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Therapeutic targeting of inflammation and tryptophan metabolism in colon and gastrointestinal cancer

Srikanth Santhanam[a,*], **David M. Alvarado**[a,*], and **Matthew A. Ciorba**[a,#]

[a]Division of Gastroenterology, Washington University School of Medicine, Saint Louis, Missouri 63110, USA

Abstract

Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer death in the United States. Cytotoxic therapies cause significant side effects for most patients and do not offer cure in many advanced cases of CRC. Immunotherapies are a promising new approach to harness the body's own immune system and inflammatory response to attack and clear the cancer. Tryptophan metabolism along the kynurenine pathway is a particularly promising target for immunotherapy. Indoleamine 2,3 dioxygenase 1 (IDO1) is the most well studied of the enzymes that initiate this pathway and it is commonly overexpressed in CRC. Herein, we provide an in-depth review of how tryptophan metabolism and kynurenine pathway metabolites shape factors important to CRC pathogenesis including the host mucosal immune system, pivotal transcriptional pathways of neoplastic growth and luminal microbiota. This pathway's role in other gastrointestinal malignancies such as gastric, pancreatic, esophageal and gastrointestinal stromal tumors (GIST) is also discussed. Finally, we highlight how currently available small molecule inhibitors and emerging methods for therapeutic targeting of IDO1 might be applied to colon, rectal and colitis associated cancer.

Keywords

IDO2; TDO; gastrointestinal disease; gut; inflammation; metabolome; metabolomics; Crohn's disease; ulcerative colitis; biomarker; TLR; microbiome; colorectal

INTRODUCTION

Inflammation is a common feature of colorectal cancer (CRC).[1] Despite immune cell infiltration, CRCs evade immune surveillance and resist immune mediated destruction. Metabolism of the essential amino acid tryptophan along the kynurenine pathway (KP) is

[#]Corresponding author: Matthew A. Ciorba, MD., 660 South Euclid Avenue, Box 8124, Saint Louis, Missouri 63110, USA. mciorba@dom.wustl.edu, Telephone: 1-314-362-1026.

[*]Authors contributed equally to this work.

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one potential explanation for this phenomenon and is recognized as an important link between inflammation and neoplastic progression in many cancers.

Indoleamine 2,3 dioxygenase 1 (IDO1) is the most well studied of the enzymes that initiate tryptophan's catabolism to kynurenine. High IDO1 expression is present in a subset of human CRC where it portends a worse prognosis.[2, 3] IDO1 is also one of the most highly upregulated genes in human inflammatory bowel disease (IBD), a precancerous condition.[4, 5] Local tryptophan depletion and the biologically active KP metabolites exert potent immunomodulatory effects to shape the tumor microenvironment and contribute to tumor immune escape.[6] In CRC, IDO1 also directly supports tumor growth independent of effect on adaptive immunity.[2] These features establish IDO1 and the KP as highly promising targets for immunotherapy of cancers including those of the gastrointestinal (GI) tract.

Herein, we provide an in-depth review of how tryptophan metabolism and KP metabolites shape factors important to CRC pathogenesis including the mucosal immune system, luminal microbiota and pivotal transcriptional pathways for neoplastic growth. More limited coverage is provided on how this pathway affects other GI malignancies. Finally, we highlight how currently available agents and emerging methods for therapeutic targeting of the IDO1-KP might be applied to CRC.

BACKGROUND ON COLON CANCER AND INFLAMMATION

CRC is the third most common cancer worldwide,[7] and in the United States is the second leading cause of cancer related death (almost 50,000 per year).[8] In most cases, the transition from normal colon epithelium to cancer is influenced by the acquisition of somatic mutations and environmental factors including diet and lifestyle.[9, 10] The adenomatous polyposis coli (APC) gene is a key component to Wnt signaling and is mutated in most CRCs.[10]

Chronic inflammation is also a risk factor for CRC. Colitis-associated cancer (CAC) is a form of CRC that develops in patients with chronic inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis.[5] In IBD, dysregulated activation of the gut mucosal immune system driven by genetic susceptibility loci and environmental factors leads to chronic inflammation.[11] The risk of developing CAC correlates with the duration, extent and severity of IBD activity.[5] While estimates vary, ~2% of individuals diagnosed with ulcerative colitis will develop CAC by 10 years after symptoms emerge, and 18% by 30 years, verses a 5.2% lifetime risk of developing CRC for the US population[12, 13]. Importantly, CAC often develops earlier in life and progresses more quickly than sporadic CRC, frequently affecting young persons in their prime productive years.

Several recent and excellent reviews highlight differences between CAC and sporadic CRC.[5, 14, 15] Notably the molecular steps and sequence of acquired genetic mutations vary between the two. For example, the loss of APC gene function is considered a crucial early step in sporadic CRC, but occurs late in CAC tumors.[10] In CAC, reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced by both immune cells and the inflamed epithelium are a primary sources of DNA damage.[9] Ultimately, mutations in the Wnt/APC and/or inflammatory signaling pathways such as PI3K/AKT support dysregulated β-catenin

activity which in turn leads to the transcription of genes such as Cyclins, Axin2 and c-Myc that promote proliferation and tumor growth.[16] While CAC is a model disease to examine links between chronic inflammation and neoplasia, inflammation and immune cell infiltration also comprise a component of all CRC.[1] Once colon neoplasia begins to form, immune cells are invariably recruited to the tumor site. This immune cell infiltration can be somewhat paradoxical, as immune cells both contribute to tumorigenesis and participate in clearance of the tumor. Cytokines produced by the tumor-associated immune cells, including TNF-α, IFNγ, IL-1β and IL-6, induce ROS and RNS in the tumor which potentiate genetic damage and tumor progression.[9] Conversely, the presence of natural killer and T lymphocytes in tumors correlates with better clinical outcome and longer survival in patients with CRC[17–19]. Despite the infiltration of natural killer and T cells, tumors are often able to evade cytolysis, indicating that mechanisms of immune suppression are present in the tumor milieu.[20] These findings highlight as an important focus in CRC research to target interruption of this process, known as immunoediting, with *immunotherapies*.

