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Review Article

Involvement of platelet signaling pathways in colorectal cancer and new therapeutic targets

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Abstract: Colorectal cancer (CRC) is one of the most widespread tumor types, and it stands as the second leading cause of disease-related mortality globally. Due to its adverse effects, which lead to low patient adherence, new alternatives to conventional chemotherapy and radiotherapy treatments are being studied. Since, in most cases, platelets are positively involved in the persistence and progression of CRC, several elements of the platelet signaling pathway have been considered possible therapeutic targets. The present study assembles the main treatments for CRC and investigates the cellular mechanisms involved in the interaction between blood platelets and cancer cells. Additionally, this review cites other articles that propose possible therapeutic targets in the platelet activation pathways to be explored. Despite the reported benefits of antithrombotic therapy on CRC progression, some studies have warned about an increased bleeding risk and CRC incidence and highlight the importance of controlling this therapy through diagnostic tests. However, their high cost is still a significant obstacle to the population's access from low Human Development Index (HDI) countries. Many research groups have studied platelet signaling pathways in depth to develop a safer, more effective, and affordable therapy for the population.

Keywords: Colorectal cancer, antineoplastic agents, tumor microenvironment, blood platelets, platelet activation, platelet aggregation inhibitors

Introduction

Colorectal cancer

Epidemiology, etiology, histological aspects: According to the World Health Organization (WHO), in 2018, there were 18.1 million cancer cases globally, with 9.6 million deaths, 90% due to metastasis. Cancer incidence and mortality rates are higher in males (9.5 million new cases; 5.4 million deaths) compared to females (8.6 million new cases; 4.2 million deaths). Colorectal cancer (CRC) is one of the most prevalent cancers in both sexes, ranking third in incidence (10.2%) after breast (11.6%) and lung cancers (11.6%), and second in mortality (9.2%) after lung cancer (18.4%) [1, 2]. In Brazil, from 2005 to 2018, CRC incidence tripled (14.6 to 51.4 per 100,000 inhabitants), particularly among those aged 50-69. Incidence increased in those under 50 and over 70 due to health policies and lifestyle changes. The Human Development Index (HDI) inversely correlates with CRC occurrence, with the Brazilian Northeast having a lower HDI, showing the second-highest CRC incidence [3].

CRC originates from polyps in the colon or rectum, developing 10-35 years after their appearance [4-6]. Polyps smaller than 1 cm resemble normal cells, while larger ones have a higher cancer risk [4, 5]. Inflammatory bowel diseases (IBD), like Crohn's disease or ulcerative colitis, increase the risk of colitis-associated cancer (CAC), with an 18-20% higher risk in long-term ulcerative colitis patients [6]. While most CRC tumors are not inflammation-induced, inflammatory processes can trigger CRC of sporadic or hereditary origin, and anti-inflammatory drugs may prevent or delay the disease. Furthermore, in the consensus molecular subtype (CMS) 1, which is the immunogenic subtype and

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encompasses the majority of tumors with microsatellite instability (MSI), and the CMS 4 (mesenchymal subtype) cancers, a significant increase in the presence of lymphoid and myeloid-specific genes were observed [7]. Factors like tissue damage, mucus consistency changes, and certain bacteria (e.g., *Clostridia*) can cause inflammation and neoplasms through carcinogenic metabolites and pro-inflammatory cytokines (e.g., IL-17, IL-10) [6].

The predisposing hereditary factor is also an important cause of CRC, with Lynch Syndrome (LS) being the main cause of hereditary CRC. LS is an autosomal dominant disorder caused by germline missense, nonsense, or frameshift mutations with decreased expression of one or more genes responsible for the DNA mismatch repair (MMR) mechanism (*MLH1, MSH2, MSH6, PMS2,* and *EPCAM*), which leads to MSI [8-13]. The MMR (Mismatch Repair) proteins form heterodimeric complexes that detect and bind to DNA regions containing mispaired nucleotides, facilitating the repair of mismatches. The integrity of MMR proteins, as well as their functional loss, is modulated by microRNAs (miRNAs). Specifically, miR-155 has been shown to negatively regulate MSH2-MSH6 and MLH1-PMS2 complexes, contributing to the emergence of a mutant phenotype [9, 11].

The loss of function of one of the MMR proteins causes an accumulation of errors, such as insertions and deletions in the microsatellite region of DNA, leading to genetic instability. MSI may have oncogenic potential when it occurs in the genome's coding region with genes essential for cellular function or negative regulation of cancer (e.g., *MRE11A, TGFBRII, BAX*). Heterozygous mutations of MMR genes can also increase the susceptibility of the normal alleles to a secondary somatic mutation [8-13]. Germline mutations in the *MLH1* and *MSH2* genes account for approximately 70% of all hereditary mutations. Beyond traditional germline mutations, *MLH1* and *MSH2* are also susceptible to constitutional epimutations. Somatic alterations in the *APC* gene are more frequently observed in MSH2-associated tumors than those involving MLH1. Conversely, pathogenic variants in the *CTNNB1 (catenin beta-1)* gene are more prevalent in MLH1-related tumors. Differences in the development pathways of screen-identifiable and non-identifiable colorectal tumors were also observed. Mutations in the *MLH1* and *MSH2* genes present a greater risk for the development of CRC (30%-97% risk) and the early onset of the disease (27-42 years). Patients with LS are also at risk for the incidence of synchronous and metachronous tumors (e.g., endometrial cancer, gastric cancer) [8, 9].

CRC causes macroscopic and microscopic tissue changes, including diminished mucus production and lesions like aberrant crypt foci (ACF). These ACFs, which can potentially progress into adenomas and carcinomas, are comprised of hyperplastic and dysplastic cells. Genetic differences distinguish hyperplastic ACFs from dysplastic ones, with some studies suggesting that hyperplastic ACFs may progress to dysplastic lesions. Additionally, the histological characteristics of tumors differ between the proximal (poorly differentiated cells) and distal (medium to high differentiation adenomas and adenocarcinomas) regions of the intestine [14].

Colorectal tumors contain tumor-initiating cells with stem cell-like characteristics, leading to therapy resistance through IL-22-induced STAT3 phosphorylation. Moreover, Paneth cell dysfunction in the small and proximal large intestine can trigger inflammation and cancer [7]. The non-muscleβ and γ-actin fibers are crucial for CRC cell migration and invasion. These fibers are found in submembrane rings, pseudopods, and proteins like ezrin and myosin [15].

Molecular characteristics: Seventy-five percent of colorectal cancer (CRC) cases occur due to the inactivation of suppressor genes *p53*, *adenomatous polyposis coli (APC)*, and *deleted in colon carcinoma (DCC)* through loss of heterozygosity. Fifty percent results from point mutations in "*ras*" family proto-oncogenes, particularly *K-ras*, and a few from amplifying the *myc* proto-oncogene [14, 16]. Additional mutations or increased expression of genes such as *β-catenin*, *K-ras*, *transforming growth factor beta (TGF-β)*, and *metalloproteinase 9 (MMP-9)* are also observed, along with increased fatty acid synthesis and oxidation due to enzymes like iNOS, COX-2, and fatty acid synthase [14, 17].

In the early stages of CRC, mutations in *APC* and *ras* genes are observed in polyps, while

mutations in *DCC* and *p53* appear later in malignant cells [18, 19]. Proteins such as p53 induce apoptosis in cells with the potential to become cancerous. According to Ma et al., 2013 [20], *p53 reduces* the expression of proteins that promote cell survival (e.g., survivin) and cell death through an RNA fragment named miR-16. In CRC, miR-16 levels are reduced, and its expression is inversely correlated with the degree of cell differentiation. *p53* increases protein levels of miR-16, which binds to the 3'untranslated region (3'UTR) of survivin, preventing its synthesis. Ultimately, increased expression of miR-16 eventually leads to *p53* downregulation.

