Published OnlineFirst January 24, 2019; DOI: 10.1158/0008-5472.CAN-18-0668

Tumor Biology and Immunology

Cancer Research

IDO1 and Kynurenine Pathway Metabolites Activate PI3K-Akt Signaling in the Neoplastic Colon Epithelium to Promote Cancer Cell Proliferation and Inhibit Apoptosis

Kumar S. Bishnupuri, David M. Alvarado, Alexander N. Khouri, Mark Shabsovich, Baosheng Chen, Brian K. Dieckgraefe, and Matthew A. Ciorba

Abstract

The tryptophan-metabolizing enzyme indoleamine 2,3 dioxygenase 1 (IDO1) is frequently overexpressed in epithelial-derived malignancies, where it plays a recognized role in promoting tumor immune tolerance. We previously demonstrated that the IDO1–kynurenine pathway (KP) also directly supports colorectal cancer growth by promoting activation of β-catenin and driving neoplastic growth in mice lacking intact adaptive immunity. In this study, we sought to delineate the specific role of epithelial IDO1 in colon tumorigenesis and define how IDO1 and KP metabolites interact with pivotal neoplastic signaling pathways of the colon epithelium. We generated a novel intestinal epithelial–specific IDO1 knockout mouse and utilized established colorectal cancer cell lines containing β-catenin–stabilizing mutations, human colorectal cancer samples, and human-derived epithelial organoids (colonoids and tumoroids). Mice with intestinal epithelial–specific knockout of IDO1 developed fewer and smaller tumors than wild-type littermates in a model of inflammation-driven colon tumorigenesis. Moreover, their tumors exhibited reduced nuclear β-catenin and neoplastic proliferation but increased apoptosis. Mechanistically, KP metabolites (except kynurenic acid) rapidly activated PI3K-Akt signaling in the neoplastic epithelium to promote nuclear translocation of β-catenin, cellular proliferation, and resistance to apoptosis. Together, these data define a novel cell-autonomous function and mechanism by which IDO1 activity promotes colorectal cancer progression. These findings may have implications for the rational design of new clinical trials that exploit a synergy of IDO1 inhibitors with conventional cancer therapies for which Akt activation provides resistance such as radiation.

Significance: This study identifies a new mechanistic link between IDO1 activity and PI3K/AKT signaling, both of which are important pathways involved in cancer growth and resistance to cancer therapy.

Introduction

Several types of cancer pathologically exploit tryptophan metabolism along the kynurenine pathway (KP) to promote growth and escape immune surveillance (1–3). Indoleamine 2, 3 dioxygenase 1 (IDO1) is the most widely studied of tryptophan-metabolizing enzymes that catalyze the initial step of the KP. Tumor overexpression of IDO1 increases local kynurenine (Kyn) concentrations and depletes tryptophan levels. These changes promote an immune-tolerant tumor microenvironment by several mechanisms including suppressing the tumor-reactive effector T-cell responses and NK-cell responses, promoting T-regulatory cell differentiation as well as the expansion and activation of myeloid-derived suppressor cells (2, 4, 5). In addition, a nonenzymatic, protolerance function is also attributed to IDO1 via its interactions with TGFβ (6).

Human and animal studies illustrate the importance of IDO1 in cancer. In preclinical models, IDO1 expression promotes greater tumor burden of several cancers including those of the colon, lung, skin, pancreas, and breast (7–14). In humans, increased IDO1 expression is associated with poor clinical prognosis across several solid tumor types (7, 15, 16). On the basis of these findings, IDO1 inhibition is currently under evaluation in clinical trials for numerous cancer types (17, 18). The IDO1 functional ortholog, tryptophan dioxygenase (TDO), and evolutionary paralog, IDO2, also metabolize tryptophan and contribute to neoplastic pathogenesis in some models; however, the potential for therapeutic targeting remain less well developed than for IDO1.

IDO1 overexpression is a common feature of human colorectal cancer, the second leading cause of cancer-related death in the United States. Pathology studies show that IDO1 expression localizes to colorectal cancer–infiltrating myeloid-derived cells as well as in the neoplastic colon epithelium (3, 19, 20). Patients with colorectal cancer also exhibit reduced serum tryptophan levels and increased KP metabolites, indicating increased IDO1 activity (21–23). Furthermore, high epithelial IDO1 expression at the tumor invasion front is an independent adverse prognostic

Division of Gastroenterology and the Inflammatory Bowel Diseases Center, Washington University School of Medicine, St. Louis, Missouri.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Corresponding Authors: Matthew A. Ciorba, Washington University School of Medicine, Campus Box 8124, 660 South Euclid Ave, St Louis, MO 63110. Phone: 314-362-9054; Fax: 314-362-8959; E-mail: mciorba@wustl.edu; and Kumar S. Bishnupuri, E-mail: kbishnup@wustl.edu

doi: 10.1158/0008-5472.CAN-18-0668

Published OnlineFirst January 24, 2019; DOI: 10.1158/0008-5472.CAN-18-0668

factor for overall survival and metachronous colorectal cancer metastases, whereas high density of IDO1-expressing cells in the tumor-draining lymph nodes was associated with a reduced 5-year survival rates in patients with colon cancer (16, 20, 24). These findings, which are reviewed more thoroughly elsewhere (25, 26), highlight the relevance of IDO1 as a therapeutic target in human colorectal cancer.

We recently examined the role of IDO1 in a model of colitis-associated cancer and in the cell lines derived from patients with sporadic colorectal cancer. This study demonstrated that IDO1 is highly expressed in the neoplastic colon epithelium and that promoted tumor growth (14). Germline genetic deletion of IDO1 and administration of the first-generation IDO1 inhibitor (1-methyl tryptophan) decreased tumorigenesis. Two novel mechanistic observations arose from these studies. First, IDO1 blockade reduced tumorigenesis even in the mice lacking mature adaptive immunity. Second, IDO1 activity promoted nuclear translocation of epithelial cell β-catenin, a pivotal transcriptional regulator in colorectal cancer. Together these findings provided initial evidence that IDO1 expression is a pathogenic driver of colorectal cancer progression by a mechanism involving the neoplastic epithelium and one that is complementary to its ability to promote immune tolerance through T-cell suppression.

In this study, we sought to delineate the role of epithelial IDO1 in colon tumorigenesis and to define the regulators and mechanism of its immune-independent protumorigenic effect. To address this, we developed an epithelial-specific IDO1 knockout mouse and examined the human colorectal cancer samples, established cell lines, and human-derived colonoids and tumoroids. The data presented herein reveal that epithelial cell IDO1 is key to colon tumorigenesis and that KP metabolites rapidly activate PI3K-Akt signaling in the neoplastic epithelium to promote cellular proliferation and resistance to apoptosis. These findings are highly relevant to IDO1 inhibitors as they move through clinical trials and to the important unmet therapeutic need in advanced colorectal cancer.

Materials and Methods

Mice and *in vivo* modeling of colon cancer

All mice used in this study were on C57BL/6J background. Heterozygous Ido1 "knockout-first" tm1A allele mice; $Ido1^{tm1a(EUCOMM)Wtsi}$ were initially acquired as heterozygotes via Material Transfer Agreement from the Wellcome Trust Sanger Institute [Ido1 (MDCT; EPD0198_1_F02)]. A schematic of the targeting vector used to create the Ido1 "knockout-first" allele (tm1a) is shown in Fig. 1A. As the figure illustrates, subsequent breeding with FLPeR mice and Cre mice with under the control of villin promoter generated mice with intestinal epithelium–specific Ido1 knockout (IDO1-iKO). A detailed protocol is given in the Supplementary Materials and Methods. Cre-littermates were used as wild-type (WT) controls.

Age (6–12 weeks)-matched littermates were used in all experiments with approximately equal representation of male and female mice. Animals were housed in specific pathogen-free barrier facility and all the protocols were performed under the regulations of and approval of Washington University's (St Louis, MO) Institutional Animal Care and Use Committee. Induction of colon carcinogenesis by azoxymethane (AOM) followed by cycles of dextran sodium sulfate (DSS), preparation of tissue section for histology and assessment of disease activity index were done as per earlier published protocols (14, 27, 28). Detailed mouse phenotyping data are shown in Fig. 1A. Additional phenotyping data and detailed procedures data are provided in Supplementary Methods and Methods and Supplementary Fig. S1A–S1D.

Organoid culture

The collection and use of human tissue for establishing primary epithelial cell culture or organoid culture was approved by the Washington University human research protection office (Institutional Review Board) and collected by the Washington University Digestive Diseases Research Core Center Biobank Core. Informed consent was obtained from all patients. Organoid cultures were established from human and mouse tissues and maintained in Matrigel (catalog no. 354234; Corning Life Sciences) as described previously by our group and others (29–31). Human tumoroids were derived from three colorectal cancer specimens from patients with known APC mutations and familial adenomatous polyposis syndrome. Mouse normal colonoids were established from WT and iKO mice, whereas tumoroids were established from colon tumors of WT mice. Differentiation was induced in normal organoids by maintaining them in 1:10 diluted L-WRN conditioned media with Advanced DMEM/F12 supplemented with 10 μmol/L Y-27632 (ROCK inhibitor; Tocris Bioscience, R&D Systems) before using them for experiments (29). Tumor organoids were maintained in Advanced DMEM/F12 supplemented with 10 μmol/L Y-27632 (Tocris Bioscience) and 10 μmol/L SB 431542 (TGFBR1 inhibitor; Tocris Bioscience, R&D Systems). For experiments comparing human and mouse normal organoids with tumor organoids, tumor organoids were maintained in 1:10 diluted L-WRN conditioned media to match the growth conditions of normal organoids.