CURRENT THERAPEUTICS ILLUSTRATE A LINK BETWEEN INFLAMMATION AND COLON CANCER

In setting the stage for a detailed examination of tryptophan metabolism's role in inflammation and CRC, it is useful to have perspective on how current therapeutics fit into this paradigm. To date no *immunotherapy* is approved for CRC. However, observations from currently available therapeutics provide support for this concept and insight into the complex relationship between colon inflammation and cancer. Aspirin and NSAIDs, common anti-inflammatory drugs, reduce the risk of sporadic CRC in some individuals.[21] Aminosalycilates (mesalamine), integral maintenance anti-inflammatories for IBD, decrease the risk of CAC.[22] NF-κB modulation, may contribute to mesalamine's chemo-preventative properties. TNFα inhibitors, another potent mainstay of IBD therapy, reduces tumorigenesis in experimental CAC models,[23] but it is not yet clear if they do so in humans.

Stimulating the immune system is also recently recognized as an important additional function of some cytotoxic CRC therapies. Chemotherapy regimens for cancer proximal to the rectum are based on the pyrimidine analog 5-Fluorouracil (5-FU) and leucovorin. Radiation therapy is added for rectal cancers and works in synergy with 5-FU. 5-FU was recently demonstrated to also shape tumor immunity by reducing myeloid derived suppressor cells which are known to promote colon tumorigenesis.[24–26] The recently reported "abscopal effect" of radiation therapy in melanoma suggests that locally applied radiation can enhance anti-tumor immunity against metastatic sites.[27] These findings suggest that combining cytotoxic therapies with immunotherapy may be the key to unlocking the potential of both therapies. This concept is discussed again later in this review.

TRYPTOPHAN METABOLISM AND INFLAMMATION

Tryptophan metabolizing enzymes

Tryptophan metabolism is one important mechanism exploited by cancers to evade immune surveillance.[28–31] Tryptophan is the most essential amino acid and 95% of dietary

tryptophan is metabolized along the kynurenine pathway (KP, Figure 1).[32] Indoleamine 2,3 dioxygenase 1 (IDO1), the first and rate limiting step in this pathway, has important roles in limiting adaptive immune responses in a variety of both inflammatory and malignant diseases.

Two other enzymes, indoleamine 2, 3 dioxygenase 2 (IDO2) and tryptophan dioxygenase (TDO or TDO2) also metabolize tryptophan along the KP.[33] IDO1 and IDO2 are similar in both structure, while TDO is structurally unique[34]. At baseline, IDO1 is widely expressed across most tissue and cells types, while tissue distribution is much more limited for IDO2 (epididymis and kidney) and TDO (Liver). However, all three have been shown to be expressed in a variety of cancers[35–37]. As IDO1 is the most well studied of these enzymes and is the principle enzyme expressed in the inflamed and malignant gut, it will be the primary focus of this review.

IDO1: the immune modulator

IDO1 as a mediator of immune tolerance was first described for its role at the maternal-fetal interface by Munn *et al.* in 1998.[38, 39]. This group described the now classical mechanism whereby IDO1 expression in professional antigen presenting cells (APCs, monocytes/macrophages, dendritic cells) reduces local tryptophan concentrations, which reduces T cell proliferation and thus inhibits T cell-mediated immune response (Figure 2). Mechanistic studies eventually determined that local tryptophan depletion is itself immunosuppressive, but that these effects are also mediated by several KP metabolites[30, 40]. Low levels of tryptophan in the local microenvironment activate stress-response pathways, including GCN2 kinase and mTOR[41, 42]. Some KP metabolites bind the aryl hydrocarbon receptor (AHR) to promote forkhead box (FOX) $P3^+$ regulatory T cell (Tregs) differentiation[43–46]. The function of kynurenines as promoters of tolerance is a theme reflected in numerous immunological contexts, including protection of transplanted tissues and reduction in immune response to pathogen infection[47–50].

A *non-enzymatic* mechanism by which IDO1 functions as a tolerogenic signaling molecule in plasmacytoid DCs has also been described[46, 51]. This mechanism is mediated by phosphorylation of IDO1's immunoreceptor tyrosine-based inhibitory motifs by Src family kinases and requires co-stimulation with TGF-β[46, 51, 52]. These signals in turn activate non-canonical NF-κB signaling to induce type I IFNs[46, 52].