The Wnt signaling cascade is another pathway involved in many functions that affect the living organism, ranging from physiological processes to pathological conditions such as inflammation and cancer. This pathway supports regeneration and cell survival, facilitates the epithelial-to-mesenchymal transition (EMT) promoting metastasis, and modulates interactions between neoplastic cells and elements in the microenvironment, such as leukocytes and fibroblasts. A wide variety of cytokines activates the Wnt pathway, which is divided into two branches: (1) the Wnt/β-catenin pathway or classical pathway, which is involved in cell survival, differentiation, proliferation, and migration, and (2) the non-canonical β-catenin-independent pathway, which is encompassed by the pathway involved in planar cell polarity (Wnt-PCP) and the Wnt-Ca^{2+} pathway, which in turn modulate cell polarity and migration. When Wnt does not stimulate cells, β-catenin is phosphorylated by glycogen synthase kinase 3β (GSK-3β) and linked to a ubiquitin-dependent proteolytic pathway by the *APC* and *Axin* proteins [21-23].

In the classical pathway, Wnt binds to a complex of LRP5 or LRP6 receptors and proteins of the *Fzd* family. This binding leads to the recruitment of cytosolic proteins and disruption or prevention of the formation of the β-catenin/GSK-3β/Axin complex/*APC*. Consequently, there is an accumulation of β-catenin in the cytoplasm, facilitating its translocation to the nucleus, which regulates the expression of Wnt target genes. The Wnt/β-catenin pathway governs the division of the cells that form the intestinal crypts, and most colorectal tumors occur through modifications that increase the activation of this pathway. Furthermore, the Wnt/β-catenin pathway can synergize with the TGF-β and Notch pathways, favoring tumorigenesis and disseminating neoplastic cells throughout the body. The β-catenin-independent pathways contributing to CRC progression include (a) the receptor tyrosine kinase-like orphan receptor (ROR) signaling pathway; (b) the activation of the Wnt/β-catenin pathway by the transcription factor SP1 through the circular RNA circ_0026628; (c) the p38MAPK activation pathway; and (d) the *MyD88*-induced Wnt/β-catenin activation pathway. Additionally, there are inhibitory pathways that favor the tumor cell. Circular RNAs such as circ_0026628 and circ_0082182 capture micro RNAs, miR-346, miR-411, and miR-1205, which play negative regulatory roles in tumorigenesis. Another counterbalanced pathway involves the decreased expression of long non-coding RNAs such as ADAMTS9-AS1, which are negative modulators of the Wnt/β-catenin pathway [21, 24-27].

β-catenin, a critical transcription factor for cell proliferation and adhesion, is negatively regulated by the *APC* gene and GSK-3β. β-catenin also inhibits the mechanisms that lead to cell apoptosis, such as the formation of autophagosomes and the synthesis of the p62/SQSTM1 protein through the TCF4 factor. In some conditions, such as hypoxia, it forms a complex with the LC3 protein, leading to the degradation and reduction of serum levels. Due to *APC* and β-catenin mutations and the increased activation of the signaling pathway induced by the Wnt protein, β-catenin accumulates in the nucleus where it associates with T cell factor (TCF) and promotes the activation of cyclin D1, *c-myc*, and PPAR-δ [14, 21].

The *K-ras* gene mutation activates the PI3K/Akt and MEK/MAPK/ERK pathways, which are associated with increased expression of cyclin D1 and the c-erbB-2 receptor, as well as decreased expression of GTPase-activating protein expression. TGF-β, produced by adenomas and adenocarcinomas, facilitates bloodstream entry and negatively regulates leukocyte function [14]. Additionally, other factors, such as platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), are also increased, promoting angiogenesis, cell proliferation, and glycolysis [28].

Differences in cellular kinetics, sensitivity to carcinogens and chemotherapeutics, and gene expression (*K-ras, COX-2, PPAR-δ*) are noted between the proximal and distal colon [14]. One distinction between the majority of CRCs and CAC is that, in the CRCs, mutations leading to the activation of the Wnt/β-catenin pathway, such as the inhibition of *APC*, GSK-3β, and mutations in β-catenin, often occur at an early stage of the disease's progression. In contrast, in CAC, dysplasia is suspected to be caused by ROS produced during inflammation, and activation of the Wnt/β-catenin pathway occurs later, following mutations in the *p53* and *K-ras* genes [6, 21].

In both IBD and CRC, an increase in the activation of nuclear factor kappa B (NF-kB), a factor mainly regulated by the Tumor Necrosis Factor (TNF), is observed. This cytokine signaling pathway promotes the stabilization of the Snail factor, which is essential for tumor epithelial-mesenchymal transition (EMT) [29]. Moreover, in an inflammatory model induced by lipopolysaccharide (LPS), tumor cells benefit from activating the TNF-α/NF-kB pathway to survive and metastasize [30]. This evidence emphasizes that the inflammatory process is an essential factor in the positive modulation of the disease.

Interaction between CRC cells and the tumoral microenvironment: The tumor microenvironment (TME) comprises a dynamic interplay among various cell types, including immune cells (e.g., macrophages, CD4 cells, CD8 cells, Treg cells, and NK cells), extracellular matrix components, endothelial cells, and platelets. Inflammation attracts these cells, leading to an increase in the secretion of cytokines and chemokines. Cancer cells can connect to and regulate constituents of the TME in order to help them grow and evade the immune system [31, 32]. In CRC, increased expression of NADPH oxidase 1 (NOX1) and generation of H_2O_2 by intestinal bacteria promotes neoplastic cell proliferation through activation of the Wnt/β-catenin pathway. Generation of CD45RO cells, a memory cell subpopulation of CD4 T lymphocytes, is directly proportional to tumor cell survival. The events that lead to increased generation of this population of cells are mainly microsatellite instabilities and *LINE-1* gene methylation [32].

Regulatory T cells, another immune system element, can also positively modulate inflammation and tumor development by synthesizing and releasing the RAR-related orphan receptor type γ (RORγt) via the Wnt/β-catenin pathway. In addition, stromal cells can activate this signaling pathway and induce tumor cell proliferation by secreting the R-spondin-3 (RSPO3) protein. Conversely, CRC cells can produce phosphatidic acid through PLD2 activation, which causes the senescence of fibroblasts in the microenvironment, favoring cancer cell proliferation. Furthermore, the TME can inhibit the maturation of dendritic cells, causing them to assume a regulatory phenotype and promote tumor survival [21, 32]. However, not all TME elements support the survival and proliferation of neoplastic cells; some play a crucial role in the defense against these cells. For example, in CRC, macrophage infiltration is related to a good prognosis. This is attributed to the disease regression being directly linked to an increase in the M1 macrophage subpopulation, exerting an anti-tumorigenic effect. Stage III CRC patients treated with the chemotherapy drug fluorouracil (5-FU) led to increased M1 macrophage infiltration [32].

Role of the platelets in the colorectal cancer

Platelet's function

Platelets are the most minor functioning units, originating from megakaryocytes in the bone marrow, and are crucial for maintaining hemostasis. The production of platelets depends on the activity of thrombopoietin and the size of the megakaryocyte nucleus. Inflammatory mediators such as interleukin (IL)-6, IL-1, and TNF-α also activate platelet precursors. Under physiological conditions, when platelet levels in the peripheral circulation increase, the liver reduces the secretion of thrombopoietin, leading megakaryocytes to produce platelets through the fragmentation of their cytoplasm. However, in inflammatory conditions, cytokines like IL-6 stimulate increased platelet production by promoting the generation of thrombopoietin and its direct contact with megakaryocytes [33].

Platelets have various receptors that allow them to respond to different agonists associated with endothelial injury and interact with one another. They have GPIbα and GPVI, which are

involved in thrombus formation. The GPIIbIIIa receptor, or $\alpha_{IIb}\beta_3$ integrin, participates in platelet adhesion and aggregation. Additionally, platelets contain other receptors, such as the toll-like receptor (TLR), essential in eliminating microorganisms [34]. In the submembrane region, actin filaments are responsible for platelet shape change, release of substances, and translocation of receptors in the cytoplasm to the cell surface [35]. Platelets are in constant contact with mediators in the plasma through the canalicular system. This channel is a means for releasing granular contents and assists in agonist-induced cell shape change. Platelets have three compartments in their cytoplasm: α, dense, and lysosomal granules. These granules contain important factors for coagulation and hemostasis (e.g., ADP, P-selectin, factor V, VWF), which, upon activation, are released into the cytoplasm or are transferred to the cell membrane [34, 35].