Reagents and assays

Human colorectal cancer cell lines HT-29 (ATCC HTB-38; MSS; KRAS WT), DLD1 (ATCC CCL-221; MSI; KRAS mutant), and HCT116 (ATCC CCL-247; MSI; KRAS and B-Catenin mutant) were purchased from ATCC at study initiation. Cells were grown in DMEM (Gibco, Thermo Fisher Scientific) with high glucose supplemented with 10% FBS (Gibco) and 1% penicillin/streptomycin (Corning Life Sciences). Data presented use cell lines from passage 3 and 30 at approximately 70%–80% confluence. Cell lines were checked for *Mycoplasma* contamination in September 2016 at the Tissue Culture Support Center in Washington University School of Medicine (St Louis, MO). Kyn, 3-hydroxyanthranillic acid (3HAA), quinolinic acid (QA), picolinic acid (PA), kynurenic acid (KA), xanthurenic acid (XA), and anthranilic acid (AA) were purchased from Millipore Sigma. The 3-hydroxykynurenine (3HK) was acquired from Santa Cruz Biotechnology. Each were solubilized according to manufacturer's recommendation. Recombinant Human DKK-1 (catalog no. GF170) was purchased from Millipore. Total protein was extracted from colorectal cancer cell lines using Cell Lysis Buffer (Cell Signaling Technology) as per manufacturer's recommendation. Total protein was extracted from organoids using RIPA Buffer (Sigma) as per published protocols (29). Cytoplasmic and nuclear proteins were extracted from HT-29 cells using NE-PER (Thermo Fisher Scientific). Total protein was estimated using BCA Protein Assay Kit (Thermo Fisher Scientific) before subjecting them to Western blot analyses. All

Published OnlineFirst January 24, 2019; DOI: 10.1158/0008-5472.CAN-18-0668

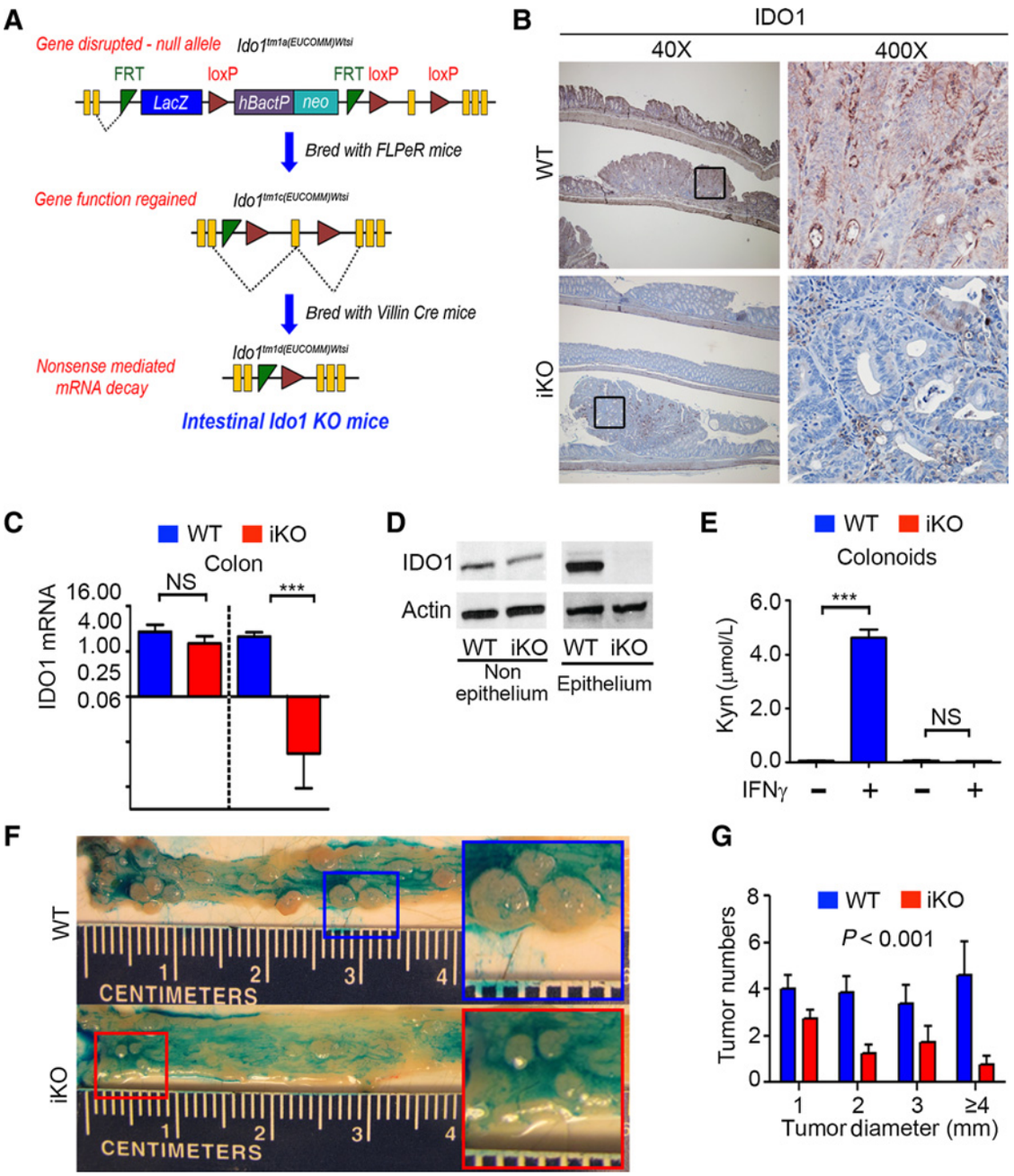

Figure 1.

Epithelial IDO1 promotes tumor growth and neoplastic proliferation in the colon. Mice with intestinal epithelial specific Ido1 deletion (IDO1-iKO) and WT (Cre-) littermate controls were examined for gene expression and propensity for colon tumorigenesis when exposed to the AOM/DSS protocol. Mice received 10 mg/kg AOM followed by two week-long cycles of 2.25% DSS in drinking water separated by sterile water for 2 weeks. **A,** Schematic for generating iKO mice. **B,** IHC for IDO1 on AOM/DSS–induced tumors in iKO and WT mice. **C** and **D,** mRNA and IDO1 protein expression from isolated tracts of nonepithelial and epithelial cellular compartments of mice 48 hours after IDO1 induction with 20 μg immunoprecipitation of CpG DNA. **E,** Measurement of Kyn production in the supernatants of colon organoid culture from WT and iKO mice. **F,** Representative colon morphology with tumors highlighted by topically applied Alcian blue. Magnified image of tumors shown in inserts to highlight the difference in the sizes of tumors. **G,** Tumor quantification showing reduced tumor size and number in IDO1-iKO mice. Statistical comparison by two-way ANOVA (**D**) or Student *t* test (**F**). $N = 5$–6 mice/group, 2–4 tumors counted/mouse.

phosphorylation-specific and corresponding total antibodies used in this study were purchased from Cell Signaling Technology, unless specified. Supplementary Table S1A lists all the antibodies used in this study.

Signal transduction assays

Colorectal cancer cells at approximately 75% confluency and serum were starved for 24 hours then incubated with KP metabolites for defined time points followed by total protein or

cytoplasmic and nuclear protein extraction. For experiments involving signaling pathway inhibition, HT-29 cells were incubated with 20 μmol/L PI3K (GDC-0941, Selleckchem) or Akt (MK2206, Selleckchem) inhibitor 4 hours prior to KP metabolite incubation. Furthermore, to determine whether the activation of Akt signaling by Kyn is intracellular, a putative Kyn transporter, L-amino acid transporter 1 (LAT1), was blocked using specific inhibitors BCH and JPH203 overnight prior to KP metabolite incubation. Organoids were first isolated from Matrigel using cell recovery solution (catalog no. 354253; Corning Life Sciences) after overnight serum depletion, then incubated with KP metabolites in serum-free DMEM/F12 followed by total protein extraction.

Proliferation and cell viability assay

Colorectal cancer cell lines and organoids were plated in flat-bottom 96-well plates. Eight hours after plating, colorectal cancer cells were serum starved overnight followed by incubation with 100 μmol/L KP metabolites in DMEM containing 1% FBS. Organoids were plated in Matrigel and maintained in L-WRN (20% FBS) media for 12 hours after plating. The media were then changed to 1% FBS containing differentiation media and maintained for 12 hours followed by the addition of 100 μmol/L KP metabolites in 1% FBS differentiation media. Tumor organoids were maintained in advanced DMEM (20% FBS) for 12 hours followed by maintenance in 1% FBS-containing differentiation media for 12 hours. After 12 hours, 100 μmol/L KP metabolites were added in 1% FBS containing differentiation media. Proliferation was measured using CCK-8 assay (Dojindo Laboratories) after 72 hours of incubation with KP metabolites in both colorectal cancer cell lines and organoids.

Apoptosis assessments

Expression of cleaved PARP was used as a measure of apoptosis by Western blotting and immunofluorescence. HT-29 cells were grown to approximately 75% confluency before serum starving overnight. To induce apoptosis, cells with treated with 100 ng/mL TNFα (PeproTech) or 2 μmol/L Staurosporine (Cell Signaling Technology) in DMEM containing 1% FBS for 12 hours with or without combinations of Akt inhibitor (MK2206) and 100 μmol/L Kyn. Cells were pretreated with 5 ng/mL cycloheximide for 1 hour before incubating with TNFα or staurosporine (STP). Cleaved PARP was determined using rabbit anti-cleaved PARP mAb (Cell Signaling Technology) by Western blot analysis.

For immunofluorescence, cells were seeded on coverslips in 6-well plates and grown to approximately 75% confluency. Media were aspirated and coverslips were washed in cold PBS then fixed in fresh 4% paraformaldehyde in PBS for 20 minutes at room temperature. Cells were permeabilized (0.1% Triton X-100 in PBS for 10 minutes), washed in cold PBS, and then blocked with 1% BSA containing 1% normal goat serum for 60 minutes at room temperature. Cells on coverslips were then probed with anti-cleaved PARP mAb (Cell Signaling Technology) at 1:400 dilution in blocking buffer at 4°C overnight. After washing in cold PBS, goat anti-rabbit AlexaFluor 488 (Thermo Fisher Scientific) at 1:600 dilution was added in blocking buffer for 60 minutes at room temperature and mounted with Southern Biotech DAPI Fluoromount-G.