IDO1 in colitis

The colon is a site of high IDO1 expression even in the homeostatic state. In this basal state, IDO1 is predominately in myeloid derived cells of the lamina propria. IDO1 expression is strongly induced by inflammatory cytokines including IFNγ, TNFα and IL-1β and, as such, is thus one of the most upregulated genes in mouse models of colitis and human inflammatory bowel disease (IBD)[4]. In the inflamed colon, epithelial cells become a major IDO1 expressing cell type. Functionally relevant IDO1 gene polymorphisms correlate with a more severe disease phenotype in Crohn's disease.[53] Genetic or pharmacological inhibition of IDO1 worsens the severity of colitis in mouse models[54, 55]. Induction of IDO1 by a toll-like receptor (TLR) 9 agonist limits murine disease severity in both the T-cell driven 2,4,6-

Trinitrobenzenesulfonic acid (TNBS) and epithelial cell disruption driven dextran sodium sulfate (DSS) models of colitis[56]. These studies indicate IDO1 expression acts as a natural brake in limiting colitis. However, mice with germline deletion of IDO1 do not demonstrate spontaneous colitis, suggested the existence of complementary tolerance promoting pathways exist.

In human IBD, IDO1 activity tracks with disease activity and is expressed in both epithelial and myeloid derived cells.[57] In IBD, chronic inflammation can cause DNA damage by oxidative and nitrosative stress, resulting in genetic and epigenetic alterations[10, 58] placing these patients at a higher risk for developing CAC[9, 10]. As such, in IBD patients with chronic uncontrolled colitis, chronic IDO1 overexpression may take on a pathogenic function to promote CAC.

TRYPTOPHAN METABOLISM PROMOTES TUMOR IMMUNE ESCAPE

IDO1's function in tempering host response to both allogenic and pathogenic antigens hastened the discovery of its role in tumoral escape from immune surveillance. The majority of tumors are infiltrated by immune cells, indicating tumor cells are able to illicit an immune response[19]. However, tumor cells are often able to evade cytolysis, suggesting mechanisms of immune suppression are present in the tumor milieu[59]. IDO1 expression has been described as one such mechanism in several cancers[60–62]. IDO1-expressing tumor-associated dendritic cells inhibit cytotoxic T lymphocyte proliferation and activity[62, 63]. In dendritic cells of tumor-draining lymph nodes, the transcription factor FOXO3 induces expression of both IDO1 and TGF-β, indicating a tolerance pathway parallel to normal immune function[63]. In ovarian cancer, tumor-associated macrophages were shown to produce CCL-22, which recruits Tregs to the tumor microenvironment[64]. Additionally, expression of cytotoxic T-lymphocyte associated protein 4 (CTLA-4) by Tregs has been shown to induce IDO1 expression in DCs, suggesting a potential immunosuppressive feedback loop in cancer-associated immune cells[64, 65].

Inhibition of IDO1 increases anti-tumor immunity in a number of tumor models[60, 61, 66, 67]. Inhibition of IDO1 by 1-methyltryptophan (1mT) reverses T cell inhibition in dendritic cells derived from tumor-draining lymph nodes in a murine model of melanoma and breast cancer[68]. These effects have also been reported for TDO and for the IDO2 inhibitor or D-1mT (indoximod).[6, 31]

IDO1 expression in cancer is not limited to tumor-associated immune cells. A survey of both cancer cell lines and human cancer tissue sections revealed that the majority of carcinoma cells also express IDO1[66]. Theate and colleagues recapitulated these findings by surveying a collection of normal and cancerous human tissues, although their findings illustrate that the cellular source of IDO1 in tumors can include cells from the stroma, endothelium, and the primary neoplastic epithelial cells[69]. IDO1 expression in these tumors are predicted to contribute to an immunosuppressive tumor microenvironment by tryptophan depletion[60, 66, 69, 70].

Tryptophan metabolism in colon cancer

Colon cancers frequently exhibit IDO1 expression in primary tumor and infiltrating myeloid derived cells[3, 66, 69]. Several studies have shown reduced tryptophan levels and increased kynurenine pathway metabolites in colon cancer patients, indicating an increased IDO1 activity[71–73]. High IDO1 expression at the tumor invasion front is an independent adverse prognostic factor for overall survival and metachronous CRC metastases, and high density of IDO1 expressing cells in the tumor draining lymph nodes was associated with a reduced 5 year survival rates in colon cancer patients[3, 74, 75].

IDO1 expression appears to be an important early event in the development of colon dysplasia and may be driven by the genetic changes associated with CRC. Rats exposed to the carcinogen azoxymethane (AOM) express IDO1 in early aberrant crypt foci (ACF).[76] The development of these tumor precursor lesions was reduced with 1mT. KRAS and β-Catenin mutations are common in AOM-induced pre-neoplastic lesions in rats. Though it is not known if these mutations drive the expression of IDO1[77] in CRC, this has been in a model of KRAS driven lung carcinoma.[78]

Constitutive expression of IDO1 is also present in some CRC cell lines like HCT-116 and HT-29, but not in Caco-2[2, 74, 79]. As this expression occurs in the absence of an inflammatory tumor milieu, it suggests IDO1 expression may be related to the genetic changes that drive CRC.[80, 81] In support of this, our lab has found that IDO1 expression is higher in the colon epithelial cells from $Apc^{min/+}$ mice compared to WT controls (unpublished data). Studies performed on tumor tissues with genetic alterations in APC, KRAS or p53 alone could help identify the precise regulation of IDO1 by these genetic alterations[82].