Although they do not possess a nucleus, platelets have 3000-6000 transcripts among strands of pre-messenger RNA (pre-mRNA) and messenger RNA (mRNA) that are used in protein synthesis through the mTOR signaling pathway. Furthermore, they have non-coding fragments of RNA (miRNA), important in gene regulation and pathological processes. These fragments also lead to other effects, such as decreased ICAM-1 expression in the endothelium during myocardial infarction [36-38].

Platelets circulate in the plasma alone but activate and form aggregates in response to endothelial injury. GPIbα and GPVI bind to von Willebrand factor (VWF) and collagen in the exposed subendothelial layer, stabilizing the platelet adhesion. This process triggers a range of events that culminate in the propagation of the platelet activation signal through the secretion of agonists (e.g., ADP, TXA2), which promote cell activation through autocrine and paracrine pathways [34]. Many processes that promote activation and constitute the inside-out pathway are induced by G protein-coupled receptors G13, Gq, and Gi, such as ADP (P2Y1, P2Y12) and thrombin (PAR1, PAR4) receptors. In turn, the G13 protein promotes the activation of the GTPase RhoA, which inhibits the phosphatase of the myosin light chain kinase and increases its phosphorylation, leading to platelet shape change [39-42]. Gq protein participates in the activation of phospholipase C beta (PLC β), which converts phosphatidylinositol 4,5-bisphosphate (PIP2) from the plasma membrane into inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 binds to the dense tubular system, promoting Ca^{2+} release from the intracellular reserves, which leads to Ca^{2+} influx via the "store-operated calcium entry" (SOCE) pathway. DAG, in turn, activates protein kinase C (PKC), which leads to granular secretion [43, 44].

The activation of the Gi protein results in adenylyl cyclase (AC) inhibition with a consequent decrease in cAMP synthesis, positively contributing to platelet activation [45]. Activation of the Gi protein also leads to the activation of the PI3K enzyme, which, together with its substrates PIP2 and PIP3, is responsible for the translocation of the Akt protein to the membrane, where it is phosphorylated on serine 473 (Ser473) by mTORC2 [46, 47]. Subsequently, intracellular calcium, PKC, and Akt are essential for activating GPIIbIIIa. Integrin phosphorylation triggers the outside pathway, which results in the cytoskeleton reorganization and stabilization of the platelet aggregate [45, 48-53]. **Figure 1** illustrates these pathways.

Beyond their role in regulating coagulation, platelets are also involved in other processes, such as cell proliferation and inflammation. This involvement has been well-documented in conditions like sepsis and atherosclerosis [54-56].

Platelets and cancer

Armand Trousseau established a link between platelets and cancer in 1865 [57]. Recent studies have confirmed that daily use of aspirin at a dose indicated for preventing cardiovascular diseases (75-100 mg) reduced the incidence and recurrence of various types of tumors. Moreover, one of the isoforms of COX, COX-2, is the primary source of prostanoid generation in inflammatory conditions and cancer [8, 10, 38, 58]. However, the observed effect of the interaction between platelets and tumor cells remains controversial.

On the one hand, platelets contribute to cancer progression by negatively modulating leukocytes, directly and indirectly, and by secreting metalloproteinases and pro-angiogenic factors

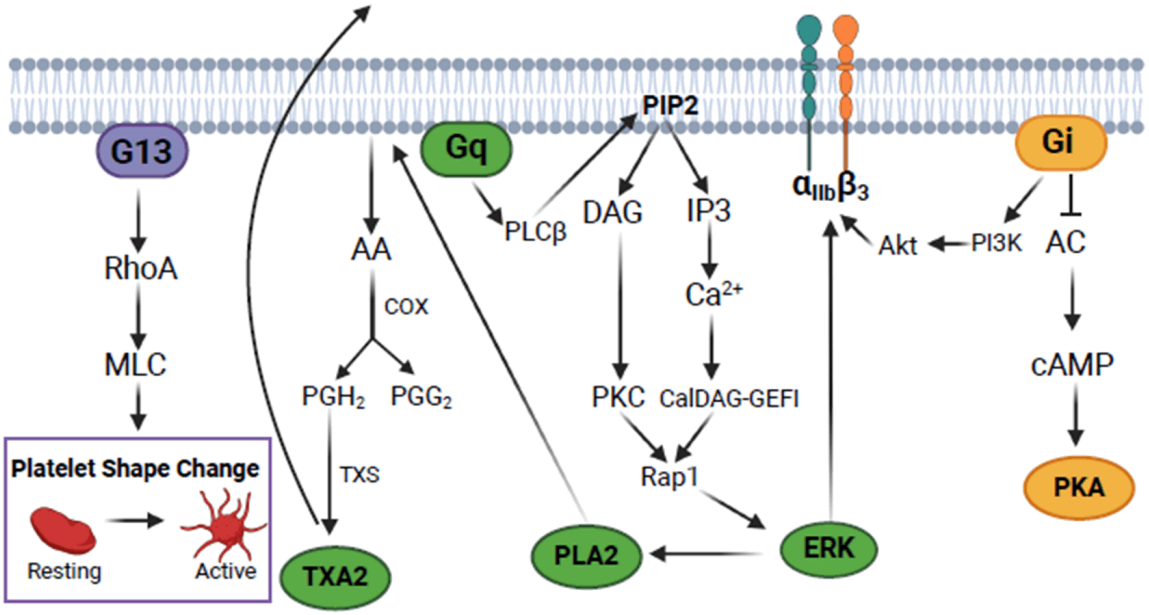

Figure 1. Platelet inside-out signaling triggered by activation of G protein-coupled receptors. AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; PKA, cAMP-dependent protein kinase; PI3K, phosphoinositide 3-kinase; PLCβ, phospholipase C beta; PIP2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; PKC, protein kinase C; IP3, inositol triphosphate; CalDAG-GEFI, calcium and diacylglycerol-regulated guanine exchange factor 1; Rap1, ras-associated protein 1; ERK, extracellular signal-regulated kinase; PLA2, phospholipase A2; AA, arachidonic acid; COX, cyclooxygenase; PGH2, prostaglandin H2; PGG2, prostaglandin G2; TXS, thromboxane synthase; TXA2, thromboxane A2; RhoA, ras homolog family member A; MLC, myosin light chain. The figure was created with BioRender.com.

[57]. On the other hand, tumor cells can activate circulating platelets, which then support the tumor in spreading (**Figure 2**). This interaction occurs through direct interaction involving integrins $\alpha_{IIb}\beta_3$, αvβ3, and α6β1, as well selectins (e.g., P-selectin) present in the platelet's membrane and tumor cells, or indirectly, through the release of angiogenic and procoagulant substances (e.g., ADP, thromboxane, VEGF) by neoplastic cells. Tumor cells can lead to thrombosis by several mechanisms, such as the tissue Factor (TF) and microparticles released from tumor cells that activate the coagulation cascade, and the platelets form the thrombus via the extrinsic route. Active platelets and phospholipids cleaved by tumor cells can also stimulate coagulation via the intrinsic pathway [34, 59].