For analyzing cell viability after apoptosis induction, the CCK-8 assay was used 24 hours after TNFα or STP incubation with or without 100 μmol/L Kyn cotreatment. Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay was performed to determine apoptosis in mouse tumors using *In Situ* Cell Death Detection Kit (Roche Applied Science).Immunofluorescence images of cleaved PARP and TUNEL assay were captured with a Zeiss Axioskop 2 MOT microscope equipped with an Apotome module. Immunofluorescence was quantified in ImageJ as follows: black and white images of the blue channel were converted to binary and nuclei were defined using nuclei watershed separation setting, followed by particle analysis with size exclusion of 0.005 in^2-infinity. Green channel image thresholds were adjusted until individual nuclei were visible, and particle analysis was performed as above. Data are expressed as percent of particles in green channel (positive nuclei) divided by number of particles in blue channel (total nuclei) × 100.

IHC

Murine colon tissues were fixed in formalin and stabilized in 2% agar before embedding in paraffin as described previously (27). All antibodies are described in Supplementary Table S1. Ido1 immunostaining with rat anti-mouse IDO1 antibody (BioLegend) was used at 1:50 dilution after antigen retrieval by steaming in sodium citrate buffer (pH 6.0) for 30 minutes. Trilogy one step antigen retrieval (Millipore Sigma) was performed as per the manufacturer's instructions for pAkt SER473, cyclin D1, β-catenin, and survivin immunostaining (Cell Signaling Technology at 1:50 dilution) on mouse tissue. Tissue sections from postirradiated, surgically resected human colon adenocarcinoma were obtained from Siteman Cancer Center histopathology core. Trilogy one step heat-mediated antigen retrieval was performed for immunostaining IDO1 and pAkt SER473. The relative intensity of immunostaining for human IDO1 and pAkt SER473 was graded on the basis of a point system (no staining = 0, low expression = 1, high expression = 2). Colorectal cancer pathology samples from 5 patients were analyzed by selecting neoplastic crypts (>10/specimen, 141 total) followed by sequential evaluation of IDO1 staining and pAKT SER473. The grading was confirmed by two contributing scientists. Using this data, a correlation curve between IDO1 and pAkt SER473 expression was calculated.

Statistical analysis

Animal experiments included 5 to 10 mice in each group and were repeated twice. Cell proliferation assays involved data accumulation from five to eight wells/group and experiments were repeated at least twice. Wound healing assays included data from three wells in each group and the experiments were performed twice. All data were represented as average ± SEM. *P* values were calculated using two-way ANOVA or Student *t* test and a value less than 0.05 was considered as significantly different between groups. The Spearman correlation coefficient was used to analyze the correlation between IDO1 and Akt SER473 based on their IHC staining scores. Figures and statistical analysis were done using GraphPad Prism version 5.00 for Windows (GraphPad Software, www.graphpad.com).

Results

IDO1 expression by the neoplastic intestinal epithelium promotes colon tumorigenesis

We previously showed that germline deletion of Ido1 reduces tumor burden in a mouse model of colorectal cancer and that

Published OnlineFirst January 24, 2019; DOI: 10.1158/0008-5472.CAN-18-0668

inhibition of IDO1 activity reduces colorectal cancer cell proliferation *in vitro*. These findings suggest that the neoplastic epithelium may be a key cellular source of IDO1 activity for driving colorectal cancer growth. To directly examine this issue, we generated a mouse with genetic knockout of Ido1 specifically in the intestinal epithelium (IDO1-iKO) using cre-lox recombination technology (Fig. 1A). Mice with a floxed Ido1 gene were crossed to mice expressing cre behind the intestinal epithelial-specific villin promoter. The resulting mouse line demonstrated effective knockout of Ido1 protein expression in the colonic epithelium, but not nonepithelial lamina propria cells (Fig. 1B–D). Colonoids established from normal colons of WT, but not iKO mice, showed change in Kyn production after IFNγ treatment (Fig. 1E), illustrating the functional knockout of Ido1 and the relative importance of IDO1 (vs. IDO2 or TDO) to the KP in the colon epithelium. Additional details on mouse modeling and Ido1 expression data are presented in Supplementary Fig. S1A–S1D.

Colon tumorigenesis was induced in IDO1-iKO and littermate WT control mice using the AOM-DSS protocol. No difference in weight loss was observed between the two groups and the disease activity index differed only briefly during the first DSS cycle (Supplementary Fig. S1B and S1C). However, IDO1-iKO mice were found to develop fewer and smaller tumors compared with WT mice (Fig. 1F and G). Nuclear β-catenin was visibly reduced in the tumors of IDO1-iKO versus WT mice, as was expression of the target gene cyclin D1 and the tumor cell proliferation index by 16% (Fig. 2A–C). These effects were not significant in the adjacent normal colon epithelium, although the colonic crypt epithelial proliferation index trended lower in the IDO1-iKO mice. Tumors from IDO1-iKO mice also demonstrated increased apoptosis (TUNEL staining) and reduced expression of Survivin (a key protein that regulates mitosis and apoptosis; Fig. 2D and E). Together, these data illustrate the importance of epithelial-based IDO1 expression as a driver of neoplastic proliferation, apoptotic resistance, and colon tumorigenesis.

KP metabolites activate β-catenin through Akt signaling in colorectal cancer cells

We next sought to examine the signaling mechanism by which IDO1 activity promotes neoplastic epithelial proliferation, nuclear translocation of β-catenin, and resistance to apoptosis. In these experiments we examined the impact of Kyn on colorectal cancer cells using *in vitro* methods. We found that both Kyn rapidly promoted nuclear translocation of β-catenin from the cytoplasm in a time dependent manner (Fig. 3A and B).

Unchecked nuclear β-catenin–mediated transcriptional activity is a common feature of colorectal cancer and usually attributable to genetically driven aberrant signaling along the canonical Wnt pathway (32). Therefore, we examined whether KP metabolites act as endogenous Wnt ligands. Two approaches were taken, but neither confirmed this mechanism. First, exogenous Kyn did not promote activation of LRP5/6 (lipoprotein receptor–related protein), a coreceptor that is phosphorylated in the presence of Wnt ligands. Second, exogenous Kyn promoted enhanced colorectal cancer cell proliferation even in the presence of the Wnt antagonist Dickkopf (Fig. 3C and D).

We next evaluated whether KP metabolites activate protein kinase B (Akt), a key signaling intermediate known to inhibit apoptosis while promoting β-catenin nuclear translocation and transcriptional activity. This mechanism involves phosphorylation of Akt at Ser473 and Thr308 residues leading to increased phosphorylation of β-catenin at Ser552 and has been linked to inflammation in colorectal cancer (33, 34). Indeed, we found that exogenous Kyn application to serum-starved cells promoted rapid and dose-dependent Akt activation evidenced by increases in pAKT S472 and phosphorylated PRAS40 pT246, a direct target Akt activity (Fig. 3E).

To address the physiologic relevance of these concentrations, we assessed the intracellular Kyn levels in two colorectal cancer cell lines used in this study and found them to reach upwards of 75 μmol/L (Fig. 3F). Supporting these findings, prior studies have demonstrated intracellular concentrations of Kyn to exceed 2 mmol/L in malignant myeloid-derived cells (such as those that infiltrate the tumor) and to reach at least 100 μmol/L in WiDr colorectal cancer cells (35). On the basis of these data, we used 100 μmol/L of Kyn henceforth to examine the cell signaling pathway.

Intracellular Kyn destabilizes GSK3β and activates Akt via PI3K

We next sought to understand the pathways upstream and downstream to Kyn-mediated Akt activation as relevant to colorectal cancer and to β-catenin activation. Upon activation, Akt signaling is known to interact with β-catenin indirectly by inactivating GSK3β (36), an enzyme that when in its active state, destabilizes β-catenin towards degradation. Indeed, we identified that initial Kyn and terminal (QA) KP metabolites rapidly inactivate GSK3β by inducing its phosphorylation at Ser9. This mechanism also applied to other colorectal cancer cell lines despite known β-catenin stabilizing genetic mutations and regardless of status for microsatellite instability or KRAS mutation (Fig. 4A). Together, these data suggest a positive synergy between IDO1 activity in the neoplastic epithelium and the β-catenin stabilizing genetic mutations that drive colon cancer development and progression.

We also aimed to define signaling upstream from Akt activation. First, we identified that intracellular Kyn was required to activate Akt as inhibition of the LAT1 prevented this effect (Fig. 4B). LAT1 is an active transporter of Kyn and known to be highly upregulated in colorectal cancer (37, 38). Akt resides at the intersection of numerous signaling pathways including PI3K, PTEN, and positive feedback from mTOR. We did find that Kyn promoted mTOR phosphorylation (pmTor Ser2448) in colorectal cancer cells, a form that binds to both raptor and rictor and is shown to be overexpressed in human colorectal cancer (39, 40). However, this occurred only after 15 minutes, suggesting this was secondary (rather than proximate) to Akt activation (Supplementary Fig. S2A). Although PTEN is a negative regulator of Akt activation, (PI3K directly activates Akt. We confirmed the involvement of PI3K with inhibitor studies and found that Kyn/QA did not induce β-catenin, GSK3β, or PRAS40 phosphorylation in the presence of Akt or PI3K inhibition (Fig. 4C). Finally, although activation of the aryl hydrocarbon receptor (AHR) activation is implicated in mediating some of the biologic effects of the IDO1-KP metabolites, we found that rapid Akt activation occurred despite AHR antagonism (Fig. 4D). Altogether, these results indicate that Kyn promote β-catenin activity via a PI3K/AKT/GSK3β signaling event, rather than AHR-mediated transcriptional activity.

