Signal transducer and activator of transcription 6 as a target in colon cancer therapy (Review)

YAEL DELGADO-RAMIREZ[1], VANEESA COLLY[1,2],
GIOVANNI VILLANUEVA GONZALEZ[2] and SONIA LEON-CABRERA[1,2]

[1]Laboratory of Oncoimmunology, Biomedical Research Unit; [2]Medical School, Faculty of Superior
Studies Iztacala, National Autonomous University of Mexico, Tlalnepantla, CP 54090, Mexico

Abstract. Signal transducer and activator of transcription 6 (STAT6) is a member of the STAT family of proteins that serve key roles in the initiation of tumorigenesis and malignant transformation. STAT6 is highly expressed in several types of cancer, including breast, pancreatic, prostate and colorectal cancer. STAT6 transduces signals in response to the binding of interleukin (IL)-4 and IL-13 to their receptors and regulates the expression of genes involved in the immune response, cell survival, tumor proliferation and metastasis. Patients with colorectal cancer exhibit high STAT6 activity in the colonic epithelium, and STAT6 expression is associated with lower survival rates, lymph node metastasis, changes in the epithelial barrier function and alterations in the inflammatory response. A number of studies investigating experimental models and cancer cell lines have revealed that STAT6 is associated with tumor growth and development, as well as with increased invasion and metastasis, suggesting that STAT6 inhibition may serve as a novel therapeutic strategy in colon cancer. The present review summarizes the evidence with regard to the implications of STAT6 in cancer biology and the direct and indirect effects on colon tumor transformation. Furthermore, the current treatment strategies targeting the IL-4/IL-13/STAT6 axis in colon cancer are discussed.

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Correspondence to: Dr Sonia Leon-Cabrera, Laboratory of Oncoimmunology, Biomedical Research Unit, National Autonomous University of Mexico, Avenida De los Barrios 1, Los Reyes Iztacala, Tlalnepantla, CP 54090, Mexico
E-mail: soleonca@comunidad.unam.mx

1. Introduction

Colorectal cancer (CRC) has the second highest cancer-associated mortality rate worldwide and is the third most commonly diagnosed neoplasm; a total of 881,000 CRC-associated mortalities and 1.8 million newly diagnosed cases were reported in 2018 (1). Sporadic CRC may be caused by a hereditary mutation, such as in patients with familial adenomatous polyposis with bi-allelic inactivation of the adenomatous polyposis coli (APC) gene or in patients with Lynch syndrome. The remaining cases exhibit activating mutations in components of the Wnt signaling pathway that promote malignant cell proliferation (2). Additionally, sustained inflammation in the intestine, such as in patients with inflammatory bowel disease (IBD), favors the development of colitis-associated cancer (CAC).

During chronic inflammation in the intestine, barrier dysfunction increases susceptibility to bacterial infection. Furthermore, mucosal inflammation results in the accumulation of mutations that can result in the development of colon cancer. In CAC, inflammatory cytokines are persistently present in the intestinal tissue, and in patients with sporadic cancer, the administration of anti-inflammatory drugs can prevent or delay the disease, suggesting that inflammatory processes are involved in the initiation of tumorigenesis (3).

Cytokines serve as a means of communication among immune, cancer and non-transformed stromal cells in the tumor microenvironment. The signal transducer and activator of transcription (STAT) family of proteins serve an important role in the initiation of malignant transformation and in tumor establishment, and have been widely studied in experimental models and in patients with cancer (4). Upon cytokine binding to its receptor, Janus kinases (JAKs) mediate STAT phosphorylation. In the nucleus, STAT binds to specific DNA sequences that result in the transcription of target genes (5). Interleukin

(IL)-6, IL-10 and IL-23 signaling is mediated by JAK1 and recruits STAT3. Conversely, STAT6 functions as a transcription factor in the nucleus in response to IL-4 and IL-13 receptor binding after the activation of JAK1 and JAK3 (5). STAT6 orchestrates numerous processes beyond immune response, including cancer cell proliferation, apoptosis resistance, metastasis, epithelial cell function, chromatin compaction, and DNA damage and repair (Table I). STAT6 signaling is frequently activated in malignant cells and regulates several genes crucial for the immune response, inflammation and proliferation (5). Persistent activation of STAT6 in different types of cancer results in proliferation, survival and metastasis, as well as in decreased antitumor immunity. STAT6 polymorphisms have been identified in a subgroup of Malaysian patients suffering from Crohn's disease (CD), as well as in a patient cohort in Germany (6-8), demonstrating the importance of this gene in inflammatory processes in the colon.

Despite the advances in the diagnosis and treatment of CRC, the mortality rates remain high. Therefore, there is a requirement for the development of alternative therapeutic strategies for this disease. Interfering with the activity of STAT6 could be a potential strategy to target the action of IL-4 and IL-13, and the activation of other signaling pathways involved in tumorigenesis.

The present review describes the direct and indirect effects of STAT6 on colon tumor transformation and discusses the role of STAT6 in mucosal biology. Additionally, the current treatment strategies that target the IL-4/IL-13/STAT6 axis in colon cancer are summarized.

2. Overview of the IL-4/IL-13/STAT6 signaling pathway

STAT6 serves important roles in signal transduction throughout the cytoplasm and is a transcription factor in the nucleus. Cytokines and growth factors bind to their cognate receptors and activate the JAK family. Once activated, JAKs phosphorylate docking sites in the SH2 domain of STAT molecules. After phosphorylation, STATs form homodimers or heterodimers, which are transported into the nucleus where they regulate the expression of genes (5).