CRC can induce a hypercoagulable state, increasing the risk of venous thromboembolism (VTE), which may manifest as deep vein thrombosis or pulmonary embolism. This pathological condition is linked to the expression of TF by CRC cells. TF expression correlates with a worse prognosis, as evidenced by the significantly lower 3-year survival rate in TF-positive CRC patients compared to TF-negative patients (39% vs. 88%, respectively). Alternatively, TF is an important prognostic, predictive marker of CRC, as well as recurrent VTE within the context of cancer. Thromboembolism is a challenging condition to treat and has high morbidity and mortality. Patients with CRC have a 5% risk of developing VTE in the first six months after diagnosis. Neoplasia also increases the VTE recurrence risk compared to healthy individuals [60]. Patients who have a positive diagnosis of cancer at the time of VTE occurrence possess a lower one-year survival rate than those who do not have cancer [61]. The main factors influencing VTE incidence are the interaction between neoplastic cells, the hemostatic system, and the patient's characteristics [62].

Tumor cells can also activate platelets through the activation of COX-1 and the synthesis of TXA2, which can contribute to tumorigenesis [38]. Sciulli et al., 2005 [63] observed that patients with CRC history had high urinary levels of the TXA2 metabolite, 11-dehydro-TXB2, significantly reduced with aspirin. There was

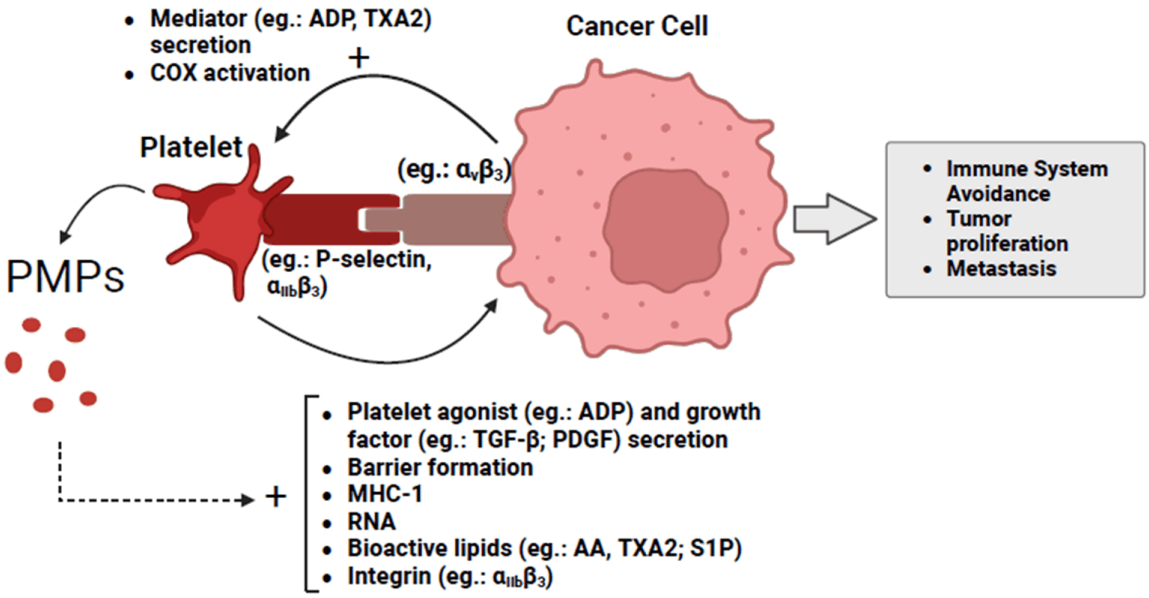

Figure 2. Cancer-activated platelets help neoplastic cells avoid the immune system and promotes tumor growth and dissemination. PMPs, platelet microparticles; ADP, adenosine diphosphate; AA, arachidonic acid; TXA2, thromboxane A2; COX, cyclooxygenase; TGF-β, transforming growth factor beta; PDGF, platelet-derived growth factor; MHC-1, major histocompatibility complex class 1; S1P, sphingosine-1-phosphate. The figure was created with BioRender.com.

also an increase in COX-2 expression in tumor cells, which led to increased activation of circulating platelets and tumorigenesis. COX-1 and COX-2 have the same substrates and produce the same types of prostanoids, but the latter requires a smaller substrate for this synthesis. For this reason, COX-2 has emerged as a promising target for treating CRC. A COX-2 inhibitor in patients can have a chemopreventive effect [38]. Additionally, the pro-angiogenic factor, podoplanin, is highly expressed in CRC cells, and its interaction with the platelet C-type lectin-like receptor 2 (CLEC-2) promotes platelet activation, contributing to tumor progression and metastasis formation [57].

ADP, TXA2, and thrombin promote platelet activation and contribute to tumor growth and angiogenesis. Furthermore, TF expressed by cancer cells enhances thrombin generation by bidding with the VIIa factor, forming a complex that promotes the activation of IX and X factors [57]. Circulating platelets activate and release VEGF upon binding to TF expressed on endothelial cells. Along with platelet microparticles (PMP), this factor promotes angiogenesis and induces tumor cells to express pro-angiogenic factors [34].

Platelets challenge the recognition and elimination of tumor cells by leukocytes. This is achieved by forming a protective barrier around the cancer cell, transferring Major Histocompatibility Complex Class 1 (MHC-I) to the neoplastic cell, and secretion of TGF-β. TGF-β, in turn, contributes to a reduction in the expression of the NKG2D receptor in natural killer (NK) cells. Additionally, platelets inhibit neutrophil function, which typically induces apoptosis of tumor cells through synthesizing H_2O_2. Platelet surface receptors also facilitate the adhesion of tumor cells to adhere to distant sites in the body, favoring metastasis. Platelet-secreted TGF-β also supports angiogenesis and the formation of premetastatic niches. The inhibition of TGF-β is related to decreased tumor growth by recruiting CD8 T lymphocytes, macrophages, neutrophils, and NK cells [34, 38, 57, 58, 64, 65]. Conversely, platelets activate the TGF-β and NF-kB pathways in tumor cells, leading to EMT and metastasis [64].

Neoplastic cells can trigger the release of PMPs, which display a range of integrins on their surface, including $\alpha_{IIb}\beta_3$, and contain several bioactive lipids such as arachidonic acid (AA) and sphingosine one phosphate (S1P). The interaction between PMPs and specific cells

can activate both platelets and leukocytes. Indeed, PMPs play a role in transferring genetic material to cancer cells, which can lead to increased invasiveness and allow them to evade tumor suppressive mechanisms [57, 58, 64]. Cancer cells can also transfer their RNA to platelets, creating what is known as tumor-educated platelets (TEP). This incorporated genetic material reduces the expression of genes related to immunity and protein synthesis while increasing the expression of genes linked to cancer [34]. Rodriguez-Martinez et al., 2022 [65] observed that the co-culture of SW480 cells with platelets resulted in a higher rate of substances from platelets to the neoplastic cells. This process appears dependent on physical contact between two cells. Furthermore, the co-culture of colon cells with platelets led to a decrease in the expression of epithelial cell adhesion molecule (EpCAM) after 24 h, and an increase in the expression of genes associated in EMT (e.g., *VIMENTIN*, *SNAIL-1/2*) and those which support the cellular structure (e.g., *REX1*, *OCT4*, and *NANOG*) after one hour of incubation [58].

During the colitis phase, Servais et al., 2018 [59] observed an increase in the expression of P-selectin and $\alpha_{IIb}\beta_3$ integrin on the platelet membrane, which normalized when cancer developed. Moreover, using clopidogrel reduced dysplasia, formation of adenomas and adenocarcinomas, disease activity index (DAI), diarrhea, and bleeding in the stool. This platelet inhibitor also reduced the accumulation of myeloid-derived suppressor cells (MDSC) in the blood and the expression of the *Tgfb* and *Cebpb* genes, which are related to the immunosuppressive activities of these cells. Haemmerle et al., 2017 [66] showed that incubation of ovarian and colon cancer cell lines with platelets for a period of 24-48 h led to a decrease in programmed cell death related to the lack of adhesion (anoikis) of neoplastic cells through S127 and S397 dephosphorylation and activation of the yes-associated protein 1 (YAP1) transcription factor, which is translocated to the nucleus. This process was mediated by the RhoA GTPase that interacted with the PP1 phosphatase at myosin phosphatase target subunit 1 (MYPT1), reducing inhibitory phosphorylation at T696 and T853 and leading to dephosphorylation of YAP1. This indicates that platelets are essential tools in the survival and spread of tumor cells throughout the body and participate in the inflammatory process that originates CRC.