Published OnlineFirst January 24, 2019; DOI: 10.1158/0008-5472.CAN-18-0668

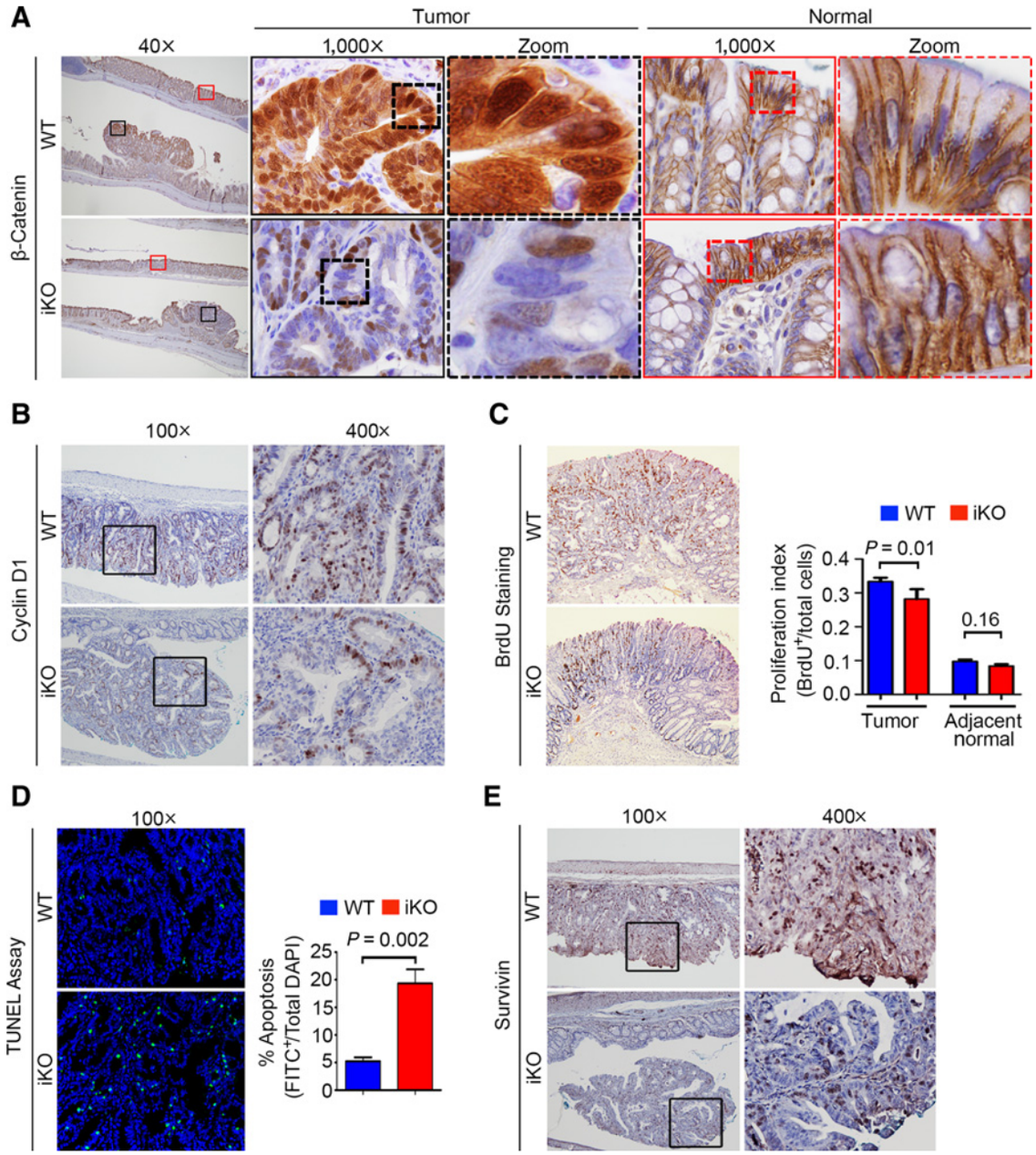

Figure 2.

Epithelial IDO1 promotes nuclear β-catenin activation, increases epithelial proliferation, and protects from apoptosis. **A,** Decreased nuclear β-catenin staining in IDO1-iKO tumors compared with WT. Magnified images of β-catenin staining by IHC in tumors and adjacent normal areas in WT and iKO mice. **B,** Representative IHC images of β-catenin target gene cyclin D1. **C,** Epithelial proliferation by BrdU staining, with its quantitation represented as epithelial proliferation index in tumors and adjacent normal epithelium. **D,** Apoptosis by TUNEL assay including representative immunofluorescence images quantitation. **E,** Representative images of survivin IHC.

IDO1 and activated Akt coexpress in murine and human colorectal cancer cells

Having shown that KP metabolites rapidly activate Akt signaling in colorectal cancer cell lines, we then sought to determine whether this mechanism extended in tumor epithelium *in vivo*. Indeed, we found expression of pAkt Ser473 to be reduced in the neoplastic colon epithelium of iKO versus WT mice (Fig. 5A). We next evaluated a series of five human colorectal cancer samples and found that crypt staining for IDO1 mirrored and significantly correlated with that of pAkt Ser473 staining (Fig. 5B and C).

KP metabolites except for KA activate Akt/β-catenin and promote colorectal cancer proliferation

IDO1 catalyzes the initial and rate-limiting step in tryptophan metabolism to Kyn initiating a cascade that generates

Published OnlineFirst January 24, 2019; DOI: 10.1158/0008-5472.CAN-18-0668

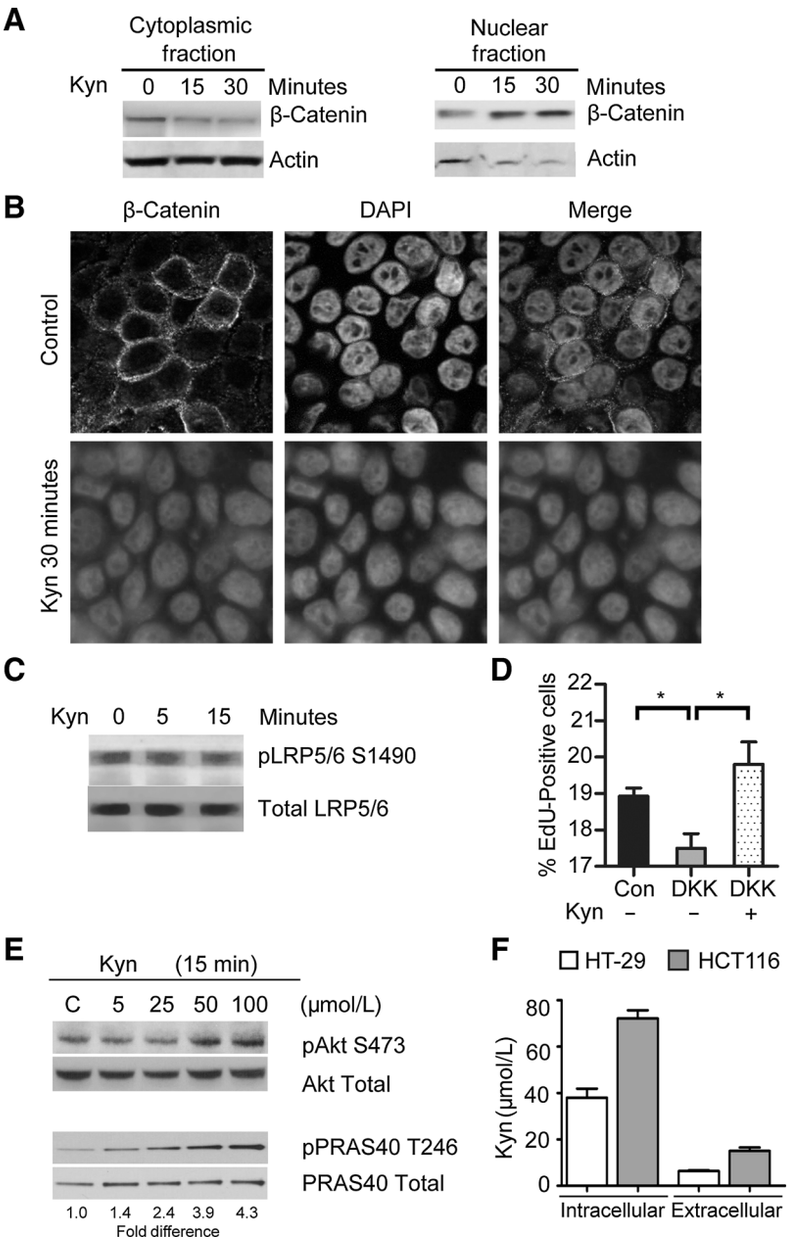

Figure 3.

Kyn rapidly induces β-catenin nuclear translocation and activates Akt in colorectal cancer cells. Experiments were completed with HT29 cells treated with Kyn at 100 μmol/L or at concentrations shown. The impact of Kyn on β-catenin cellular location was assessed by Western blot analysis (**A**) and immunofluorescence (**B**). Kyn did not activate Wnt pathway as evidenced by no observed change in the frizzled coreceptor activation as measured by pLRP5/6 S1490 levels (**C**) and Kyn increasing EdU incorporation as a marker of proliferation in the presence of Wnt signaling inhibitor DKK1 (**D**). *, $P < 0.05$. **E** and **F,** Kyn did induce dose-dependent activation of Akt (pS473) and Akt phosphorylation target PRAS40 at physiologic concentrations of Kyn (**E**) as measured by intracellular and extracellular levels in colorectal cancer cell lines (**F**).

several bioactive downstream KP metabolites (Supplementary Fig. S3A). We next evaluated the capacity of each of these metabolites to activate the Akt/GSK3β/β-catenin pathway and to increase colorectal cancer cell proliferation. The majority of KP metabolites activated the Akt/GSK3β/β-catenin pathway and promoted colorectal cancer proliferation. Only KA did not activate Akt and pPRAS40 T246 and conversely suppressed colorectal cancer proliferation (Supplementary Fig. S3B–S3D). These findings were confirmed in DLD1 colorectal cancer cells as well (Supplementary Fig. S3E and S3F). Consistent with our findings of KP metabolites potentiating malignant colorectal cancer activity, we also confirmed that Kyn and QA promote wound closure (Supplementary Fig. S4A and S4B).