STAT6 activation occurs by IL-4 or IL-13 binding to their receptors. Currently, three types of receptors have been described. The IL-4 type I receptor is composed of the IL-4 receptor α (IL-4Rα) chain and the common γ chain (γc). The IL-4Rα chain has a high affinity for IL-4, while the γc is a component of receptors that bind IL-2 and IL-9. IL-4 binds to the IL-4Rα chain, resulting in dimerization with the γc chain and receptor activation (9). However, in human colon carcinoma cells, it has been reported that the IL-4Rα chain does not bind with the γc (10).

The type II receptor is composed of the IL-4Rα and IL-13 receptor α variant 1 (IL-13Rα1) chains, and is able to bind to both IL-4 and IL-13. IL-13 binds to the monomer IL-13Rα1 with low affinity, and requires heterodimerization with IL-4Rα to form a high-affinity bond. This complex recruits JAKs and leads to the downstream activation of STAT6 (9).

Additionally, IL-13 can bind to the IL-13 receptor α variant 2 (IL-13Rα2) chain; this receptor is a monomer that binds IL-13 with higher affinity than IL-13Rα1 and is expressed in T cells, B cells and endothelial cells, among others, where it has

been demonstrated to promote tumorigenesis (11). IL-13Rα2 is considered as a decoy receptor, as it is able to bind IL-13 and prevent it from binding to IL-13Rα1 (11).

After stimulation of the IL-4Rα receptor, JAK1-3 and tyrosine kinase 2 (Tyk2) are phosphorylated in the cytoplasmic tails of the receptor. Once active, JAKs phosphorylate the tyrosine residues Y575, Y603 and Y631 on the receptor, generating docking sites for STAT6. When monomers of STAT6 become phosphorylated on tyrosine Y641, the C-terminal of the SH2 domain usually forms homodimers (12) that are translocated to the nucleus, where they can bind DNA and activate or repress target genes. Therefore, the activity of STAT6 depends on tyrosine phosphorylation. However, the proximity of subunits may influence heterodimer formation. The stimulation of B cells with interferon (IFN) type 1 promotes the generation of STAT2:STAT6 heterodimers (13).

Proper regulation of STAT6 signaling is crucial, and specific molecules have been identified to regulate this signaling pathway. Suppressor of cytokine signaling (SOCS) proteins, particularly SOCS1, repress the activation of JAK1/3 and STAT6, and affect IL-4-induced proliferation (14). STAT6 signaling induces SOCS1 expression in a negative feedback loop, inhibiting the expression of STAT6-responsive genes (14). Additionally, the IL-4/STAT6 signaling pathway induces SOCS3 expression in intestinal epithelial cells. Increased SOCS3 levels in patients with ulcerative colitis (UC) are indicative of disease exacerbation (15). STAT6 activation is responsible for SOCS3 induction and affects the regulation of STAT1 and STAT3 signaling (15).

Protein tyrosine phosphatase (SHP1) negatively regulates IL-4/IL-13 signaling and STAT6 gene induction (16). Studies investigating the overexpression of SHP1 demonstrated a decrease in IL-4/IL-13 signaling, suggesting that tyrosine phosphorylation of STAT6 is regulated by SHP1 (16). Protein tyrosine phosphatase 1B (PTP1B) has been demonstrated to dephosphorylate cytoplasmic and nuclear STAT6, and attenuate its signaling. In diffuse large B-cell lymphomas, PTP1B contributes to STAT6 dephosphorylation, thereby enhancing tumorigenesis and inflammatory processes (17). Additionally, STAT6 function is regulated by methylation. When STAT6 is methylated on arginine at the N-terminus, decreased phosphorylation, nuclear translocation and DNA-binding activity are observed in response to IL-4 (18).

Several target genes of STAT6 have been identified and characterized. The STAT6 signal transduction pathway serves an important role in mediating the biological functions of IL-4 and IL-13 in processes associated with Th2 immune responses. During allergic reactions, STAT6 mediates T helper 2 (Th2) cells and eosinophil recruitment within sites of allergic inflammation, and is involved in immunoglobulin (Ig) class switching to produce IgE (5).

However, abnormal STAT6 activation may contribute to the pathology of cancer by increasing the expression of proteins involved in proliferation, migration and invasion. The IL-4/STAT6 signaling pathway increases nuclear survivin in CRC stem cells, allowing them to evade cell death (19). Platelet-derived growth factors (PDGFs) are secreted by human vascular endothelial cells, epithelial cells and fibroblasts. PDGF is a survival factor that inhibits apoptosis, promotes proliferation and stimulates mesenchymal cell proliferation

Table I. Different roles of STAT6 in colon cancer.