Other studies also identify links between cancer associated genes and IDO1 expression. In a mouse model of esophageal carcinoma, overexpression of the extracellular matrix protein periostin and a mutant p53 induced STAT1, which in turn induced IDO1[83]. IDO1 is also a known genetic target of the transcriptional repressor BIN1[67]. Only one published study has evaluated this relationship in CRC and did not identify a significant correlation.[75] Understanding the mechanisms responsible for IDO1 expression in CRC should facilitate the identification of novel ways to target this pathway in cancer and thus deserves further investigation.

In CRC and CAC, inflammatory pathways likely also drive IDO1 expression. The IDO1 promoter region contains two interferon-sensitive response elements and two interferon-γ activated sites, and therefore is upregulated in response to a number of inflammatory stimuli[84, 85]. Litzenburger and colleagues recently identified an autocrine signaling loop whereby IDO1 sustains its own expression via AHR-mediated IL-6 expression and STAT3 activation[85]. Though not this study's focus, this pathway is certainly relevant in CRC and especially so in CAC.

IDO1 expression directly promotes proliferation of colon cancer

We identified a key role for IDO1 in promoting tumorigenesis in the AOM/DSS mouse model of CAC.[2] Germline $IDO1^{-/-}$ mice developed tumors that were smaller, had a lower

tumor proliferation index and reduced nuclear β-catenin. These findings were recapitulated in mice treated with the IDO1 inhibitor 1mT. We went on to identify that this effect was independent of IDO1's impact on adaptive immunity as the phenotype was also observed Rag1 null mice. Moreover, we found a specific role for epithelial IDO1 by demonstrating that gene silencing reduced proliferation in CRC cell lines which constitutively expressed IDO1. Proliferation could be normalized by addition of exogenous kynurenines, though the upstream signaling events were not yet identified. These findings together support a *new paradigm for dual functions of IDO1* in colon neoplasia to both promote adaptive immune-tolerance and to directly promote tumor cell growth.

IDO1 functions at the host-microbe interface

Luminal microbiota can have a profound influence GI health including the development and progression of colitis and colon cancer.[86, 87] Resident microbes interact with host cells directly by binding pattern recognition receptors (PRRs) and indirectly by secreted metabolites[88, 89]. An increasing body of evidence indicates IDO1 is an important player in host-microbe crosstalk, which may impact the pathophysiology of gastrointestinal cancer. One way IDO1 is involved in host-microbe interactions is at the level of PRRs. Expression of IDO1 is induced in response to activation of several PRRs, especially TLR4 and TLR9[56, 90]. CpG oligonucleotides (CpG-ODNs), a Toll-like receptor (TLR) 9 agonist induces IDO1 throughout the GI tract and protect from murine colitis[56]. Additionally, activation of TLR4 by lipopolysaccharide is important in promoting a pro-tolerance environment dependent on IDO1 expression[52, 91].

However, the physiologic impact of IDO1 and TLR signaling is dependent on both cell type and disease context. For example, contrary to these findings, in a mouse model of sepsis, IDO1 activity exacerbates inflammation and disease activity by increasing the sensitivity of TLR4[92]. This is important in considering the suggested role of TLR signaling in cancer. One study of colon polyps from 70 human patients revealed that, while TLR7 and TLR9 expression is higher in hyperplastic or adenomatous polyps, TLR9 expression in polyps from patients which formed CRC was decreased[93]. Treatment with CpG-ODNs has also been shown to enhance the efficacy of chemotherapeutic agents in several mouse tumor models[90, 94, 95]. Additionally, treatment with an IDO1 inhibitor augments the anti-tumor effects of the TLR7 agonist imiquimod in mice inoculated with colon carcinoma cells[96]. These findings suggest TLR signaling, and perhaps the associated IDO1 expression, has differential roles in normal and cancer tissues[90, 97].

Secreted bioactive metabolites represent another way in which microbial populations interact with the host and has likely relevance to CRC[88, 89, 98–100]. Two classes of microbial metabolites with particular relevance to tryptophan metabolism and CRC are short chain fatty acids and indole compounds[101]. Short chain fatty acids such as acetic, propionic, and butyric acid play significant roles in intestinal homeostasis and resistance to tumorigenesis[102–105]. Butyrate-producing microbial populations are reduced in CRC-associated microbial dysbiosis[106]. While not yet evaluated in CRC, butyrate reduces IDO1 expression in some epithelial carcinomas which could contribute to its tumor suppressive properties[102, 104]. Indole compounds are generated from dietary tryptophan by commensal

colonic microbiota. Several of these indole metabolites can in turn activate the aryl hydrocarbon receptor (AHR)[89, 107], which has important roles in several cancers and is discussed in greater detail below. The emerging field of microbial metabolomics further highlights the importance of the gut microbiota in gastrointestinal cancer pathophysiology[108]. Figure 2 illustrates potential mechanisms by which IDO1 may be involved in host-microbial interactions.

COLON CANCER RELEVANT PATHWAYS THAT INTERSECT WITH IDO1

The aryl hydrocarbon receptor

First identified as a dioxin detoxifying enzyme, AHR is a cytoplasmic transcription factor which is activated by a variety of compounds[109]. A number of tryptophan metabolites produced by both host and microbes act as agonists for this receptor[35, 36]. Activation of AHR is an important mechanism by which IDO1 expression promotes immune tolerance[43, 44]. AHR effect on pathology appears to depends on the cancer type[109]. Constitutive AHR activation leads to the spontaneous gastric tumors[110]. In brain tumors, kynurenines derived from TDO2 activate AHR to promote clonogenic survival and malignant progression[35, 111]. A number of studies have shown that AHR activation leads to enhanced tumor cell proliferation[109]. However, the role of ligand-activated AHR in colon cancer pathogenesis is less clear.[107, 112] One study suggested that AHR may actually be a tumor suppressor in CRC as its genetic ablation led to β-catenin accumulation and increased cecal tumor formation in both wildtype and $Apc^{Min/+}$ mice[107]. Finally, it is intriguing to note that in breast and ovarian cancer AHR activation and IDO1 induction appear to be in a feedback loop with one another (Figure 2)[43, 85]. Further defining of the interactions between IDO1 and AHR in CRC is warranted[113, 114].