Kyn protects colorectal cancer cells from apoptosis

PI3K/Akt pathway activation is a recognized mechanism by which colorectal cancer cells resist stress-induced apoptosis (41–43). We thus sought to determine whether KP metabolites offer protection against apoptosis in colorectal cancer cells. Indeed, Kyn cotreatment reduced TNFα-induced apoptosis as measured by cleaved PARP and enhanced colorectal cancer cell viability (Fig. 6A–D). Importantly, we found that Kyn did not prevent TNFα-induced apoptosis in the presence of an Akt inhibitor. We also found that Kyn reduced staurosporine-induced apoptosis (Supplementary Fig. S5A–S5D). These results complement the *in vivo* data presented in Fig. 2 and collectively indicate that KP metabolites promote tumor progression by promoting Akt-induced resistance to apoptosis

Published OnlineFirst January 24, 2019; DOI: 10.1158/0008-5472.CAN-18-0668

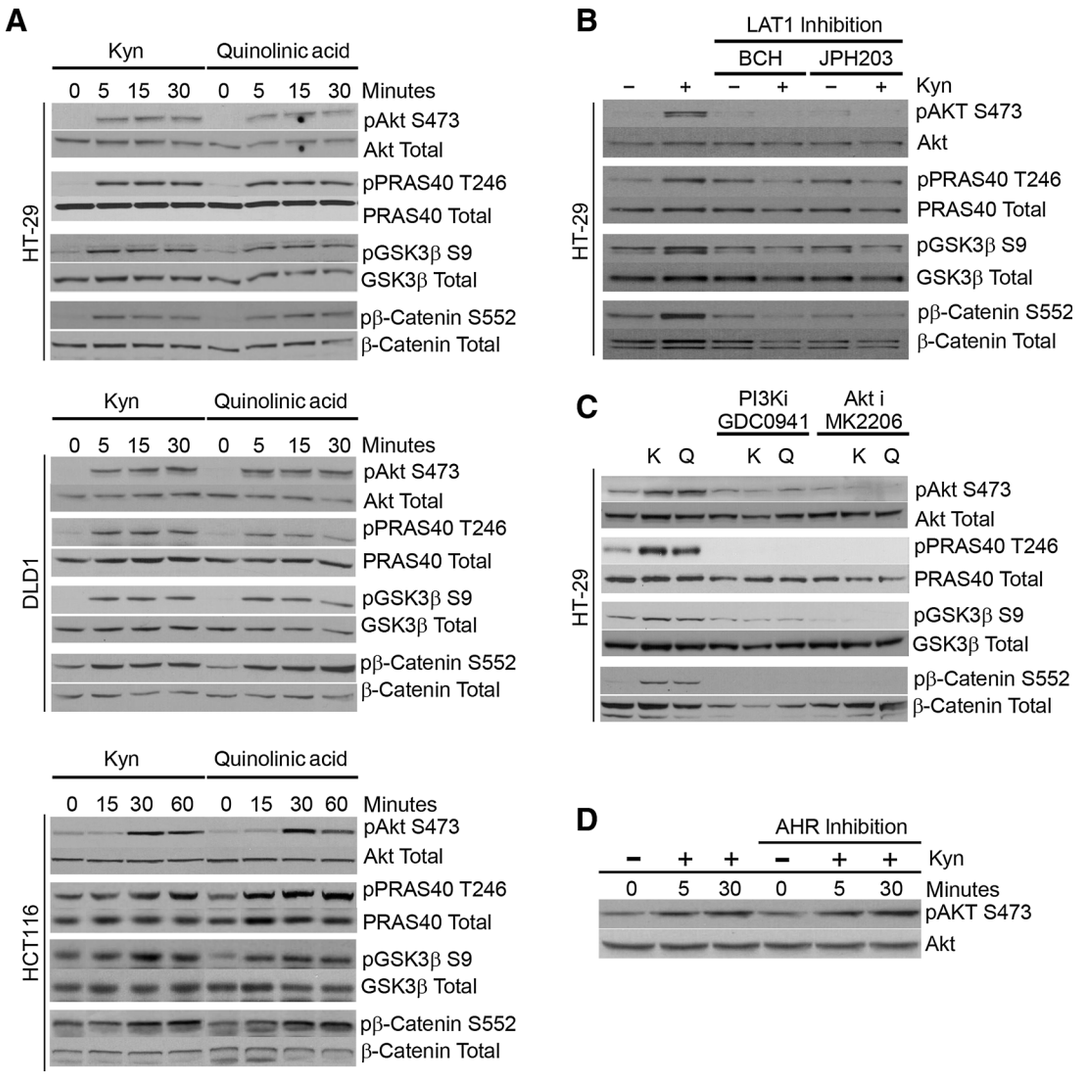

Figure 4.

Kyn metabolites rapidly activate β-catenin via intracellular activation of PI3K/Akt pathway. **A,** HT29, DLD1, and HCT116 colon cancer cells were treated with Kyn or QA for specified time points, followed by whole-cell protein extraction. Western blot analyses were performed to analyze Akt-Ser473 (activated), pPRAS40-T246 (activated), GSK3β-Ser9 (inactivated), and β-catenin Ser552 (activated) phosphorylation levels. **B,** Inhibition of the LAT1 amino acid transporter by either BCH or JPH203 blocks Kyn-mediated Akt pathway activation. **C,** Inhibition of Akt (MK2206) and PI3K (GDC0941) blocks Kyn-mediated phosphorylation of Akt at S473 and prevents downstream targets GSK3β from inactivation (pSer9) and β-catenin from activation (pSer552) in HT-29 cells. **D,** Inhibition of AHR with CH-223191 does not prevent Akt activation in HT-29 colorectal cancer cells.

and increasing neoplastic survival during inflammatory and cellular stress.

Kyn-mediated Akt activation differentiates tumor cells from normal cells

In IDO1-iKO mice, the epithelial cell proliferation index was significantly reduced in the tumor, but the difference was less pronounced in adjacent normal crypts (Fig. 2C). To further explore potential differences in how KP metabolites impact normal versus neoplastic epithelial cells and to extend the findings further to human disease, we used human-derived colon tumoroid and normal colonoid cultures. Similar to our observations in monolayer colorectal cancer cell cultures, Kyn and QA induced rapid Akt/GSK3β/β-catenin signaling in human colorectal cancer–derived tumoroids (Fig. 7A). However, in the colonoids derived from normal colon epithelial crypts, Kyn and QA provoked no rapid response in phosphorylation. Enhanced proliferation and cyclin D1 levels were also observed in colorectal cancer–derived tumoroids compared with minimal change in proliferation in normal colonoids (Fig. 7B and C). These data indicate that neoplastic transformation drives an augmented response of the colon epithelium to IDO1 pathway activity. A summary illustration of the overall findings is shown in Fig. 7D.

Discussion

The immunometabolic IDO1 pathway has emerged as an important therapeutic immunooncology target for solid tumors

Published OnlineFirst January 24, 2019; DOI: 10.1158/0008-5472.CAN-18-0668

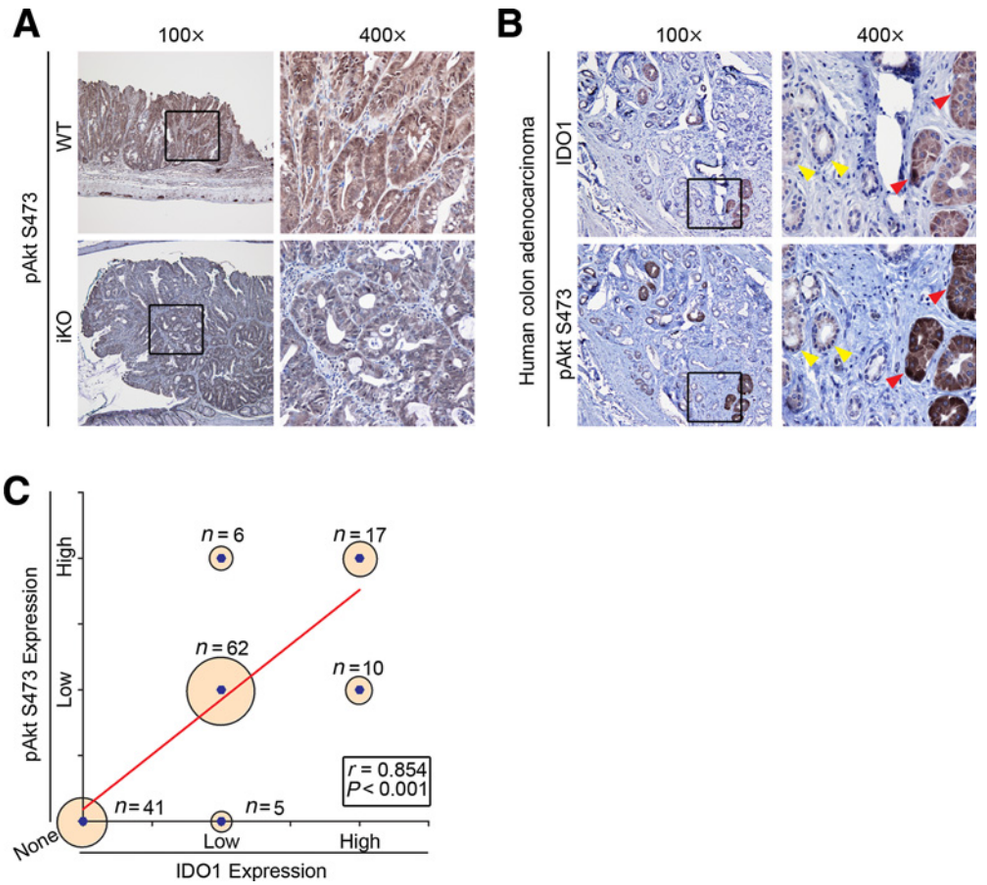

Figure 5.
IDO1 and activated Akt colocalize in neoplastic epithelium. **A,** Tumors and normal epithelium of IDO1-iKO mice demonstrate diminished pAkt S473 immunostaining versus WT. Representative figure from immunostaining in 5 mice/group. **B,** Representative images of pAkt S473 costaining with IDO1 immunostaining in human colon tumors. Arrows indicate dual negative crypts (yellow) and dual-positive crypts (red) from immunostaining for 5 tumor tissues. **C,** Positive correlation between pAkt and IDO1 immunostaining–based colocalization in human colorectal cancer. Five colorectal cancers with a total of 141 crypts compared.