System/process affected	Mechanism implicated	First author, year (ref.)
Immune system	IL-4, IL-13, IL-4R and IL-13R are differentially expressed in the colonic mucosa of patients with CRC according to the metastasis and survival rates.	Formentini et al, 2012 (28)
	T cells, macrophages and natural killer T cells exhibit increased STAT6 phosphorylation during colitis development. STAT6^{-/-} mice exhibit decreased secretion of IL-4, IL-5, IL-13 and interferon-γ accompanied by disease amelioration.	Rosen et al, 2013 (29)
	STAT6 signaling contributes to the pathogenesis of colitis by inducing B cell-dependent mast cell activation.	Hoving et al, 2012 (30)
	STAT6^{-/-} mice exhibit reduced tumorigenicity associated with decreased inflammation and low mRNA levels of IL-17A and tumor necrosis factor-α in the azoxymethane/DSS model of CAC.	Leon-Cabrera et al, 2017 (33)
	STAT6 orchestrates epithelial cell proliferation and myeloid-derived suppressor cell expansion by decreasing the cytotoxic activity of CD8 T cells in a mouse model of adenomatous polyposis.	Jayakumar and Bothwell, 2017 (34)
	The STAT6-specific inhibitor AS1517499 decreases the number of tumors and circulating inflammatory monocytes and granulocytes in experimental CAC.	Leon-Cabrera et al, 2017 (33)
Apoptosis	In DSS-induced colitis, STAT6 deficiency alters chromatin compaction with direct repercussions in the apoptosis of epithelial cells and mucosal damage.	De Oliveira et al, 2019 (35)
	IL-4-dependent STAT6 signaling pathway favors the expression of anti-apoptotic proteins and epithelial cell growth in colon cancer in vivo and in vitro.	Todaro et al, 2008 (59)
	Abnormal IL-4/STAT6 activation induces survivin expression that in turn increases apoptosis resistance in colon cancer stem cells.	Di Stefano et al, 2010 (19)
Epithelial cells	IL-13 drives STAT6 phosphorylation and alters epithelial barrier function by increasing claudin-2 activity in colitis.	Rosen et al, 2013 (29)
	IL-13/STAT6 signaling pathway serves a pivotal role in reducing matriptase and prostasin expression, two proteins involved in epithelial barrier dysfunction.	Buzza et al, 2017 (31)
	Administration of IL-13 increases production of colonic serotonin and colitis severity in DSS-induced colitis.	Shajib et al, 2013 (32)
	The exposure of the colon cancer HT29 and SW480 cell lines to IL-13 enhances EMT-promoting factor zinc finger E-box-binding homeobox 1 expression.	Cao et al, 2017 (37)
	E2F transcription factor 1 increases STAT6 expression in colon cancer cell lines that in turn leads to expression of EMT drivers in CRC cells.	Chen et al, 2018 (40)
	IL-4 downregulates E-cadherin expression, altering cell-cell adhesion and favoring metastasis in CRC.	Kanai et al, 2000 (43)
	STAT6^{-/-} mice with acute murine colitis exhibit impaired wound healing and low mucosal expression of Wnt ligands, which are important mediators in response to epithelial injury.	Cosin-Roger et al, 2016 (52)
Proliferation	STAT6^{-/-} mice exhibit reduced colonic expression of cyclooxygenase-2 and nuclear β-catenin in experimentally induced colon cancer.	Leon-Cabrera et al, 2017 (33)
	Patients with CAC exhibit a shift from pSTAT6 to pSTAT3 in colonic epithelial and mucosal immune cells during neoplastic transformation.	Wick et al, 2012 (36)

Table I. Continued.

System/process affected	Mechanism implicated	First author, year (ref.)
Metastasis	High STAT6 expression levels concur with lymph node metastasis and low survival rates in patients with CCR patients.	Wang et al, 2010 (26)
	IL-13Rα2 signaling favors 11β-hydroxysteroid dehydrogenase type 2 expression, a key enzyme involved in liver metastasis during CCR development.	Jiang et al, 2016 (38)
	Activation of PI3K, Akt and SRC in response to IL-13 is associated with increased levels of IL-13Rα2 in highly metastatic CRC cells.	Barderas et al, 2012 (39)
DNA damage	Increasing IL-4 abundance contributes to NADPH oxidase 1 expression along with production of reactive oxygen species in human colon cancer cells.	Liu et al, 2017 (42)

CRC, colorectal cancer; CAC, colitis-associated cancer; DSS, dextran sulfate sodium; EMT, epithelial-to-mesenchymal transition; IL-R, interleukin (receptor); pSTAT, phosphorylated signal transducer and activator of transcription.

and migration (20). A previous study suggested that PDGF and STAT6 have related functions. PDGF is able to activate STAT6 in fibroblasts to promote their proliferation (21). The IL-13/STAT6 signaling pathway upregulates PDGF mRNA expression levels in lung fibroblasts, and STAT6 is required for PDGF-A and PDGF-C gene expression, with important implications in tumor development (22). Therefore, STAT6 is a key regulatory molecule with tumor proliferating functions that may aid the identification of molecular targets for the treatment of colon cancer.

3. STAT6 and its role in CRC

Clinically detectable IBD increases the risk of CAC; patients with UC have an increased risk (2.4-fold) of developing CAC (23), while patients with CD have an increased risk (2.59-fold) of developing CAC in the next two decades, compared with the general population (24).

Expression of the constitutively activated IL-4/IL-13/STAT6 axis during UC, CD and in colon cancer tissues suggests that this pathway may contribute to the underlying pathology of these diseases. In a previous study, immunohistochemistry analyses were performed to detect phosphorylated STAT6 (pSTAT6) in colonic tissues of pediatric subjects with UC or CD, and it was demonstrated that nuclear pSTAT6 was significantly upregulated in the colonic epithelium (25). Furthermore, STAT6 was significantly upregulated in neoplastic tissues of patients with CRC, and its expression was associated with lower survival rates and with lymph node metastasis (26). Gene expression levels of IL-4, IL-5 and IL-13 were significantly upregulated in tumors compared with those in normal tissues of patients with CRC; however, no apparent effect on clinical outcome was observed (27). By contrast, a study performed by Formentini *et al* (28) revealed that IL-4, IL-13, IL-4R and IL-13R were expressed in CRC specimens, and that high expression levels of IL-4, IL-4R and IL-13R were associated with a lower frequency of lymph node metastasis, while IL-13 expression was associated with a better overall survival rate.