Quinolinic acid dependent NAD^+ production offers resistance to apoptosis

An important characteristic of cancer cells is their ability to counter cellular stress in the setting of hypoxia and high proliferative activity. Nicotinamide adenine dinucleotide (NAD +) plays a key role as both a substrate and an enzyme cofactor in maintaining cellular integrity under these conditions.[115] As such, cancer cells rapidly consume NAD+ and require alternative synthesis pathways (other than via nicotinamide). The tryptophan-kynurenine pathway generates Quinolinic Acid (QA) is an alternative substrate for NAD+ synthesis in a step involving the enzyme quinolinate phosphoribosyltransferase (QPRT). QA, as a precursor to NAD+, has recently been identified as an important factor in conferring resistance of gliomas to oxidative stress.[116] QPRT expression was induced in glioblastoma patients who underwent chemotherapy. High QPRT expression was associated with poor prognosis, indicating glioma cells resisted apoptosis to chemotherapy by inducing *de novo* NAD^+ synthesis[116]. Colon carcinoma cells also express QPRT[117]. If colon carcinoma cells produce NAD^+ from QA, antitumor efficacy of IDO1 inhibitors could potentially be enhanced by concurrent blockade of other enzymes involved in NAD^+ synthesis such as nicotinamide phosphoribosyltransferase using the specific inhibitor FK866.

Inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2)

iNOS and COX-2 are known mediators of colon carcinoma progression[118–121]. iNOS expression is reported in aberrant crypt foci (ACF) of both rats and humans[121, 122]. In humans, enhanced iNOS staining was found in ACF transitioning from hyperplasia to dysplasia[78]. Several studies have shown that iNOS can up-regulate COX-2 expression[123–127] and COX-2 has been shown to upregulate IDO1 expression in some cases[128–130]. Clarifying the relationship between the complementary pathways may lead to the identification of novel and synergistic was to target CRC.

OTHER GASTROINTESTINAL CANCERS

Tryptophan metabolism along the kynurenine pathway is relevant in other gastrointestinal tumors, though the data is less well developed. We will briefly discuss this pathway in gastric, pancreatic, esophageal and stromal tumors. As each of these tumors is typically diagnosed at a late stage and has a poor prognosis, it is exciting to think that inhibitors of this pathway may have a role in improving therapeutic outcomes.

Gastric carcinoma

IDO1 is overexpressed in up to 90% of human gastric carcinomas[66, 131]. Zhang *et al* reported the expression of IDO1 correlated significantly with tumor invasion depth, lymph node positivity and reduced tumor infiltration with $CD4^+$ and $CD8^+$ T cells[132]. Other human specimen and cell culture studies indicate that gastric tumor cells may promote immune escape via IDO1 activity[133, 134]. One of these studies intriguingly tied expression of FoxP3 in gastric carcinoma cells to IDO1 expression.[133]

Pancreatic carcinoma

Enhanced expression of IDO1 is reported in pancreatic carcinoma in humans[66]. Overexpression of IDO1 in metastatic pancreatic ductal adenocarcinoma (PDA) was shown to be associated with increased T cell numbers[135]. This study also reported that IDO1 expression was present in both primary and metastatic tumors in all the patients with lymph node metastasis, and suggested that PDA use IDO1 mediated immune escape mechanisms to survive in the lymph node. The same group later published another study showing that IDO2 was also expressed in PDA and should be considered as a target for inhibition along with IDO1[136]. IDO1 inhibitor INCB023483 was shown to inhibit the growth of murine PAN02 pancreatic cell-derived tumors in WT and $IDO1^{-/-}$ mice, highlighting that tumor-derived IDO1 contributes to pancreatic tumor growth and immune evasion.[137]

Esophageal carcinoma

Enhanced expression of IDO1 is observed in esophageal carcinoma and its expression correlates with poor survival[138, 139]. IDO1 expression correlated with a lower number of tumor infiltrating lymphocytes[139]. In a mouse model of p53 mutant esophageal cancer, the expression of IDO1 was shown to be associated with expression of periostin, an extracellular matrix protein involved in esophageal carcinoma metastasis. In this model, periostin induced expression of IDO1 via STAT1 activation, indicating an intersection between inflammatory signaling pathways and malignancy in esophageal carcinoma[83].

Gastrointestinal stromal tumors (GIST)

GIST account for less than 1% of all GI cancers but are the most common non-epithelial GI tumor. Imatinib is a drug which targets mutated KIT oncoproteins and produces clinical response in 80% of patients. In a mouse model of spontaneous GIST, activation of CD8+ T cells was found to be key to the antitumor effects of Imatinib and that this effect involved IDO1[140]. Moreover, concomitant CTLA-4 blockade of T cell co-stimulation further enhanced Imatinib efficacy suggesting a role for dual immunotherapies as a strategy for GIST.