including colon cancer (17). The recognized capacity of this enzyme to suppress antitumoral immunity provides the mechanistic basis for current clinical trials of IDO1 inhibitors. In this study, we define a novel, epithelial cell–centric mechanism and signaling cascade by which IDO1, through Kyn, interacts directly with the signaling pathways of the neoplastic cells to promote tumor growth. Using cell culture, animal models, and human colorectal cancer tissues, we illustrate that epithelial IDO1 activity and several KP metabolites directly promote colon tumorigenesis by activating the proproliferative/antiapoptotic PI3K/Akt pathway in the neoplastic epithelium. These findings provide mechanistic insight and strengthen justification for clinical trials evaluating IDO1 inhibitors as adjuvant therapeutics in colon cancer. Furthermore, these findings may have implications for the rational design of future clinical trials that would exploit a synergy of IDO1 inhibitors and conventional cancer therapies where Akt activation provides resistance such as radiation.

Linking IDO1 activity and KP metabolites to PI3K/Akt signaling provides a new mechanistic insight to how inflammation fuels neoplastic growth. Activation of Akt signaling or impaired expression of PTEN (a negative regulator of Akt) is reported in a majority of human colon cancers, whereas inhibitors of PI3K/Akt signaling are considered potential therapeutic agents (44). We demonstrated that intracellular KP activation rapidly initiates several Akt regulated protumorigenic effects including β-catenin activation and proliferative cell-cycle signaling, prevention of apoptosis from internal and extrinsic sources of cellular stress, and activation of the mTOR signaling pathway. These findings also define the upstream signaling pathway for our previous observations linking IDO1 to β-catenin activation as being mediated through PI3K/Akt signaling, which is implicated in colitis and colorectal cancer (34, 45). The precise mechanism by which KPs rapidly activate PI3K is an area of future study with our preliminary investigations not implicating the common activator Src (Supplementary Fig. S6A). However, the rapidity of these events (initiated within 5 minutes) do point to activation of cell signaling cascades rather than activating translational regulators such as AHR or induction of COX2, which Kyn has been tied to in inflammation and malignancy (46, 47). Regarding COX2, we confirmed the recent observation that Kyn induced its expression, but required a longer time course ranging from hours, rather than minutes (Supplementary Fig. S6B).

Published OnlineFirst January 24, 2019; DOI: 10.1158/0008-5472.CAN-18-0668

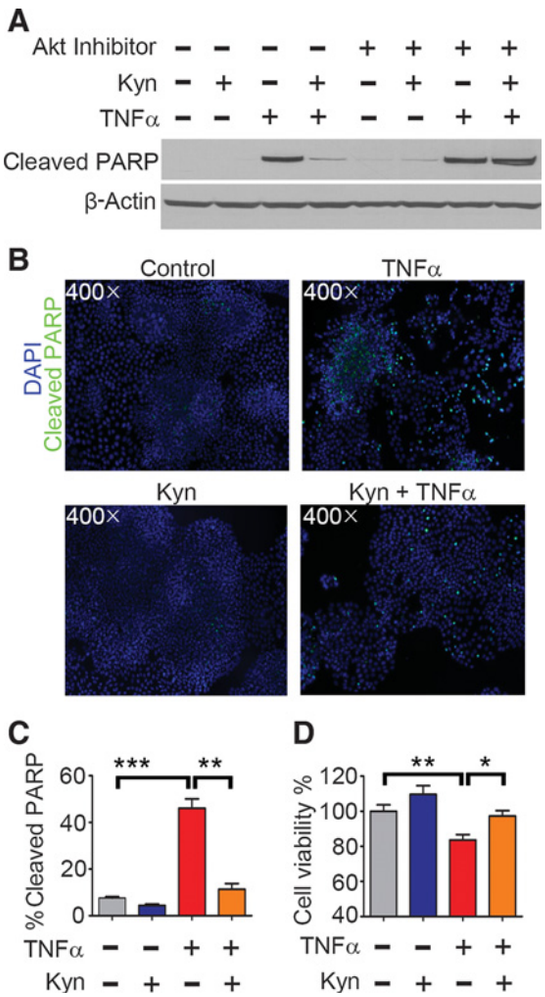

Figure 6.

Kyn protects colorectal cancer cells from apoptosis and promotes viability during stress. **A,** Apoptosis was induced in HT29 cells by TNFα (100 ng/mL) in the presence and absence of Kyn (100 μmol/L) and the Akt inhibitor MK-2206. Apoptosis was measured by cleaved PARP expression 12 hours after listed treatment by Western blotting. **B** and **C,** Immunofluorescence staining images and quantitation of cleaved PARP as a percentage of DAPI positive cells. **D,** Cell viability 24 hours after treatment measured by WST-8 assay (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

To our knowledge, this is the first study to demonstrate a specific functional role for epithelial-based IDO1 as an independent promoter of tumorigenesis using tissue-specific mouse modeling. Mice with genetic deletion of colon epithelial IDO1 demonstrate fewer tumors, significantly reduced tumor proliferation and showed higher apoptosis than WT mice. Furthermore, KP metabolites directly promoted colorectal cancer cell proliferation, resistance to apoptosis and restoration after injury. Given that the levels of intracellular Kyn within cancer cells can reach high micromolar to low millimolar concentrations (35), it should not be surprising that they drive biologic effects within the neoplastic cells. Our own analysis shows on intracellular concentrations of Kyn show that the levels can reach >70 μmol/L, which when applied exogenously activated Akt (Fig. 3). Thus, together with our published work showing IDO1 inhibition directly suppresses proliferation in colorectal cancer cells *in vitro*. These findings support the hypothesis that epithelial IDO1 and Kyn are both sufficient and necessary to drive colon tumor growth. The protumorigenic, epithelial cell–intrinsic effects of IDO1 activity no doubt augment the pathogenic immunosuppressive effect of IDO1 expressing bone marrow–derived monocytic cells and tumor cells.

Differences in IDO1-mediated effects between normal and neoplastic epithelial cells are also highlighted by the current data. *In vivo*, tumor cell proliferation is higher in WT than IDO1-iKO mice, but not significantly higher in the adjacent normal epithelial crypts. *In vitro*, KP metabolites robustly activate Akt and potentiate β-catenin activity to promote proliferation in colorectal cancer cell lines and colon tumoroids derived from patients with APC mutations. However, only delayed and modest effects were observed in colonoids derived from normal tissue. Prior studies had already identified that expression of IDO1 and other Kyn-producing enzymes is often high and constitutively active after neoplastic transformation, compared with the nontransformed state (3, 25, 26, 46). Intriguing studies have implicated COX2 and inflammation-associated AHR signaling as regulators of this phenomenon (47, 48). Our studies now illustrate a functional significance to this observed difference in expression by demonstrating that the progrowth, epithelial cell–centric effects of IDO1 activity are also more pronounced in neoplastic over normal epithelial cells. Taken together, these data suggest a positive synergy between acquisition of constitutive IDO1 activity and the genetic mutations that drive colon cancer progression, including those that promote stabilized β-catenin. Thus, although the *in vivo* AOM/DSS model of colitis-associated cancer was used in this study, we predict that the phenotype extends to APC and β-catenin mutation-driven sporadic colon cancers as well.

As an immunomodulatory pathway, there is existing rationale and clinical precedence for examining IDO1 inhibition in colorectal cancer. High tumor IDO1 expression is observed in a significant subset of with colorectal cancer and high IDO1 expression at the tumor invasion front is an independent adverse prognostic factor for overall survival and metachronous metastases (16, 20, 25, 26). As promising as this sounds, IDO1 inhibition as a monotherapy appears inadequate in colorectal and other cancers. A recently published phase I trial examined a novel hydroxyamidine small-molecule IDO1 inhibitor (Epacadostat, Incyte Corp.; refs. 11, 49) in individuals with advanced solid tumors failing prior therapies. Twenty-nine of 52 patients enrolled (56%) had colorectal cancer (50). Although the study met safety and biochemical efficacy endpoints, no patients achieved complete or partial response. Current studies with Epacadostat are examining it in combination with other immune-checkpoint inhibitors targeting CTLA-4 and the programmed cell death (PD-1) pathway.

Our findings provide new mechanistic rationale for targeting IDO1 inhibition in colorectal cancer and potentially add directionality to future clinical studies. IDO1 inhibitors may synergize with or augment the effects of cytotoxic chemo- or radiation therapy as the Akt pathway is intimately involved in preventing apoptotic or radiation-induced cell death in colorectal cancer (51). Moreover, the specificity of IDO1 expression in neoplastic versus normal colon cells may provide a target that could enhance therapeutic efficacy without enhancing the toxicity. Also relevant to cytotoxic cancer therapies, the IDO1–Kyn pathway provides an important source for *de novo* generation of NAD^+ (52).