IL-13-induced activation of STAT6 has been implicated in mediating the host inflammatory cascade in UC. A mouse model of oxazolone-induced UC demonstrated that IL-13 is responsible for inducing the expression of the pore-forming tight junction protein claudin-2, increasing epithelial barrier permeability and compromising gastrointestinal barrier function (29). In addition, epithelial cells, T cells, macrophages and natural killer T cells exhibit increased STAT6 phosphorylation during colitis development (29). When colitis was induced in STAT6-deficient mice, decreased pathology, claudin-2 expression, and secretion of IL-4, IL-5, IL-13 and IFN-γ were observed (29). CD4+ Th2 cells produce IL-13, which favors IgE production by B cells, and induce mast cell activation, suggesting that STAT6 contributes to the pathogenesis of colitis (30). Therefore, IL-13 seems to drive STAT6 phosphorylation, alter epithelial barrier function, regulate Th2 cytokine production and mediate the inflammatory response to colitis.

The IL-13/STAT6 signaling pathway may be detrimental for intestinal epithelial cell function. The downregulation of matriptase and prostasin, two membrane-anchored serine proteases important for the epithelial barrier development and for homeostasis, was observed in a dextran sulfate sodium
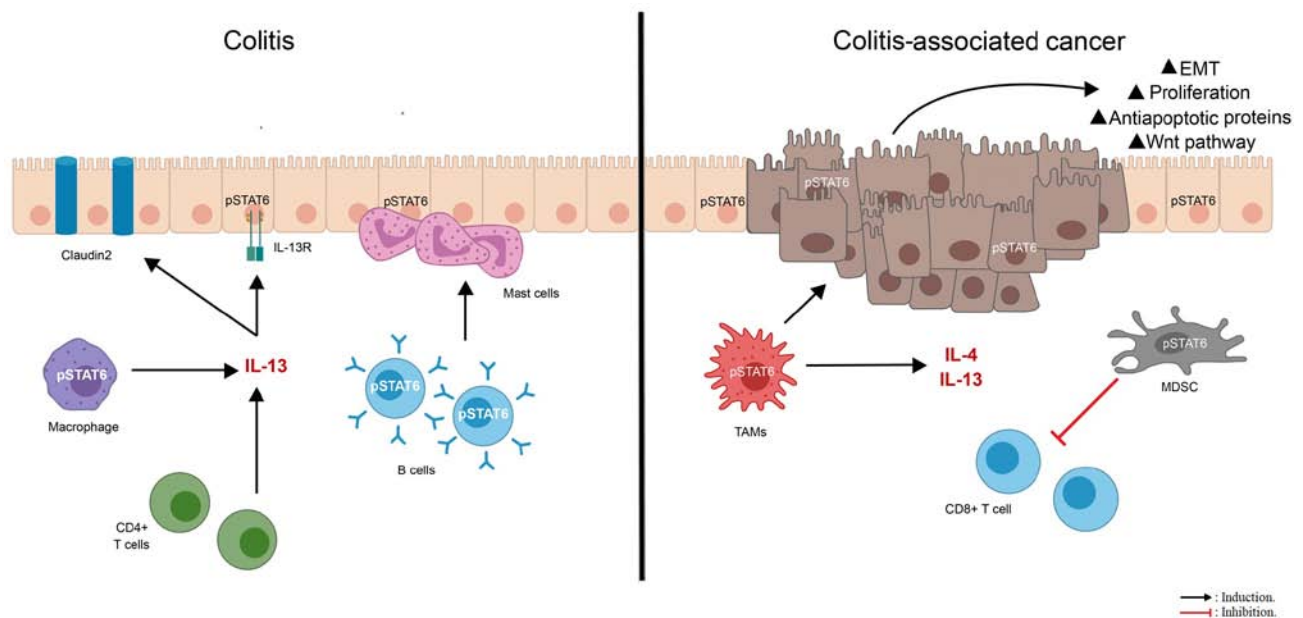
Figure 1. STAT6 modulates pro-colitis and pro-cancer responses. The IL-13/STAT6 signaling pathway appears to be detrimental to the function of intestinal epithelial cells in subjects with ulcerative colitis or Crohn's disease, in which nuclear pSTAT6 is significantly increased in the colonic epithelium. IL-13 signaling alters the expression of proteins, including the pore-forming tight junction protein claudin-2, matriptase, prostasin and serotonin, involved in epithelial barrier permeability and gastrointestinal barrier function via STAT6. In addition, IL-13 produced by CD4^{+} T helper 2 cells favors STAT6 phosphorylation in mast cells, B cells and macrophages during the development of colitis, suggesting that STAT6 signaling in immune cells favors the progression of the disease. In the tumor microenvironment, STAT6 coordinates both polyp cell proliferation and myeloid-derived suppressor cell expansion. Furthermore, the IL-4/STAT6 signaling pathway induces the expression of anti-apoptotic proteins and the growth of epithelial colon cancer cells. STAT6 activation prompts the differentiation of infiltrating macrophages into a tumor-associated phenotype with pro-tumoral functions; these M2 macrophages promote mucosal repair through the activation of the Wnt signaling pathway. However, mutations that cause aberrant Wnt signaling are responsible for polyp development in the small intestine and colon. Additionally, the exposure to IL-13 enhances the expression of epithelial-to-mesenchymal transition-promoting factors, resulting in a decrease in adhesion, migration, invasion and metastatic colonization. pSTAT6, phosphorylated signal transducer and activator of transcription 6; IL-R, interleukin receptor; TAMs, tumor-associated macrophages; MDSC, myeloid-derived suppressor cell.