THERAPEUTIC TARGETING OF TRYPTOPHAN METABOLISM PATHWAYS FOR COLORECTAL CANCER

IDO1 is a well-established target for drug discovery in cancer immunotherapy. Outstanding recent reviews have detailed IDO1 inhibitors and other inhibitors of tryptophan metabolism.[31] Here we will briefly summarize the topic with a focus on application to CRC. We also highlight that IDO1 inhibitors' role in clinical oncology will most likely be as a combined, rather than monotherapy.

Pharmacologic Inhibitors

The most commonly used inhibitor of IDO1 in preclinical studies is the methylated tryptophan molecule termed 1-methyl Tryptophan (1mT). Studies have used the L and D enantiomers alone or in combination. While the L-enantiomer is more specific for blocking IDO1 activity, its practical implementation for human clinical applications it limited by low potency, poor solubility and likely off target effects.[4, 31] The D-enantiomer of 1mT is being evaluated in clinical trials (indoximod, NLG8189). IDO2 appears to be its main target as an enzymatic inhibitor.[79, 141] However, IDO2 has also been demonstrated to impact IDO1 function and T-cell suppression.[142] NewLink Genetics also has an IDO1 inhibitor, NLG919, which increased the efficacy of Indoximod against a preclinical model of melanoma.

A novel hydroxyamidine small molecule (INCB24360) was recently described as a potent specific inhibitor of IDO1. It does not affect IDO2, TDO or tryptophan transporter THP-1 and has a more favorable pharmacokinetic profile than 1mT with better oral bioavailability.[137, 143] INCYTE, the maker of this compound, sponsored a Phase I clinical trial with INCB024360 in 52 patients with advanced solid tumors which had failed prior therapies.[143, 144] 29 (55.8%) of enrolled patients had colon cancer. This compound demonstrated safety and a maximum tolerable dose was not established. However, at doses ≥300 mg BID produced biochemical efficacy by lowering the serum Kyn/Trp ratio, a marker of IDO1 activity.[143] No patients in this study demonstrated complete or partial response, but nearly 30% did demonstrate stable disease for eight weeks. Based on these results INCB24360 is currently being evaluated as a combination with Ipilimumab for melanoma in a phase Ib/2 study and advanced ovarian cancer as a monotherapy. This agent is not currently being targeted for CAC or CRC, but these results would suggest that it will likely need to be combined with another therapeutic.

TDO expression is another way by which some tumors pathogenically exploit tryptophan metabolism.[36] The TDO pathway is important because IDO1 inhibitors typically do not target its activity and it is possible that IDO1 inhibition could enhance TDO expression or function. Some studies suggest that TDO is also expressed in a subset of CRC,[35] a finding corroborated by our query of TCGA datasets[145, 146] (Table 1). However, even in CRC, the expression of TDO appears to be ~100 fold less than in the normal liver.[36] TDO specific inhibitors are described in preclinical models,[36] but have not yet moved clinical trials. Hepatotoxicity is a potential concern given that TDO is constitutively expressed in the liver where it plays a role in maintaining systemic tryptophan levels. Specific gene silencing of both IDO1 and TDO in specific cell types may be one way to get around this problem.

Recently, investigators probed CRC tissue micro-arrays using a new and proprietary L-kynurenine specific antibody.[147] Positivity was noted in the neoplastic epithelium of 20% of 69 CRC samples and was not detected in normal colon epithelial cells. Positivity of IDO1 and kynurenine staining overlapped in only 42% of cases. Immunostaining for IDO2 and TDO were not reported, though it is inferred that these may be the source of kynurenine production. Bacterial produced kynurenine may be another consideration. There are inherent limitations to immunohistochemistry based scoring and selection bias confounds interpretation of tissue micro-arrays. However, if confirmed, these findings suggest that IDO1 inhibition alone may not be sufficient to counter the pathogenic functions of the kynurenine pathway in CRC. It is conceptually intriguing that antibodies against kynurenine could fill that gap, but physiologic proof of efficacy is not yet available.

Gene silencing of IDO1

Genetic manipulation is another promising option for targeting tryptophan metabolism in cancer. Using RNA interference, Zheng and colleagues illustrated that silencing IDO1 reduced melanoma tumor growth both in vitro and in vivo.[148] This same group subsequently demonstrated that silencing IDO1 in dendritic cells which have been loaded with murine breast cancer antigens significantly enhanced the efficacy of this DC-based vaccine approach.[149] An exciting feature of this approach is that it can be targeted to specific cell types and can inhibit not only IDO1, but other tryptophan metabolizing enzymes concurrently. Furthermore, unlike pharmacologic inhibitors, gene silencing also eliminates any non-enzymatic tolerance promoting capabilities of the IDO1 protein.[46] The startup company formed based on these discoveries was recently bought by a large pharmaceutical company.