Cancer-related platelet parameters

Several platelet indicators are associated with its activation and function, as well as assisting in the early diagnosis and prognosis of various types of cancer. Usually, four key platelet indices are measured: (1) number of circulating platelets (PLT); (2) platelet crit (PCT), which indicates the percentage of blood volume that is occupied by platelets; (3) platelet distribution width (PDW) and (4) mean platelet volume (MPV) [67]. An elevated blood platelet count serves as an indicator of metastasis presence and an unfavorable prognosis. Additionally, thromboembolism was noted in 15% of patients with a confirmed cancer diagnosis. This occurrence was particularly prevalent in the initial months post-diagnosis, with an escalated risk associated with factors like hyperviscosity syndrome, chemotherapy, infections, and surgical interventions [34, 59, 64].

Qian et al., 2019 [67] showed that although surgical tumor removal followed by chemotherapy treatment reduces PLT, PCT, and PDW levels, only changes in MPV levels seem to affect the overall survival of patients with CRC. MPV is another indicator that can be used in the prognosis of IBD, such as Crohn's disease and most neoplastic disorders [33, 68-71]. This parameter determines the size of the platelet as well as its activity. Platelets with a high MPV (>15 fl) are very active young cells consumed more quickly than others. A rise in MPV level is related to an increase in platelet aggregation and the release of TXA2 and β-thromboglobulin. This circumstance is also associated with the increase in the interaction of cytokines with megakaryocytes and the induction of pro-platelet release [33]. Numerous studies have demonstrated an increase in this parameter in patients with colorectal cancer (CRC), and the reduction of MPV following tumor removal is associated with a favorable prognosis and patient recovery [67, 70, 71]. However, reduced MPV has also been observed in some neoplastic diseases. Additionally, Karagöz et al., 2010 [72] observed that, although there was an increase in the number of platelets in cancer patients, no difference was observed in MPV

values. Qian et al., 2019 [67] observed that maintaining unchanged MPV levels in CRC patients undergoing surgical procedures and chemotherapy treatment is desirable to obtain a reasonable prognosis of the pathological condition, and a decrease in MPV levels leads to the opposite effect. Complementing this data, a study showed that MPV reduction is associated with the presence of cytokines and growth factors (e.g., TNF-α, IL-1β, VEGF) secreted by several cells which cause an endothelial vascular thrombus and the fragmentation of large sized platelets [73].

Treatment of colorectal cancer

Diagnosis of Lynch syndrome-related colorectal cancer

To determine whether the adenoma or adenocarcinoma identified in routine tests is linked to a hereditary factor such as LS, until recently, the diagnosis consisted of surveying the patient's medical and family history according to the Amsterdam and Bethesda criteria (e.g., family members diagnosed with CRC before the age of 50, individuals with synchronous or metachronous cancers, tumors with MMR genes deficiency by MSI or loss of protein expression), with the adjuvant use of clinical prediction models (e.g., PREMM5) which assist in the determination of the patients who will undergo genetic screening (e.g., immunohistochemistry, MSI test, multigene test). The results obtained from biopsies collected during colonoscopy exams determine whether surgical removal will be total or segmented. Since preoperative chemotherapy and radiotherapy may lead to a false negative result for LS, it is recommended that immunohistochemistry (IHC) be performed before therapy. However, due to the inability to carry out a more detailed survey of the patient's medical and family history in clinical practice, their criteria are shown to be less accurate than those who worked on clinical trials [8, 11, 74-77]. Since 2017, the Amsterdam and Bethesda criteria for identifying and managing LS patients have been replaced by those of the National Institute for Clinical Excellence (NICE). According to this, all newly discovered CRCs should be analyzed for the identification of dMMR by IHC or the identification of MSI, and the results serve as a guide for evaluating the degree of LS; this leads to an improvement in the agility of diagnosis and the treatment efficacy [10].

Several signs are related to LS, such as skin lesions like sebaceous adenomas, sebaceous adenocarcinomas, and keratoacanthomas. These signs are most commonly associated with *MLH1* and *MSH2* mutations, and more than half of individuals with these skin lesions subsequently manifest internal malignancy [11]. LS patients have fewer precancerous polyps in the intestinal mucosa compared to individuals with other CRC-predisposing syndromes; furthermore, tumors that present the *MLH1* pathogenic variant are generally not identifiable, while individuals carrying the pathogenic variant for *MSH2* often develop adenomas that can be observed [8, 9].

The diagnosis of LS is confirmed through comprehensive microscopic and molecular analyses, including germline testing, immunohistochemistry (IHC) to detect deficient expression of MMR proteins, and the identification of MSI via polymerase chain reaction (PCR). This process involves comparing the number of nucleotides repeats between normal and tumor tissue from the same individual using a panel of five microsatellite markers provided by the National Cancer Institute (NCI). According to the presence or absence and the number of unstable mononucleotides, tumors are classified as MSI with high instability (MSI-H) (≥ two mononucleotides), MSI with low instability (MSI-L) (1 mononucleotide) or stable (MSS). Tumors that present lesions with MSS can progress to MSI when polyps grow to >8-10 mm in diameter. Patients undergo genetic testing and counseling in case of an abnormal result [9-11, 78].

The molecular analysis of MSI-H holds significant prognostic value. Patients with MSI-H tumors tend to exhibit an enhanced response to immunotherapy, such as anti-PD1 treatments, due to the elevated frequency of frameshift mutations that produce neoantigens and a heightened immune response. Consequently, these patients often experience a more favorable disease course. Also, using frameshift peptides (FSP) induces a specific cellular and humoral immune response in LS individuals, and detecting FSP-specific antibodies and T cells may be useful for the early diagnosis of cancer. Tumors can present frameshift mutations in genes such as *HLA Class 1* and *β2*

microglobulin that grant immune system evasion. However, this genetic profile is generally associated with a more benign type of cancer [8, 10, 11].

In the absence of tumor tissue, IHC can be performed on adenomas. Abnormal loss of color, polyp size (>10 mm), and the presence of high-grade dysplasia impact the Lynch Syndrome diagnosis [13, 79-81].

A pathogenic variant for MMR genes does not imply a standard diagnosis for all patients. Penetrance and expressivity impact the determination of the cancer development risk rate. Despite this, due to the high risk of early CRC onset, most guidelines for LS patients' surveillance recommend that the minimum age at which investigation for the presence of CRC should be carried out in individuals with *MLH1* and *MSH2* mutations by an imaging test (e.g., colonoscopy) is 25 years. The frequency of monitoring should be done every 1 or 2 years [10]. In addition to periodic colonoscopy, the detection of high levels of CD3, CD8, and POXP3 positive T lymphocytes, as well as mutations in the *EPCAM* gene in adenomas can help in the early diagnosis of LS-associated CRC [8, 10, 13].

In the case of CRC, unrelated to any familial history or inherited DNA mutation, identification of *MLH1* hypermethylation or mutation of the *BRAF V600E* gene can aid in early cancer diagnosis [82].

Chemotherapy

One class of adjuvant therapies frequently employed in the treatment of advanced CRC stages (III-IV), or in patients with elevated risk factors for metastasis, comprises chemotherapy agents such as capecitabine, 5-fluorouracil (5-FU), irinotecan, and oxaliplatin [83-86]. Genetic exams support the choice of the best therapeutic alternative. 5-FU is a pyrimidine analog, and it acts by inhibiting thymidylate synthase (TS), leading to the exchange of thymidine for fluorinated nucleotides in the DNA of the tumor cell, causing its death. TYMS has excellent prognostic value when determining the gene expression that encodes TS. Patients with low TS expression have a better survival rate than those with high enzyme expression [32].