(DSS)-induced model of colitis in mice and in colonic tissues from human subjects with active UC and CD (31). When STAT6 was inhibited by suberoylanilide hydroxamic acid (SAHA), the expression levels of matriptase and prostasin were restored and barrier dysfunction was decreased, implicating STAT6 signaling in the loss of the barrier-protective protease pathway (31). Enterochromaffin cells, which are responsible for synthesizing serotonin to increase epithelial cell secretion, express IL-13R (32). In a model of DSS-induced colitis, the administration of IL-13 increased colonic serotonin production and exacerbated the severity of colitis (32). It is unclear whether these effects are unique to colitis or increase the risk of developing CRC. Previous studies have revealed that STAT6 serves important roles in the early steps of colitis-associated carcinogenesis and that it modulates inflammatory responses, as well as controlling cell recruitment and proliferation (33,34). CAC induction in STAT6-deficient mice (STAT6^{-/-}) in a azoxymethane/DSS model resulted in reduced tumorigenicity, associated with reduced inflammation, decreased concentrations of cyclooxygenase-2 (COX2) and nuclear β-catenin protein in the colon, and decreased mRNA expression levels of cytokines IL-17A and tumor necrosis factor-α (TNF-α) (33). In addition, the number of circulating inflammatory monocytes and granulocytes was decreased in STAT6^{-/-} mice (33). Furthermore, STAT6 deletion in the Apc[Min/+] mouse model reduced the incidence of polyps in the small intestine and decreased the proliferation of polyp epithelial cells; this effect was attributed to an expansion of myeloid-derived suppressor cells (MDSCs), implying regulation of the antitumor T-cell response in a STAT6-dependent manner (34). Therefore, STAT6 may serve a broad role in coordinating both polyp cell proliferation and MDSC expansion. By contrast, Oliveira et al (35) reported that STAT6-deficient mice are more susceptible to mucosal damage during DSS-induced colitis, and exhibit enhanced intestinal epithelial cell apoptosis, tissue injury and inflammatory responses. Chromatin condensation in intestinal epithelial cells is affected by STAT6 signaling and may protect cells from apoptosis and severe tissue damage (35). However, the role of STAT6 during different stages of colitis and tumor cell proliferation has not been fully elucidated.

The activation and function of STAT6 in colitis along the continuum of inactive disease to CAC are dynamic. In a previous study, immunohistochemistry to detect pSTAT1, pSTAT6 and pSTAT3 in colonic epithelial and mucosal immune cells in patients with UC and CAC was performed. A shift from predominant STAT6 activation in immune cells to STAT3 activation accompanied the onset of dysplasia with a concomitant increase in epithelial cell STAT3 activation in low- and high-grade tumors. STAT6 expression was frequently detected in normal tissues, but not in CAC tissues (36). The decrease in pSTAT6 expression in both immune cells and intestinal epithelial cells indicates that STAT6 signaling may be detrimental in the transition from colitis to cancer. Nevertheless, abnormal STAT6 signaling has been implicated in pro-metastatic processes, including
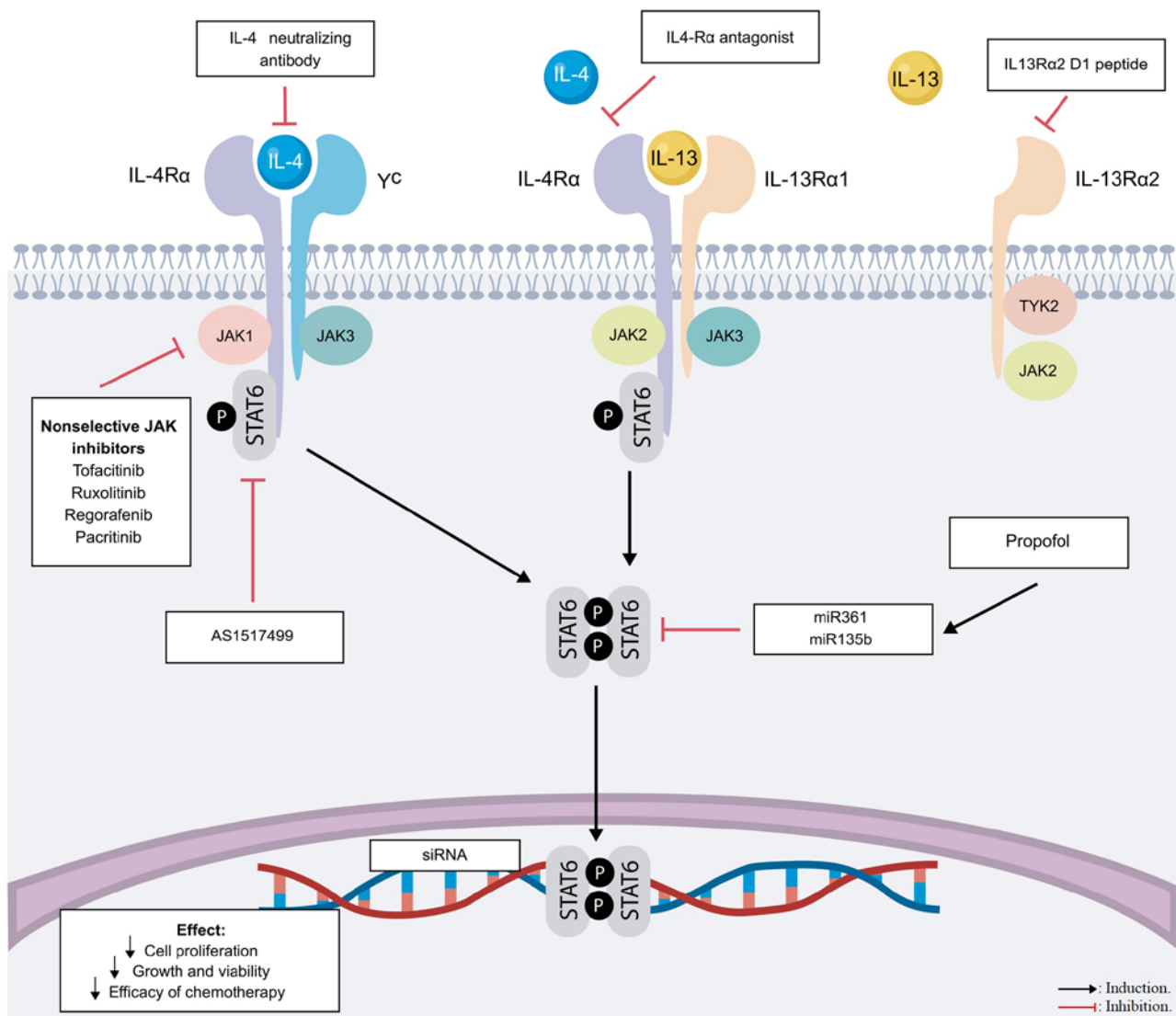
Figure 2. Targeting the IL-4/IL-13/STAT6 signaling pathway for cancer therapy. Upon cytokine-receptor binding, JAK1, JAK2 or tyrosine kinase 2 are recruited and activated, inducing the phosphorylation of STAT6. STAT6 homodimers translocate to the nucleus and bind DNA sequences at genes involved in apoptosis, proliferation and the immune response. Various drug targets have been evaluated to block IL-4, IL-4R, IL-13Rα1 or STAT6. Points of potential therapeutic inhibition of the pathway are indicated. STAT6, signal transducer and activator of transcription 6; IL-R, interleukin receptor; JAK, Janus kinase; miR, microRNA; siRNA, small interfering RNA; γc, γ chain.