IDO inhibitors as Combinatorial Therapy

How IDO1 inhibitors will most effectively be employed in the clinical setting is an active area of investigation. It is likely that these agents will be most effective when used in combination with other immune checkpoint inhibitors or with traditional cytotoxic therapies. In most preclinical models, just as with the Phase I clinical trials, IDO1 inhibition alone slowed neoplastic growth but did not eliminate or prevent the tumors. This slowing of tumor growth was found in a preclinical model of a CRC using a syngeneic tumor implant and IDO1 inhibition with INCB024360.[137]

There are currently no clinical trials specifically targeting IDO1 in combination with other cytotoxic or immunotherapies for CRC. However, in breast cancer cell lines IDO1 gene silencing enhanced the effect of radiation therapy, suggesting this may be an effective option.[150] Additionally, INCB24360 is being evaluated in combination with programmed cell death (PD-1) inhibitors in ongoing Phase I/II clinical trials for other solid tumors. One of these two trials does include metastatic CRC patients who have progression on or intolerance of approved standard therapies. The recent report identifying that an anti-PD1 inhibitor had some effect in subgroups of sporadic CRC suggests promise for this approach[151].

Alternatives Approaches to Targeting IDO1

Beyond direct inhibition of IDO1, Platten and colleagues recently proposed five conceptually distinct hubs that may serve as potential therapeutic targets for interfering with tryptophan catabolism in the context of cancer.[31] These include: 1) TDO and IDO2 inhibitors which may have enhanced function when IDO1 is inhibited. 2) Upstream regulators of IDO1 such as KIT, STAT3 and BIN1 3) Downstream targets affected by IDO1 mediated tryptophan depletion including mTOR and GCN2. 4) Kyurenine pathway receptors such as AHR and GPR35. 5) Cell membrane tryptophan transporters which could modify intra-and extracellular levels of tryptophan.

CONCLUSIONS AND FUTURE DIRECTIONS

Activated tryptophan metabolism is a common feature in colorectal cancer and preclinical data indicates that this pathway promotes neoplastic disease progression. Orally administered small molecule inhibitors of IDO1, the most prevalent enzyme in this pathway, have demonstrated safety and tolerability in Phase I human trials. These agents appeared to slow, but not halt progression of solid tumors including colorectal cancer. Thus, key questions remain as to how these agents might be used in CRC. Should patients be selectively chosen based on demonstrated IDO1 overexpression? Could quantitative grading of IDO1 expression in tumor biopsy specimens be used to identify the subset of patients in which inhibitors might be most effective? What is the clinical prevalence and distribution of high IDO1 expression among CRC subtypes? Could IDO1 inhibitors have enhanced efficacy by combining them with currently available cytotoxic therapies or other immune checkpoint inhibitors in development. Do alternative tryptophan metabolizing enzymes including IDO2 or TDO also have important functional roles in CRC? Clarifying the answers to these questions should guide the application of a novel and low toxicity therapeutic option for patients with advanced colon cancer.

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ABBREVIATIONS

CRC	colorectal cancer
CAC	colitis-associated cancer
ROS	reactive oxygen species
RNS	reactive nitrogen species
DNA	deoxyribonucleic acid
APC	adenomous polyposis coli
KRAS	Kirsten rat sarcoma
GSK-3β	glycogen synthase kinase 3 beta
TNF	tumor necrosis factor
IFN	interferon
IL	interleukin
DC	dendritic cell
KP	kynurenine pathway
IDO1	indoleamine 2,3 dioxygenase 1
IDO2	indoleamine 2,3 dioxygenase 2
TDO2	tryptophan dioxygenase
APCs	antigen presenting cells
GCN2	general control nonderepressible 2
mTOR	mammalian target of rapamycin
AHR	aryl hydrocarbon receptor
TGF-β	transforming growth factor β
Tregs	regulatory T cells
NF-κB	nuclear factor kappa B
TLR	toll-like receptor
TNBS	2,4,6-Trinitrobenzenesulfonic acid
DSS	dextran sodium sulfate
AOM	azoxymethane
1mT	1-methyltryptophan
CCL	CC chemokine ligand
CD	cluster of differentiation
CTLA-4	cytotoxic T-lymphocyte associated protein 4

STAT	signal transducers and activators of transcription
QPRT	quinolinate phosphoribosyltransferase
MSCs	Mesenchymal stem cells
NO	nitric oxide
iNOS	inducible nitric oxide synthase
COX-2	cyclooxygenase 2
ACF	aberrant crypt foci
PDA	pancreatic ductal adenocarcinoma

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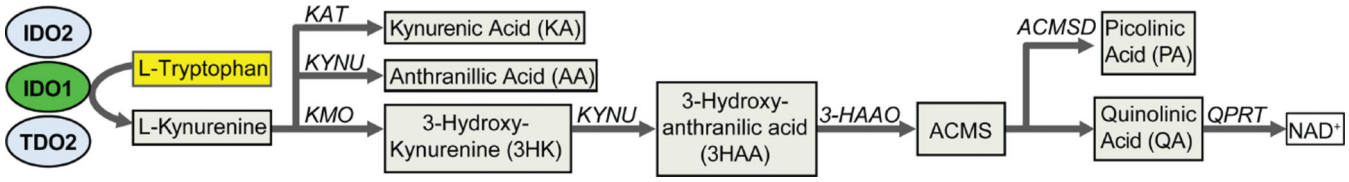

Figure 1. Tryptophan metabolism along the kynurenine pathway

The principle enzymes (IDO1, IDO2 and TDO) are shown. The bioactive metabolites downstream of kynurenine are shown with the respectively required enzymes. *KAT*, kynurenine aminotransferase; *KMO*, kynurenine 3-monooxygenase; *KYNU*, kynureninase; *3-HAAO*, 3-hydroxyanthranillic acid oxygenase; ACMS, 2-Amino-3-carboxymuconate semialdehyde; *ACMSD*, Aminocarboxymuconate-semialdehyde decarboxylase; *QPRT*, quinolinic acid phosphoribosyl transferase. NAD+, nicotinamide adenine dinucleotide (oxidized form)