Most chemotherapy drugs act on tumor cells undergoing cell division. As in CRC, there is a small fraction of dividing cells compared to leukemia and some types of lung cancer, and these cells multiply at a slower rate; this disease responds poorly to chemotherapy alone. Therefore, surgical removal of part of the tumor is performed to induce cancer cells from stage G_0 to the cell division stage [87]. The use of chemotherapy can occur in the absence or presence of radiotherapy. Such being the case, the procedure is carried out before surgery to avoid the disease's recurrence and possible damage to the intestinal mucosa that will be subjected to radiotherapy. The use of targeted therapy after the use of chemotherapy drugs has also been shown to be an effective method [32, 85, 86].

However, the disadvantage of using available chemotherapy drugs is a series of side effects such as diarrhea, nausea, vomiting, weakness, paresthesia, loss of taste, and alopecia, which lead to loss of compliance by the patient as well as the need to administer new medications, which in turn increases the total cost of treatment [85, 86].

Targeted therapy

Because cancer cells can vary significantly in appearance and behavior, therapy targeting specific genes or proteins may offer a more tailored approach. This therapy is generally used in combination with chemotherapy by advanced-stage CRC patients or by patients who are not responding to chemotherapy alone. As an advantage over chemotherapy, using specific drugs has fewer side effects, which can be easily overcome. Among the most commonly used medications are angiogenesis inhibitors (e.g., bevacizumab, regorafenib, ramucirumab), epidermal growth factor receptor (EGFR) inhibitors (e.g., cetuximab, panitumumab) and *BRAF* inhibitors (e.g., encorafenib). In rare instances of CRC with *NTRK* gene fusions, specific drugs such as larotrectinib and entrectinib can be effective treatments [32, 85].

Drug classes used for the inhibition of the Wnt/β-catenin pathway

Another important therapeutical target is the Wnt/β pathway. As in several types of cancer, dysregulation of the Wnt/β-catenin pathway is

involved in regenerating the tumor stem cell population, cell proliferation, and differentiation [21].

Therapies used for the inhibition of the Wnt/β-catenin pathway nowadays consist of the use of natural components (e.g., vitamin D, curcumin, genistein), existing drugs (e.g., NSAIDs) and small molecule inhibitors (e.g., YW2065, OMP-18R5, anthocyanins) [21]. Many drugs that are being developed for the treatment of CRC act to reduce the expression of survivin (e.g., IWR-1), inhibit the Wnt/β-catenin pathway through mediators' blockage such as CDC-like kinase (CLK) (e.g., SM08502) and porcupine (PORCN) (e.g., ETC-159) or produce genetic variants of the Wnt/β-catenin pathway that are involved in tumor cell gene suppression (e.g., SM08502) [21, 88].

CRC inhibitory small molecules are divided into three groups: (1) those that bind to Dvl proteins and affect the stability of the complex that inhibits the degradation of β-catenin and the formation of the *Axin*/GSK3/*APC* complex, (2) those that interfere with the binding of β-catenin with TCF and (3) those that act as antagonists of transcription co-activators [21].

Equally relevant, natural products have marked the history of cancer treatment, and some compounds used today, such as irinotecan and camptothecin, originate from them [89]. Sishen Wan, a medicine listed in the Chinese Pharmacopoeia, through inhibition of the Wnt/β-catenin pathway, promotes the reduction of macro and microscopic lesions, leading to a decrease in colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS). *Dendrobium officinale* polysaccharide (DOP) is another Chinese medicinal product with the polysaccharide as the main active ingredient. DOP impairs the expression of genes involved in cancer cell survival and proliferation (e.g., *Wnt2β*, *GSK3β*, *CyclinD1*, and *β-catenin*) as well as inhibits precancerous lesions of gastric cancer induced by 1-methyl-2-nitro-1-nitrosoguanidine (MNNG) through Wnt/β-catenin pathway modulation and modification of endogenous metabolites [21, 90].

Immunotherapy

Immunotherapy is another treatment option for situations where the tumor is in an inoperable condition or metastasis. The drugs assist the immune system in combating neoplastic cells that generally present microsatellite instability (MSI-H) or mismatch repair deficiency (dMMR) through inhibition of the PD-1 receptor. Some examples of immunotherapeutic drugs are pembrolizumab, nivolumab, and dostarlimab [86]. Tumors that present a high rate of mutations are more susceptible to immunotherapy since these types of cells express a large amount of neoantigens, which increase the immunogenicity of the cells. The hypothesis is that certain chemotherapy drugs or targeted therapies increase the immunogenicity of the cancer cell, which the host immune system will destroy. Neoplastic cells that are destroyed immunogenically release surface markers (e.g., calreticulin) and factors (e.g., HMGB1) that induce an adaptive immune response that can benefit from the use of checkpoint inhibitor drugs (CPI). The VEGF factor negatively modulates the maturation of dendritic cells. Therefore, VEGF inhibitor drugs such as Bevacizumab can enhance the immune response and increase patient survival. Although the development of monoclonal antibodies has improved the patient's response to treatment, the resistance of neoplastic cells to drugs still proves to be an obstacle to be overcome since, for metastasizing CRC, the 5-year survival rate is still a little more than 12% [32].

Factors such as surgical interventions can reduce the effectiveness of locally acting drugs. To overcome this issue, several groups are developing clinical trials based on bispecific antibody models that simultaneously bind to tumor proteins (e.g., CD20, Neu, EGFR) and immune system cells [32, 91-93].

Despite being considered one of the leading causes of resistance to chemotherapy therapy and disease relapse, slow-cycling cells (SCC) have received positive attention in cancer immunotherapy. Sun et al., 2012 [94] demonstrated that vaccinating colon cancer-bearing mice with SCCs led to a decrease in tumor volume and an increase in survival.

Chemotherapy drug resistance factors

Cells at the G_0 stage and cancer stem cells

Since most cancer-related deaths are linked to the appearance of metastases, the study of

new drugs that have an inhibitory effect on the progression and recurrence of the tumor has become quite common [6, 84]. A significant challenge for the healthcare team is the resistance of specific neoplastic cells to treatment. One key factor in this resistance contributing to cancer recurrence is the presence of SCCs. Although these cells are in the G_0 stage, when faced with certain stimuli, such as the stroma induction secreted protein, CoCo, they can become active and enter cell division [95, 96].

In addition to the ability to divide, the property of a neoplastic cell to originate another cell type is another aspect linked to the appearance of disease relapses. CRC tumors are built by a heterogeneous population of cells with cancer stem cells (CSC). The characteristics that make up these cells are the presence of adhesion proteins (e.g., EpCAM, DC44), the hyperactivation of the β-catenin, the TGF-β and the *Notch* and *hedgehog* genes signaling pathways, and the expression of efflux pumps similar to that belonging to the family of ATP-binding cassette transporters. Because CSCs have a low replication rate, they are not affected by treatments that eliminate their progeny and, therefore, tumors can recur. The resistance of stem cells to chemotherapy treatment and their regeneration capacity are important prognostic markers [21, 32, 97].

Genetic modifications

Changes in the karyotype, such as the presence of an extra pair of small chromosomes (double minute chromosomes) and the existence of a uniformly stained region interspersed between the places where the regular chromosomes are located, are also related to the tumor resistance to multiple antineoplastic therapies. Tumors that present a diminished MMR gene expression, like the LS-related CRCs, respond poorly to cytotoxic chemotherapy agents since these drug classes require the proper function of the MMR mechanism to induce tumor death. Specific genes, such as *mdr-1*, hinder the entry of fat-soluble drugs by encoding an ATPase [11, 98, 99]. More recent studies in CRC have shown that tumors with mutations in the *RAS* or *BRAF* genes do not respond adequately to EGFR inhibitors but are susceptible to treatment with *BRAF* inhibitors (e.g., encorafenib). On the other hand, a mutation in the G13D portion of *K-ras* is related to increased tumor sensitivity to anti-EGFR therapy [32, 85].