Published OnlineFirst January 24, 2019; DOI: 10.1158/0008-5472.CAN-18-0668

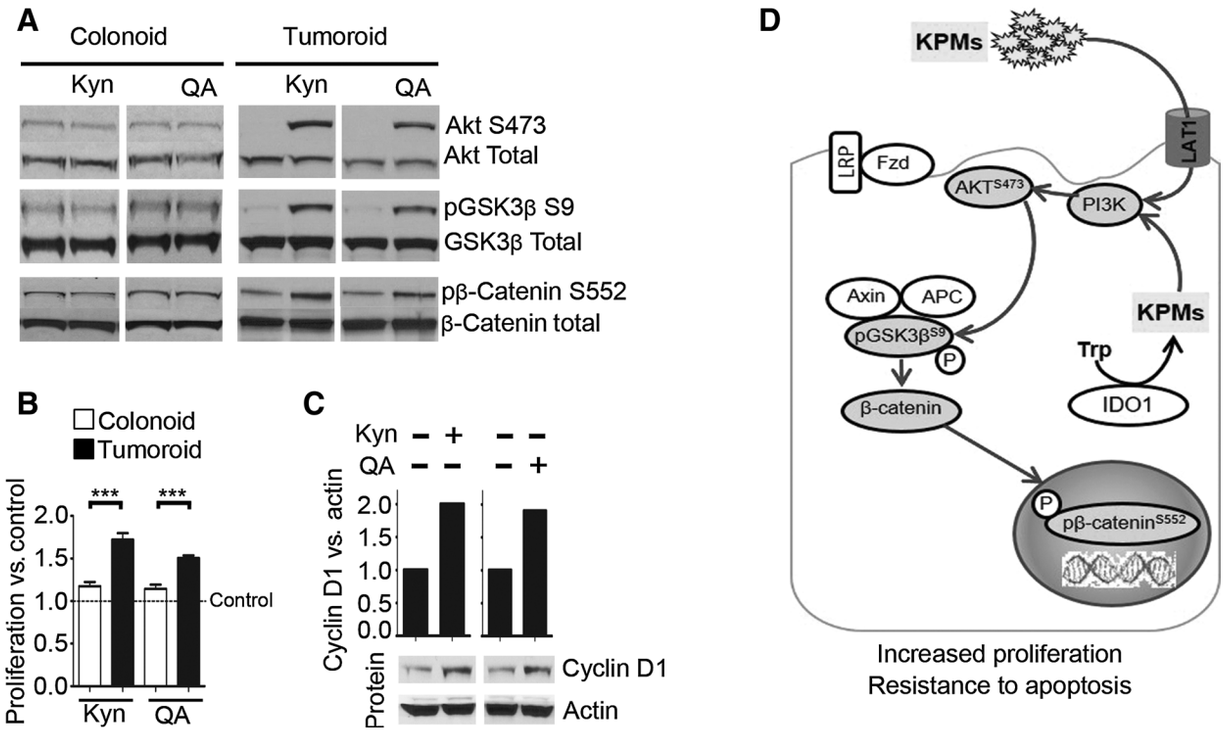

Figure 7.

Kyn-mediated rapid activation of Akt and β-catenin differentiates tumor cells from normal cells. **A,** Western blot analysis was used to detect total and phosphorylated proteins in normal human colon crypt–derived organoids (colonoids) and tumoroids derived from colon cancer tissue derived from patients with familial adenomatous polyposis. **B,** Proliferation in human colonoids and tumoroids measured by WST assay after 72 hours of treatment with Kyn or QA. **C,** Representative Western blotting of tumoroid cyclin D1 expression 12 hours after Kyn or QA treatment, with mean densitometry quantitation. **D,** Model for KP metabolite-induced PI3K/Akt activation and its effect on tumor cells and nuclear β-catenin activation. Data shown are representative data from three experiments (***, $P < 0.001$).

NAD^+ is an important enzymatic cofactor for enzymes involved in DNA repair and inhibitors of its generation are recognized targets in cancer therapy (53, 54). Finally, it is intriguing to think that IDO1 inhibitors might have a therapeutic role in colorectal cancer when combined with other well-tolerated small-molecule drugs, such as inhibitors of the COX2 pathway, with which our study also confirmed this association (47).

KA, unlike other KP metabolites, did not activate Akt and in HT29 cells decreased proliferation. This finding is consistent with published literature that suggested that KA may have chemopreventive effects in colorectal cancer after demonstrating that KA reduced PI3K/Akt signaling (55). Notably, these effects were observed using high (millimolar) doses of KA. Still, these findings are intriguing and potentially suggest that pharmacologic shunting of the Kyn pathway toward KA production and away from the Akt-activating KP metabolites may have a potentiating effect for IDO1 inhibitors or in combination with other therapeutics.

In summary, our results demonstrate a novel mechanistic link between IDO1 activity, Kyn pathway metabolites, and the PI3K/Akt proproliferative and antiapoptotic pathways in colorectal cancer including activated β-catenin signaling. These findings demonstrate the importance of epithelial IDO1 activity in driving colon tumorigenesis and extend from cell lines and mouse models to human colorectal cancer. Together, these findings underscore the value of investigating IDO1 inhibition in colorectal cancer, provide insights into mechanism of action, and may stimulate consideration for future clinical trials that may exploit a synergy of IDO1 inhibitors and therapies where Akt activation provides resistance.

Disclosure of Potential Conflicts of Interest

M.A. Ciorba reports receiving a commercial research grant and is a consultant/advisory board member for Incyte. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: K.S. Bishnupuri, D.M. Alvarado, B. Chen, M.A. Ciorba
Development of methodology: K.S. Bishnupuri, B. Chen, M.A. Ciorba
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.S. Bishnupuri, D.M. Alvarado, A.N. Khouri, M. Shabsovich, B. Chen, B.K. Dieckgraefe, M.A. Ciorba
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.S. Bishnupuri, D.M. Alvarado, B. Chen, B.K. Dieckgraefe, M.A. Ciorba
Writing, review, and/or revision of the manuscript: K.S. Bishnupuri, D.M. Alvarado, B. Chen, B.K. Dieckgraefe, M.A. Ciorba
Study supervision: M.A. Ciorba

Acknowledgments

M.A. Ciorba has support from a Crohn's and Colitis Foundation Daniel H Present Senior Research Award (Ref. 370763), NIH grants (DK109384, DK100737, and AI095776), philanthropic support from the Givin' it all for Guts Foundation, https://givinitallforguts.org/, and a Central Society for Clinical Research Early Career Development Award. B.K. Dieckgraefe was supported by I01 BX003072. Core support was from The Washington University

Digestive Diseases Research Core Center (P30 DK052574) and Siteman Cancer Center (P30 CA91842). D.M. Alvarado was supported by DK077653 and The Lawrence C. Pakula MD IBD Innovation Fellowship. We acknowledge technical assistance from Srikanth Santhanam PhD. Finally, we are grateful to Nicholas O. Davidson MD for his unwavering and enthusiastic support of gastroenterology research at Washington University and for his insightful comments on this project.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 2, 2018; revised November 27, 2018; accepted January 16, 2019; published first January 24, 2019.

