results. An immunology-based non-COX target can directly influence antitumor immune responses, leading to improved patient survival.

The report indicated that aspirin exerts anti-cancer effects by inhibiting various components of the tumor microenvironment, including platelets and the anti-inflammatory immune system. Its diverse inhibitory mechanisms enable aspirin to suppress numerous characteristics of neoplastic cells, such as their anti-apoptotic ability, as well as their capacity for migration, invasion, proliferation, and metastatic spread.

Furthermore, a shift toward precision oncology is shown in the mounting evidence in favor of non-COX targets in the treatment of colorectal cancer. Beyond what can be accomplished with COX inhibition alone, it addresses other important pathways that may enhance clinical results. Future studies should focus on discovering predictive biomarkers, optimizing combination strategies, and elucidating resistance mechanisms. Integration of non-COX targeted therapies with COX inhibitors, chemotherapy, radiotherapy, and immunotherapy may improve clinical results. Further research into these targeted pathways will be necessary to improve patient care and develop CRC therapy.

Moreover, continued investigation into these targeted pathways will be essential for the advancement of CRC therapy and optimization of patient care.

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