the proliferation and survival of colon cancer cells (37-39). During the epithelial-to-mesenchymal transition (EMT), epithelial cells transform into aggressive phenotypes with enhanced migratory capacity and invasiveness. Exposure to IL-13 enhances the expression of the EMT-promoting factor zinc finger E-box binding homeobox 1 (ZEB1) in the colon cancer HT29 and SW480 cell lines (37). When STAT6 is blocked or knocked down, the IL-13-induced EMT and ZEB1 induction in CRC cells is reversed (37). Additionally, a positive association between IL-13Rα1 and ZEB1 at the mRNA level has been observed in human CRC samples, demonstrating that the IL-13/STAT6 signaling pathway serves a critical role in promoting EMT and the aggressiveness of CRC (37). 11β-hydroxysteroid dehydrogenase type II (11βHSD2) is a key enzyme induced in an IL-13Rα2-dependent manner that promotes the expression of Akt and COX2; upon inhibiting 11βHSD2, liver metastasis is decreased, suggesting that IL-13 may regulate malignancy via 11βHSD2 during CRC (38). In

addition, highly metastatic CRC cells express high levels of IL-13Rα2 (39). IL-13Rα2 silencing results in a decrease in adhesion, migration, invasion and metastatic colonization. In a previous study, the upregulation of IL13Rα2 expression in 66% of tumor samples from patients with colon cancer was associated with late stages of progression (metastasis in lymph nodes or liver) and a poor outcome in patients with CRC. Highly metastatic CRC cells exhibit activation of PI3K, Akt and SRC proto-oncogene non-receptor tyrosine kinase in response to IL-13, supporting the role of the IL-13/IL-13R/STAT6 signaling pathway in CRC cell invasion and metastasis (39).

Additionally, STAT6 phosphorylation is induced by IL-4. E2F transcription factor 1 (E2F1), a critical transcription factor for CRC development, increases STAT6 expression in colon cancer cells and increases their susceptibility to IL-4 stimulation. E2F1 acts as an enhancer of the IL-4/STAT6 signaling pathway and increases the expression of EMT drivers in CRC cells (40). IL-4 is able to induce the expression

of anti-apoptotic proteins and the growth of epithelial colon cancer cells *in vivo* and *in vitro*. IL-4 neutralization sensitizes colon carcinoma cells to chemotherapy and to death by the TNF-related apoptosis-inducing ligand (41). Abnormal IL-4/STAT6 activation induces the expression of survivin, a protein with an important role in apoptosis resistance in colon cancer stem cells; the use of leflunomide, a STAT6 inhibitor, has been shown to decrease survivin expression and localization, thereby reducing its anti-apoptotic effect (19). An increase in IL-4 expression contributes to an oxidant milieu, thereby increasing the expression of NADPH oxidase 1 and reactive oxygen species, resulting in DNA damage and the neoplastic transformation of colon cells (42). Further evidence indicates that IL-4 is implicated in the inhibition of colon cancer cell-cell adhesions, acting as a negative regulator in the expression of E-cadherin, a key component of adherent junctions, and in maintaining epithelial cell adhesion and decreasing invasiveness (43).