Epigenetic modifications such as histone acetylation are involved in tumor resistance to irinotecan. Furthermore, inhibiting *FGF2*, *FGF9*, *MECOM*, *PLA2G4C*, and *PRKACB* seems to improve the response to chemotherapy [32]. Oxaliplatin resistance factors are linked to the genes responsible for the nucleotide excision repair pathway, the WBSCR22 protein, and the secretion of TGF-β1 by tumor microenvironment cells [32, 100]. Moreover, metastatic CRC cells show resistance against anti-VEGF therapy. This aspect is linked to an increase in the expression of the VEGFR1 receptor and a decrease in the levels of hepatocyte growth factor (HGF) [32].

Cells that comprise the tumoral microenvironment

Cells present in the microenvironment can contribute to increased tumor resistance to chemotherapy. Matrix stiffness is an essential parameter for studying the cellular response of cancer cells in the TME [101]. It was shown that in CRC-induced liver metastasis, there is the deposition of extracellular matrix and an increased fatty acid oxidation pathway activation, which is involved with increased tumor resistance to anti-angiogenic agents (e.g., bevacizumab). Inhibition of fatty acid oxidation with etomoxir improved the efficacy of anti-angiogenic drug treatment. Hepatic stellate cells are involved in matrix stiffness and fatty acid oxidation through the activation of the focal adhesion kinase (FAK)/yes-associated protein (YAP) pathway, which leads to lipolysis and fatty acid secretion [102].

Long-term treatment with oxaliplatin can cause this drug to be captured by cancer-associated fibroblasts, leading to increased TGF-β pathway activation and production of multiple factors, such as IL-11 and the *POSTNi4* gene, which enhance the tumor aggressiveness [103]. Also, when in contact with neoplastic cells, Wnt secreted by fibroblasts can cause their differentiation and increase their resistance to therapy. Also, the interaction of elements of the Wnt pathway of the neoplastic cell with the immune system cells present in the microenvironment limits their anti-tumor response and

favors the increase of the cancer cell tolerance against the immune response. However, this association is also essential for renewing the leukocyte population and maintaining damaged ones [21].

Bacteria present in the intestinal microbiota can also interfere with immunotherapy against CRC. Jiang *et al.,* 2023 [104] observed that *Fusobacterium nucleatum* can decrease the efficacy of anti-PD-1 immune checkpoint blockade therapy through succinic acid secretion, which inhibits the cyclic GMP-AMP synthase (cGAS)-interferon β pathway and decreases the CD8 cells flow to the TME. The use of metronidazole increased tumor sensitivity to anti-PD-1. Conversely, bacteria that make up the gut microbiome are essential for certain anti-tumor drugs's effects support. Studies have shown that commensal microorganisms of the *Bacteriodale*s and *Burkholderiale*s species are essential for maintaining the anti-tumor effect of ipilimumab and that antibiotics can impair its therapeutic effect [32].

*Enzymes involved in the degradation of chemotherapeutic drug*s

Endogenous enzymes are involved in the degradation of chemotherapy drugs. Therefore, they interfere with their mechanism of action. Thymidine phosphorylase, uridine phosphorylase, orotate phosphoribosyl transferase, and dihydropyrimidine dehydrogenase can carry out the metabolism and degradation of 5-FU. Similarly, carboxylesterases, uridine diphosphate glucuronosyltransferase, CYP3A, β-glucuronidase, and the ATP-binding cassette transporter protein (ABC) are involved in the sequestration and metabolism of irinotecan [32].

Drugs that affect platelet activation

Potential therapeutic approach for CRC based on inhibition of platelet activation

As seen previously, tumor cells have the ability to activate circulating platelets. This activation can benefit neoplastic cells, hindering their recognition by immune system cells, helping in their proliferation, and facilitating their dissemination throughout the body. Treatment of different types of tumors with a series of drugs already being marketed and acting on platelet activation has shown promising results. Since an increase in TXA2 synthesis was observed in CRC by identifying the 11-dihydro-TXB2 metabolite, one of the approaches was to investigate the effect of daily use of aspirin, a COX inhibitor, on the disease progression [38, 58, 63, 105]. Aspirin can reduce tumor growth by inhibiting the expression of oncoproteins on the surface of the neoplastic cell induced by platelets (e.g., c-MYC). However, the antitumor effect of aspirin may also come from interaction with other vascular cells or tumor cells themselves [106].

Determining the positive effects of aspirin on cancer prevention is a recent achievement, and some studies have presented controversial results. In 2017 and 2018, several prospective studies were carried out to determine the effect of the drug on the incidence of different types of cancer, some of the most prominent being carried out by the steering committees of the studies "Aspirin in Reducing Events in the Elderly" (ASPREE) and "Aspirin to Reduce Risk of Initial Vascular Events" (ARRIVE). Although the latter showed an increase in the incidence of cancer linked to the use of the drug, ASPREE indicated a minimal increase in the incidence of several types of cancer, including CRC, compared to the number of deaths linked to the disease and also not observed an increase in mortality in patients who already had the condition [106].

On the one hand, it was shown that the inhibition of the platelet P2Y12 receptor and the decrease in the integrin $\alpha_{IIb}\beta_3$ activation, induced by clopidogrel, was related to improving the cancer-associated colitis in C57BL/6J mice [59]. On the other hand, a study carried out by the American FDA showed a link between prasugrel, another P2Y12 receptor inhibitor, and a significant increase in the incidence of colon cancer compared to the use of clopidogrel. However, considering the number of cases, a non-significant difference was noted between the two groups (thirteen vs. four patients) [106].

Although drugs that block platelet $\alpha_{IIb}\beta_3$ integrin or its respective ligand on the tumor cell membrane are associated with a decreased incidence of metastasis [107], their chronic use is not recommended as this may lead to an increased risk of bleeding. Since the $\alpha_{IIb}\beta_3$ integrin is activated in the presence of the tumor cell, a safer and more effective approach to

treatment is to perform molecular imaging tests to identify the tumor's exact location. Another treatment option is the use of drugs that block proteins that are not essential for the execution of a physiological function of the platelet but that are involved in its interaction with tumor cells. One example is the inhibition of the signaling pathway induced by the platelet CLEC-2 receptor, which is involved in the interaction with tumor podoplanin by Btk protein inhibitors (e.g., ibrutinib) [106].

Takahashi et al., 2017 [108] performed a retrospective cohort study where they evaluated the effect of the use of antithrombotic therapy (ATT), consisting of antiplatelet drugs (aspirin, and clopidogrel) and anticoagulants (warfarin, edoxaban, rivaroxaban, apixaban, dabigatran) on the progression of CRC. The use of ATT promoted a reduction in disease recurrence and the need for chemotherapy. ATT also improved the overall survival of patients with stage 0-III CRC, which makes it positively related to the prevention of the disease and the occurrence of micrometastasis. The "clopidogrel for high atherothrombotic risk and ischemic stabilization, management and avoidance" (CHARISMA) clinical trial showed that the combined use of two antiplatelet drugs (aspirin and clopidogrel) led to more excellent prevention of cancer incidence than the use of aspirin alone [109, 110]. However, a study with the benefit of dual antiplatelet therapy, where prasugrel was one of the drugs tested, showed an increased risk of the appearance of new cancers. This probably occurred by eliminating the platelet barrier, essential for blocking the advancement of neoplastic cells in the vasculature [111].

According to Symeonidis et al., 2012 [105], in addition to aspirin and clopidogrel, the use of other antiplatelet drugs, such as ticlopidine and dipyridamole, appears to improve the early detection of CRC, as it reduces the incidence of the disease in a more advanced stage (stage IV, according to the American Joint Committee on Cancer classification). Another possible platelet target that could be explored in cancer treatment is the PKCs. Irie et al., 2012 [112] observed that the natural compound bryostatin 1 and its less lipophilic analog, along-1, suppress tumor growth by activating PKCδ.