References

1. Huang L, Mellor AL. Metabolic control of tumour progression and anti-tumour immunity. Curr Opin Oncol 2014;26:92–9.
2. Prendergast GC, Smith C, Thomas S, Mandik-Nayak L, Laury-Kleintop L, Metz R, et al. Indoleamine 2,3-dioxygenase pathways of pathogenic inflammation and immune escape in cancer. Cancer Immunol Immunother 2014;63:721–35.
3. Uyttenhove C, Pilotte L, Theate I, Stroobant V, Colau D, Parmentier N, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med 2003;9:1269–74.
4. Zhai L, Spranger S, Binder DC, Gritsina G, Lauing KL, Giles FJ, et al. Molecular pathways: targeting IDO1 and other tryptophan dioxygenases for cancer immunotherapy. Clin Cancer Res 2015;21:5427–33.
5. Holmgaard RB, Zamarin D, Li Y, Gasmi B, Munn DH, Allison JP, et al. Tumor-expressed IDO recruits and activates MDSCs in a Treg-dependent manner. Cell Rep 2015;13:412–24.
6. Fallarino F, Grohmann U, Puccetti P. Indoleamine 2,3-dioxygenase: from catalyst to signaling function. Eur J Immunol 2012;42:1932–7.
7. Munn DH. Blocking IDO activity to enhance anti-tumor immunity. Front Biosci 2012;4:734–45.
8. Muller AJ, Sharma MD, Chandler PR, Duhadaway JB, Everhart ME, Johnson BA III, et al. Chronic inflammation that facilitates tumor progression creates local immune suppression by inducing indoleamine 2,3 dioxygenase. Proc Natl Acad Sci U S A 2008;105:17073–8.
9. Smith C, Chang MY, Parker KH, Beury DW, DuHadaway JB, Flick HE, et al. IDO is a nodal pathogenic driver of lung cancer and metastasis development. Cancer Discov 2012;2:722–35.
10. Munn DH, Sharma MD, Hou D, Baban B, Lee JR, Antonia SJ, et al. Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes. J Clin Invest 2004;114:280–90.
11. Koblish HK, Hansbury MJ, Bowman KJ, Yang G, Neilan CL, Haley PJ, et al. Hydroxyamidine inhibitors of indoleamine-2,3-dioxygenase potently suppress systemic tryptophan catabolism and the growth of IDO-expressing tumors. Mol Cancer Ther 2010;9:489–98.
12. Hou DY, Muller AJ, Sharma MD, DuHadaway J, Banerjee T, Johnson M, et al. Inhibition of indoleamine 2,3-dioxygenase in dendritic cells by stereoisomers of 1-methyl-tryptophan correlates with antitumor responses. Cancer Res 2007;67:792–801.
13. Zheng X, Koropatnick J, Chen D, Velenosi T, Ling H, Zhang X, et al. Silencing IDO in dendritic cells: a novel approach to enhance cancer immunotherapy in a murine breast cancer model. Int J Cancer 2013;132:967–77.
14. Thaker AI, Rao MS, Bishnupuri KS, Kerr TA, Foster L, Marinshaw JM, et al. IDO1 metabolites activate beta-catenin signaling to promote cancer cell proliferation and colon tumorigenesis in mice. Gastroenterology 2013;145:416–25.
15. Godin-Ethier J, Hanafi L-A, Piccirillo CA, Lapointe R. Indoleamine 2,3-dioxygenase expression in human cancers: clinical and immunologic perspectives. Clin Cancer Res 2011;17:6985–91.
16. Brandacher G, Perathoner A, Ladurner R, Schneeberger S, Obrist P, Winkler C, et al. Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: effect on tumor-infiltrating T cells. Clin Cancer Res 2006;12:1144–51.
17. Prendergast GC, Malachowski WP, DuHadaway JB, Muller AJ. Discovery of IDO1 inhibitors: from bench to bedside. Cancer Res 2017;77:6795–811.
18. Hornyák L, Dobos N, Koncz G, Karányi Z, Páll D, Szabó Z, et al. The role of indoleamine-2,3-dioxygenase in cancer development, diagnostics, and therapy. Front Immunol 2018;9:151.
19. Theate I, van Baren N, Pilotte L, Moulin P, Larrieu P, Renauld JC, et al. Extensive profiling of the expression of the indoleamine 2,3-dioxygenase 1 protein in normal and tumoral human tissues. Cancer Immunol Res 2015;3:161–72.
20. Ferdinande L, Decaestecker C, Verset L, Mathieu A, Moles Lopez X, Negulescu AM, et al. Clinicopathological significance of indoleamine 2,3-dioxygenase 1 expression in colorectal cancer. Br J Cancer 2012;106:141–7.
21. Liu X, Shin N, Koblish HK, Yang G, Wang Q, Wang K, et al. Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity. Blood 2010;115:3520–30.
22. Walczak K, Dabrowski W, Langner E, Zgrajka W, Pilat J, Kocki T, et al. Kynurenic acid synthesis and kynurenine aminotransferases expression in colon derived normal and cancer cells. Scand J Gastroenterol 2011;46:903–12.
23. Engin AB, Karahalil B, Karakaya AE, Engin A. Helicobacter pylori and serum kynurenine-tryptophan ratio in patients with colorectal cancer. World J Gastroenterol 2015;21:3636–43.
24. Gao YF, Peng RQ, Li J, Ding Y, Zhang X, Wu XJ, et al. The paradoxical patterns of expression of indoleamine 2,3-dioxygenase in colon cancer. J Transl Med 2009;7:71.
25. Santhanam S, Alvarado DM, Ciorba MA. Therapeutic targeting of inflammation and tryptophan metabolism in colon and gastrointestinal cancer. Transl Res 2016;167:67–79.
26. Alvarado DM, Santhanam S, Ciorba MA. Role of kynurenine pathway in gastrointestinal diseases. In:Mittal S, editor. Targeting the broadly pathogenic kynurenine pathway. Cham, Switzerland: Springer International Publishing; 2015. p.157–67.
27. Thaker AI, Shaker A, Rao MS, Ciorba MA. Modeling colitis-associated cancer with azoxymethane (AOM) and dextran sulfate sodium (DSS). J Vis Exp 2012;11:pii: 4100.
28. Ciorba MA, Bettonville EE, McDonald KG, Metz R, Prendergast GC, Newberry RD, et al. Induction of IDO-1 by immunostimulatory DNA limits severity of experimental colitis. J Immunol 2010;184:3907–16.
29. VanDussen KL, Marinshaw JM, Shaikh N, Miyoshi H, Moon C, Tarr PI, et al. Development of an enhanced human gastrointestinal epithelial culture system to facilitate patient-based assays. Gut 2015;64:911–20.
30. Miyoshi H, Stappenbeck TS. *In vitro* expansion and genetic modification of gastrointestinal stem cells in spheroid culture. Nat Protoc 2013;8:2471–82.
31. Miyoshi H, Ajima R, Luo CT, Yamaguchi TP, Stappenbeck TS. Wnt5a potentiates TGF-beta signaling to promote colonic crypt regeneration after tissue injury. Science 2012;338:108–13.
32. Najdi R, Holcombe RF, Waterman ML. Wnt signaling and colon carcinogenesis: beyond APC. J Carcinog 2011;10:5.
33. Fang D, Hawke D, Zheng Y, Xia Y, Meisenhelder J, Nika H, et al. Phosphorylation of beta-catenin by AKT promotes beta-catenin transcriptional activity. J Biol Chem 2007;282:11221–9.
34. Lee G, Goretsky T, Managlia E, Dirisina R, Singh AP, Brown JB, et al. Phosphoinositide 3-kinase signaling mediates beta-catenin activation in intestinal epithelial stem and progenitor cells in colitis. Gastroenterology 2010;139:869–81.
35. Knowles RG, Clarkson NA, Pogson CI, Salter M, Duch DS, MP Edelstein. The role of tryptophan and kynurenine transport in the catabolism of tryptophan through indoleamine 2,3-dioxygenase. In:Schwarcz R., Young SN, Brown RR, editors. Kynurenine and serotonin pathways advances in experimental medicine and biology. Boston, MA: Springer; 1991. p.161–6.
36. Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 1995;378:785–9.

Published OnlineFirst January 24, 2019; DOI: 10.1158/0008-5472.CAN-18-0668

37. Walker AK, Wing E, Banks WA, Dantzer R. 84. Targeting the kynurenine blood-to-brain transport system to treat inflammation-induced fatigue and depression. Brain Behav Immun 2014;40:e24–e5.
38. Kaira K, Oriuchi N, Imai H, Shimizu K, Yanagitani N, Sunaga N, et al. L-type amino acid transporter 1 and CD98 expression in primary and metastatic sites of human neoplasms. Cancer Sci 2008;99:2380–6.
39. Rosner M, Siegel N, Valli A, Fuchs C, Hengstschlager M. mTOR phosphorylated at S2448 binds to raptor and rictor. Amino Acids 2010;38: 223–8.
40. Johnson SM, Gulhati P, Rampy BA, Han Y, Rychahou PG, Doan HQ, et al. Novel expression patterns of PI3K/Akt/mTOR signaling pathway components in colorectal cancer. J Am Coll Surg 2010;210: 767–76.
41. Franke TF, Hornik CP, Segev L, Shostak GA, Sugimoto C. PI3K/Akt and apoptosis: size matters. Oncogene 2003;22:8983–98.
42. Kaliszczak M, Trousil S, Ali T, Aboagye EO. AKT activation controls cell survival in response to HDAC6 inhibition. Cell Death Dis 2016;7: e2286.
43. Zhang S, Deng Z, Yao C, Huang P, Zhang Y, Cao S, et al. AT7867 inhibits human colorectal cancer cells via AKT-dependent and AKT-independent mechanisms. PLoS One 2017;12:e0169585.
44. Pandurangan AK. Potential targets for prevention of colorectal cancer: a focus on PI3K/Akt/mTOR and Wnt pathways. Asian Pac J Cancer Prev 2013; 14:2201–5.
45. Goretsky T, Bradford EM, Ye Q, Lamping OF, Vanagunas T, Moyer MP, et al. Beta-catenin cleavage enhances transcriptional activation. Sci Rep 2018;8: 671.
46. Opitz CA, Litzenburger UM, Sahm F, Ott M, Tritschler I, Trump S, et al. An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. Nature 2011;478:197–203.
47. Hennequart M, Pilotte L, Cane S, Hoffmann D, Stroobant V, De Plaen E, et al. Constitutive IDO1 expression in human tumors is driven by cyclooxygenase-2 and mediates intrinsic immune resistance. Cancer Immunol Res 2017;5:695–709.
48. Litzenburger UM, Opitz CA, Sahm F, Rauschenbach KJ, Trump S, Winter M, et al. Constitutive IDO expression in human cancer is sustained by an autocrine signaling loop involving IL-6, STAT3 and the AHR. Oncotarget 2014;5:1038–51.
49. Newton RC, Scherle PA, Bowman K, Liu X, Beatty GL, O'Dwyer PJ, et al. Pharmacodynamic assessment of INCB024360, an inhibitor of indoleamine 2,3-dioxygenase 1 (IDO1), in advanced cancer patients. J Clin Oncol 30, 2012 (suppl; abstr 2500).
50. Beatty GL, O'Dwyer PJ, Clark J, Shi JG, Bowman KJ, Scherle P, et al. First-in-human phase 1 study of the oral inhibitor of indoleamine 2,3-dioxygenase-1 Epacadostat (INCB024360) in patients with advanced solid malignancies. Clin Cancer Res 2017;23:3269–76.
51. Itoh N, Semba S, Ito M, Takeda H, Kawata S, Yamakawa M. Phosphorylation of Akt/PKB is required for suppression of cancer cell apoptosis and tumor progression in human colorectal carcinoma. Cancer 2002;94: 3127–34.
52. Sahm F, Oezen I, Opitz CA, Radlwimmer B, von Deimling A, Ahrendt T, et al. The endogenous tryptophan metabolite and NAD+ precursor quinolinic acid confers resistance of gliomas to oxidative stress. Cancer Res 2013;73:3225–34.
53. Hasmann M, Schemainda I. FK866, a highly specific noncompetitive inhibitor of nicotinamide phosphoribosyltransferase, represents a novel mechanism for induction of tumor cell apoptosis. Cancer Res 2003;63: 7436–42.
54. Espindola-Netto JM, Chini CCS, Tarrago M, Wang E, Dutta S, Pal K, et al. Preclinical efficacy of the novel competitive NAMPT inhibitor STF-118804 in pancreatic cancer. Oncotarget 2017;8:85054–67.
55. Walczak K, Turski WA, Rajtar G. Kynurenic acid inhibits colon cancer proliferation *in vitro*: effects on signaling pathways. Amino Acids 2014;46: 2393–401.

Published OnlineFirst January 24, 2019; DOI: 10.1158/0008-5472.CAN-18-0668

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

IDO1 and Kynurenine Pathway Metabolites Activate PI3K-Akt Signaling in the Neoplastic Colon Epithelium to Promote Cancer Cell Proliferation and Inhibit Apoptosis

Kumar S. Bishnupuri, David M. Alvarado, Alexander N. Khouri, et al.

Cancer Res 2019;79:1138-1150. Published OnlineFirst January 24, 2019.