Increasing evidence demonstrates that STAT6 serves an important role in the regulation of tumor immunity. The use of the STAT6-specific inhibitor AS1517499 has been shown to decrease colonic tumor load and the number of circulating inflammatory monocytes and granulocytes in experimental models of CAC (33). The reduced number of the aforementioned cells may be associated with decreased macrophage infiltration and reduced tumor growth. IL-4/IL-13 cytokines, via STAT6 activation, induce the differentiation of infiltrating macrophages to an M2 phenotype with pro-tumoral functions. The pharmacological inhibition of STAT6 with AS1517499 was shown to attenuate tumor growth and liver metastasis in an orthotopic 4T1 mammary carcinoma mouse model (44). This result was associated with a decrease in tumor-associated macrophages displaying the M2 phenotype, suggesting that IL-4 blockade inhibits tumor angiogenesis and growth by reprograming macrophages to a less aggressive phenotype (45). In addition, the deletion of the STAT6 gene was demonstrated to improve antitumor immunity in the same model via a type 1 CD4+ response (46).

Autocrine production of IL-4 by colon cancer cells protects against apoptosis and chemotherapy-induced cell death (47-50). It was found that IL-4 was highly expressed in cancer-initiating cells obtained from patients with CRC and was associated with low immunogenic profiles; blocking IL-4 increased Th1-type CD8+ T cells responses *in vitro* (51). Previous studies also revealed that STAT6 maintains mucosal homeostasis by sustaining myeloid cells. STAT6-/- mice with acute murine colitis exhibited delayed wound healing and decreased mucosal expression levels of Wnt ligands, which are important responses to epithelial injury (52). The administration of M2 macrophages to STAT6-/- mice promoted mucosal repair through the activation of the Wnt signaling pathway; however, mutations that cause aberrant Wnt signaling are responsible for polyp development in the small intestine and colon (52). In the APCmin/+ mouse model, which recapitulates the disease observed in patients with familial APC, STAT6 promoted the expansion of MDSCs and decreased the cytotoxicity of CD8 T cells, contributing to intestinal tumorigenesis (34). The identification of the direct functions of STAT6 on immunosurveillance and epithelial cell homeostasis may serve as the basis for the development of novel immunotherapy strategies.

The aforementioned results demonstrate that the IL-4/IL-13/STAT6 axis may serve a role in inflammation, tumor cell proliferation, cancer cell survival and metastasis, and that the interference of its effects in colitis or in tumor cells may present a novel strategy for the treatment of CRC (Fig. 1; Table I).

4. Targeting STAT6 for colon cancer therapy

To the best of our knowledge, the use of specific STAT6 inhibitors in preclinical and clinical studies for colon cancer has not been reported. However, in experimental models and cancer cell lines, STAT6 inhibition decreases tumor cell proliferation, survival, adhesion, invasion and metastasis, suggesting that STAT6 inhibition may serve as a therapeutic target in colon cancer.

The use of the STAT6-specific inhibitor AS1517499 reduced tumor growth and signs of the disease in mice with CRC (33,53). *In vivo*, STAT6 inhibition reduced colonic tumor load and colon tissue damage, corresponding with a decrease in STAT6 phosphorylation in the intestine (33). In an orthotopic 4T1 mammary carcinoma mouse model, the use of AS1517499 attenuated tumor growth and early liver metastasis (44). Similarly, in primary epithelial cells from patients with prostate cancer, exposure to AS1517499 decreased IL-4-induced colony formation (54). Upregulation of microRNA (miRNA/miR)-361 and miR-135b is associated with a decrease in STAT6 expression (55). The common intravenous anesthetic agent propofol has been associated with a reduction in tumor-associated inflammation and with the ability to induce miRNAs to suppress STAT6 expression (55). The treatment of the CRC SW480 and RKO cell lines with propofol was shown to increase the expression levels of miR-135b and miR-361, which are STAT6-targeting miRNAs, and decrease cell proliferation and migration, suggesting that propofol interferes with the IL-13/STAT6 signaling pathway (55). Nevertheless, the *in vivo* effect of propofol requires further investigation. A preclinical model utilizing small interfering RNA (siRNA) to specifically suppress STAT6 expression in lung epithelial cells was reported (56); the study stated that the intranasal application of STAT6 siRNAs attenuated allergic airway inflammation, demonstrating that STAT6 may be targeted in specific tissues. Therefore, further studies evaluating the possibility of suppressing STAT6 signaling in the colonic epithelium are required. The use of STAT6 inhibitors results in the inhibition of type I and type II IL-4Rs, and therefore, the negative implications for normal immune functions should be determined.

5. Targeting IL-4, IL-13, IL-4R and IL-13Rα2 in colon cancer therapy

IL-4 and IL-13 bind to their receptors and activate the JAK/STAT6 signaling pathway. Therefore, the use of molecules that can block binding or impair the signaling of these cytokines to prevent STAT6 activation may be clinically relevant. Previous studies have revealed that blocking IL-4 and IL-13, as well as their receptors, has different consequences on tumor development (Fig. 2).

The synthetic IL-13Rα2 D1 peptide inhibits IL-13-mediated STAT6 activation through IL-13Rα1, and blocks IL-13 binding to IL-13Rα2, decreasing IL-13 signaling; the administration of IL-13Rα2 D1 peptide was previously shown to repress tumor growth, invasion and proliferation in a metastatic CRC mouse model (57).