The anticoagulant heparin has also been associated with reducing metastasis by blocking the binding of platelet P-selectin to sialyl Lewis-carrying mucins present on the surface of the neoplastic cell [106]. In another sense, it can also inhibit tumor-induced thrombin release. However, it can cause angiogenesis by inhibiting VEGF, tissue factor, and platelet-activating factor (PAF) [108].

Certain tumors can trigger thrombopoiesis by causing the liver to release IL-6 and stimulating thrombopoietin secretion. It has been reported that the use of siltuximab, an antibody against IL-6, led to a decrease in the growth of ovarian tumors. Excluding situations where the patient uses myelotoxic treatment or has an immune response or infection-associated thrombocytopenia, the use of anti-thrombopoietin drugs and a decrease in the number of circulating platelets to levels still within physiological standards seems to be a safer treatment than those who use medications that have a systemic effect [106].

The use of antiplatelet drugs (e.g., aspirin, ticlopidine) is indicated for the treatment of vascular complications caused by CRC, such as VTE. However, this drug class can increase the risk of bleeding in the gastrointestinal tract, lungs, and brain, especially in cancer individuals. Therefore, developing antiplatelet drugs with fewer side effects is crucial [60, 113]. Resveratrol, a natural polyphenolic compound derived from grapes, blueberries, and peanuts, has been shown to reduce thrombus formation and stricture incidence in colon cancer patients. This occurred through the increase in cGMP synthesis and VASP phosphorylation and the inhibition of Akt and p38MAPK phosphorylation, which led to the reduction of PLC activation, DAG, and IP3 production with a consequent decrease in $[Ca^{2+}]$ and inhibition of platelet activation, adhesion and aggregation. As this inhibitory effect occurs without affecting platelet viability, this minimizes the risk of thrombocytopenia and bleeding, which makes resveratrol a safer option for VTE treatment [113].

The increase in platelet activation and the risk of thrombotic events are intricately associated not only with the progression of cancer but also with the administration of specific chemotherapy drugs, particularly those derived from platinum. New chemotherapy drugs derived from other metallic elements have been developed

to address this issue. Shyu et al., 2018 [114] developed a drug derived from Iridium, Ir-3. This drug promoted a significant but reversible decrease in collagen-induced platelet aggregation, ATP secretion, intraplatelet calcium mobilization, and P-selectin expression. Also, Ir-3 inhibited PLCγ2, impacting the activation of PKC, the phosphorylation of the Akt protein, and the activation of c-jun N-terminal protein kinase 1 (JNK1).

TRAIL, another cytokine belonging to the TNF family, presents characteristics similar to TNF-α, such as pro-apoptotic properties through the activation of DR4 and DR5 death domain-coupled receptors or pro-tumorogenic, through the activation of kinases. Wu et al., 2019 [115] observed that the treating platelets co-incubated with HT29 and HCT116 with TRAIL decreased the invasiveness of these cells. This reduction is likely due to TRAIL binding to the DR4 receptor and decreasing platelet secretion of TGF-β1. Notably, TRAIL did not affect the clotting ability of other circulating platelets, suggesting it could be a promising and safe therapeutic option for treating CRC.

Future perspectives for CRC treatment based on platelet modulation

Despite their role in facilitating the proliferation of neoplastic cells, platelets have been investigated to propose various experimental models for chemotherapy drug transport. Using platelets loaded with chemotherapy drugs (e.g., doxorubicin) combined with immunotherapy aims to enhance treatment specificity. The overarching goal is to mitigate potential side effects and to thwart tumor evasion from the immune system [116, 117].

The platelet signaling pathways of cancer are complex and have not yet been determined. The use of antiplatelet drugs as a preventive method for various types of cancer is a recent practice. Many studies have shown controversial results, with some offering a benefit from the use of these drugs and others warning about the increased risk of tumor incidence and recurrence. As there is a wide variety of CRC types with different etiologies and behaviors, this suggests that a complex web of signaling pathways encompassing other cells that are important for the protection and correct functioning of our body is involved. A significant challenge in researching new treatment alternatives for CRC is determining a pathway that is exclusively engaged in the pathogenesis of the disease. The adjuvant use of diagnostic tests can improve treatment by helping to choose the most appropriate drug and, thus, avoid possible adverse effects.

To this end, improving the population's economic conditions and public health policies can increase patient's access to these tests and, in this way, promote early detection of the disease and increase their quality of life [3]. It is also worthwhile to explore the interaction of neoplastic cells with platelets to develop new transport systems for chemotherapy drugs. Going deeper, the evidence proving the involvement of PKC in platelet activation in cancer makes it a relevant target to be explored in developing new therapies for CRC.

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None.

Abbreviations

AA, arachidonic acid; ABC, ATP-binding cassette; AC, adenylyl cyclase; ACF, aberrant crypt foci; ADP, adenosine diphosphate; *APC*, *adenomatous polyposis coli*; ARRIVE, Aspirin to Reduce Risk of Initial Vascular Events; ASPREE, Aspirin in Reducing Events in the Elderly; ATT, antithrombotic therapy; CAC, colitis-associated cancer; CalDAG-GEFI, calcium and diacylglycerol-regulated guanine exchange factor 1; cAMP, cyclic adenosine monophosphate; CHARISMA, clopidogrel for high atherothrombotic risk and ischemic stabilization, management and avoidance; CLEC-2, C-type lectin-like receptor 2; CLK, CDC-like kinase; CMS, consensus molecu-

lar subtype; COX, cyclooxygenase; CPI, checkpoint inhibitor; CRC, Colorectal cancer; CSC, cancer stem cells; DAG, diacylglycerol; DAI, disease activity index; *DCC*, *deleted in colon carcinoma*; dMMR, mismatch repair deficiency; DOP, *Dendrobium officinale* polysaccharide; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; ERK, extracellular signal-regulated kinase; 5-FU, 5-fluorouracil; GAP, GTPase-activating protein; GSK-3β, glycogen synthase kinase 3β; HDI, Human Development Index; HGF, hepatocyte growth factor; IBD, Inflammatory bowel diseases; IL, interleukin; IP3, inositol triphosphate; LPS, lipopolysaccharide; *Mdr-1, multidrug resistance 1*; MDSC, myeloid-derived suppressor cells; MHC-I, Major Histocompatibility Complex Class 1; MLC, myosin light chain; *MMP-9*, *matrix metalloproteinase 9*; MNNG, 1-methyl-2-nitro-1-nitrosoguanidine; MPV, mean platelet volume; mRNA, messenger RNA; MYPT1, myosin phosphatase target subunit 1; MSI, microsatellite instability; NF-kB, nuclear factor kappa B; NOX1, NADPH oxidase 1; PAF, platelet-activating factor; PAR, protease-activated receptor; PCP, planar cell polarity; PCT, platelet crit; PDGF, platelet-derived growth factor; PDW, platelet distribution width; PGG2, prostaglandin G2; PGH2, prostaglandin H2; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PLA2, phospholipase A2; PLC β, phospholipase C beta; PMP, platelet microparticles; PORCN, porcupine; Rap1, ras-associated protein 1; RhoA, ras homolog family member A; ROR, receptor tyrosine kinase-like orphan receptor; RORγt, RAR-related orphan receptor type γ; ROS, reactive oxygen species; RSPO3, R-spondin-3; S1P, sphingosine 1 phosphate; SCC, slow-cycling cells; Ser473, serine 473; SOCE, store-operated calcium entry; STAT, signal transducer and activator of transcription; TCF, T cell factor; TEP, tumor-educated platelets; TF, tissue factor; *TGF-β*, *transforming growth factor beta*; TLR, toll-like receptor; TME, tumor microenvironment; TNBS, 2,4,6-trinitrobenzene sulfonic acid; TNF, Tumor Necrosis Factor; TS, thymidylate synthase; TXA2, thromboxane A2; TXS, thromboxane synthase; 3'UTR, 3'untranslated region; VEGF, vascular endothelial growth factor; VWF, von Willebrand factor; WHO, World Health Organization; YAP1, yes-associated protein 1.

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