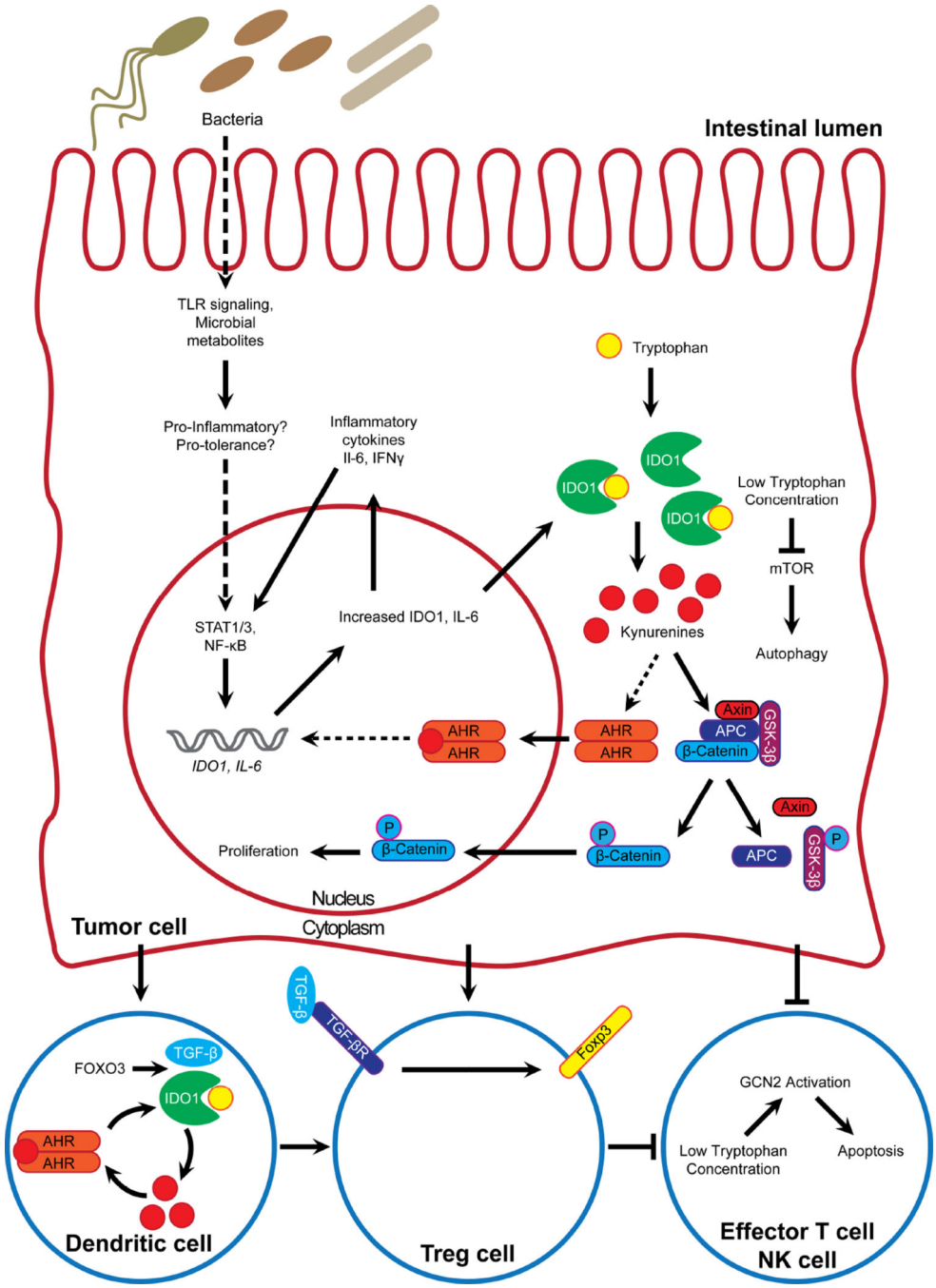

Figure 2. Schematic of confirmed and postulated roles and interactions of IDO1 in colorectal cancer

IDO1 modulates signaling pathways in both cell autonomous and non-autonomous fashion. Solid lines represent interactions demonstrated in colorectal cancer, while dotted lines represent interactions extrapolated from other cell or tissue contexts.

Table 1
Correlation of IDO1 and related gene expression in colon cancer

Gene expression profiling of colorectal adenocarcinoma samples relative to IDO1 expression. Gene expression data from colon and rectal adenocarcinomas (cbioportal) were analyzed using Graphpad Prism 5 to identify correlations in gene expression patterns relative to IDO1. Data generated by the TCGA Research Network: http://cancergenome.nih.gov/. TCGA provisional Colorectal Adenocarcinoma Dataset, Illumina HiSeq V2 RNA expression data. N=365. Two-tailed P values are reported for Pearson's correlation coefficient (r).

GENE	PEARSON (r)	CORRELATION WITH *IDO1*	P VALUE
IDO2	0.265	Positive	<0.0001
TDO2	−0.013	Negative	0.8115
IL10	0.171	Positive	0.001
STAT1	0.621	Positive	<0.0001
TGFB1	0.248	Positive	<0.0001
AHR	0.139	Positive	0.0079