The direct administration of anti-IL-4 antibody in combination with chemotherapy suppresses the growth and viability of the Caco cell line; this effect is associated with a decrease in the expression levels of the CRC stem cell marker CD133, suggesting that anti-IL-4 therapy may enhance the efficacy of chemotherapy regimens (58). When conventional drugs, such as 5-fluorouracil or oxaliplatin, are combined with an IL-4 neutralizing antibody or an inhibitory form of IL-4, the efficacy of chemotherapy is enhanced in both mature cancer cells and cancer-stem like cells (59). Similarly, it has been shown that when IL-4 and IL-13 responses are inhibited by an IL-4Rα antagonist in nude mice injected with colon cancer spheroids, the tumor response to chemotherapeutic drugs is enhanced (60). The increased production of IL-4 in cancer cells may favor a death-resistant phenotype, and limiting the production and/or signaling of IL-4 may be an alternative approach for treatment-resistant cells.

The antitumor effect of doxorubicin has been widely reported (61). In a previous study, the liposomal form of doxorubicin was conjugated with a ligand of the atherosclerotic plaque-specific peptide-1 (AP1), a peptide characterized by its ability to bind IL-4R; Yang *et al* (62) proposed that AP1-conjugated liposomal doxorubicin exhibits an increased and selective cytotoxic effect on CRC cells, and has potential as a targeted anticancer therapy. Collectively, the aforementioned studies demonstrated that the inhibition of IL-4, IL-4R, IL-13Rα1 and IL-13Rα2 may be beneficial for colon cancer.

6. Clinical trials targeting JAKs in colon cancer therapy

It is well known that IL-4R, γc and IL-13Rα1 activate JAK1/2 and Tyk2. STAT6 is phosphorylated following JAK activation and forms homodimers that translocate to the nucleus. JAK molecules have been targeted therapeutically to treat rheumatoid arthritis, psoriasis and IBD (63,64). The inhibition of the JAK/STAT inflammatory pathway may be an alternative for the treatment of CRC. However, to the best of our knowledge, JAK inhibitors have not been approved by the Food and Drug Administration (FDA) for the treatment of colon cancer.

In addition to activating STAT6, JAKs mediate a number of cytokine receptor responses (3). While several cytokines are involved in inflammatory responses that may promote cancer development, other cytokines regulate processes involved in mucosal healing, barrier function and immunosurveillance (3-5). For example, the inhibition of JAK1 may alter the signaling of IL-2, IL-7, IL-9, IL-6 and IL-10 (4). Changes in IL-10, an important anti-inflammatory cytokine, may affect intestinal homeostasis. In addition, the function of T cells and natural killer cells may be altered as a result of changes in cytokine signaling (3,4). The inhibition of JAKs in response to IL-4 mediates alterations in Akt, ERK and mTOR, which are involved in several processes (12). Therefore, it is difficult to discern and control the effect of JAK inhibition, particularly in cancer development, where the tumor and stromal cells are actively interacting through cytokines. Consequently, the use of JAK inhibitors for the treatment of colon cancer is not widespread. Ruxolitinib, an oral selective inhibitor of JAK1/2, is approved by the FDA for use in myelofibrosis (65). Ruxolitinib was tested in a phase 2 study in combination with regorafenib, an oral multi-targeted kinase inhibitor, in patients with advanced and metastatic adenocarcinoma of the colon or rectum (66). However, there was no significant difference in the overall survival rate or progression-free survival rate between the regorafenib + placebo vs. regorafenib + ruxolitinib groups (66). Nevertheless, the treatment was administered in the advanced stages of tumor development, and the trial was terminated early per sponsor decision; therefore, further investigation is required, particularly during early stages of CRC.

Pacritinib, an oral inhibitor of JAKs and other kinases, was administrated to patients with metastatic CRC; however, the study did not produce conclusive results as the trial was discontinued prior to completion (64). Additionally, the use of tofacitinib, a JAK1/3 inhibitor widely used for the treatment of rheumatoid arthritis, was found to be associated with an increase in lung metastasis accompanied with a decrease in natural killer cell number in a mouse model of colon cancer (67). Therefore, the development of selective tissue-specific inhibitors may overcome these types of complications.

7. Conclusions

Several studies have revealed that the IL-4/IL-13/STAT6 axis contributes to the pathology of IBD and colon cancer. Nuclear pSTAT6 is significantly increased in colonic epithelium and in neoplastic tissues obtained from patients with CRC, and its expression is associated with lower survival rates. Additionally, STAT6 seems to regulate mechanisms that promote the proliferation, survival, invasion and metastasis of tumor cells, as well as the suppression of antitumor immunity. Inhibiting STAT6 signaling in experimental models and cancer cell lines has resulted in a decrease in cancer-associated processes. Therefore, STAT6 may serve as a potential target in the treatment of colon cancer. However, to the best of our knowledge, STAT6 inhibitors in preclinical and clinical studies for colon cancer have not been reported. The indirect inhibition of the STAT6 signaling pathway through IL-4, IL-13 or JAKs may inhibit carcinogenesis, but it may also result in side effects as a consequence of alterations in the immune response. Therefore, preclinical and clinical studies investigating specific STAT6 inhibitors with a targeted organ distribution are required to evaluate the potential of STAT6 as a target for colon cancer therapy.

Availability of data and materials

Not applicable.

Authors' contributions

YDR and SLC contributed to the conception of the study, writing the manuscript and performing the literature search. VC and GVG collected and analyzed data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' information

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