IL10R2 Overexpression Promotes IL22/STAT3 Signaling in Colorectal Carcinogenesis

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Abstract

The mucosal immune response in the setting of intestinal inflammation contributes to colorectal cancer. IL10 signaling has a central role in gut homeostasis and is impaired in inflammatory bowel disease (IBD). Out of two IL10 receptor subunits, IL10R1 and IL10R2, the latter is shared among the IL10 family of cytokines and activates STAT signaling. STAT3 is oncogenic in colorectal cancer; however, knowledge about IL10 signaling upstream of STAT3 in colorectal cancer is lacking. Here, expression of IL10 signaling genes was examined in matched pairs from normal and tumor tissue from colorectal cancer patients showing overexpression (mRNA, protein) of IL10R2, and STAT3, respectively. IL10R2 overexpression was related to microsatellite stability.

Introduction

The risk of developing colorectal cancer increases in chronic gut inflammation associated with diseases such as inflammatory bowel disease (IBD), including ulcerative colitis and Crohn disease. The interplay of proinflammatory and anti-inflammatory cytokines affecting both immune and epithelial cells, along with genetic predisposition and environmental trigger, contributes to the process of inflammation-driven colon carcinogenesis.

IL10 is critical in limiting immunologic imbalance, and several lines of evidence emphasize an indisputable role of IL10 signaling in the maintenance of intestinal homeostasis (1–3). The induction of IL10R by inflammatory mediators such as IFNγ in polarized epithelia contributes to epithelial restitution, while its depletion impairs epithelial barrier functions and susceptibility to colitis and epithelial permeability (4). Recently, IL10R mutations causing functional impairment of IL10 signaling were implicated in early-onset enterocolitis (5). Also, the S138G variant of IL10R1 was linked to ulcerative colitis (6). This IL10R2 variant likely represents a adaptive change in the human genome as it reduces the ability of cmvIL10 to utilize the host’s IL10 signaling pathway (7). Loss of IL10, as in IL10 knockout (KO) mice, or its receptors represents a striking example of spontaneous enterocolitis and colitis-associated cancer (CAC; refs. 8, 9). The mechanism of tumor development under IL10 deficiency remains elusive; however, aberrant cytokine production, increased plasma TGFβ1 levels, and Smad3L signaling are associated with carcinogenesis in IL10 KO mice (10, 11). A recent study in mice by Zigmond and colleagues showed that IL10 production by macrophages is dispensable for gut homeostasis; however, loss of IL10R1 impaired macrophage conditioning, resulting in spontaneous development of severe colitis (12).

IL10 signaling is executed through the formation of an active complex where IL10R1 and IL10R2 recognize the same binding site on the IL10 molecule (13). This leads to activation of Janus kinase (JAK)-1 and tyrosine kinase (TYK)-2, which form docking sites for signal transducers and activators of transcription (STAT)-3 to this complex upon IL10R1 phosphorylation. These kinases also phosphorylate STAT3, causing its dimerization and nuclear translocation for its transcriptional activity (14). The two common human IL10R1 variants (S138G and G330R) are associated with impairment in STAT3 phosphorylation (6, 15). The STAT3 family of transcription factors has been conferred with oncogenic potential as these transcription factors are constitutively activated in various tumors (16, 17), causing resistance to apoptosis and facilitating tumor progression. In normal conditions, STAT3 activity remains under tight control by the family of negative modulators, including suppressor of cytokine signaling (SOCS)-3. Ligand–receptor interactions such as IL6 and IL22 are implicated in aberrant STAT3 activity in colon carcinogenesis (18–20). Besides STAT3, IL22 has been shown to activate MAPK pathways (ERK, JNK, and p38) and to promote cancer cell survival. The antiapoptotic action of IL22 is also mediated by AKT.

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

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phosphorylation. Moreover, IL22 elicited proinflammatory cytokine production and matrix-degrading molecules in IBD, suggesting its role in tissue remodeling/wound healing (21–24).

Cytokine receptors and their downstream signaling are shared by multiple cytokines, and variations in receptor expression or polymorphism can confer either protective or causative effects in the disease context. In inflammation-driven cancer, including CAC, oxidative stress mediates mutations and molecular pathways, and proinflammatory cytokines, such as TNFα and IL6, are negatively regulated by TGFβ and IL10. In spontaneous colorectal cancer, IL10 is produced by cancer cells and is mediated through activation of STAT3 via IL6 signaling to escape from immune surveillance (25). Although functional consequences of IL10 signaling have been underscored in the regulation of intestinal homeostasis, knowledge about IL10R signaling in colorectal cancer is limited. In this study, we systematically assessed the expression levels of the IL10 signaling cascade in matched pairs from normal and previous tumor tissue.

**Materials and Methods**

**Patients and tissue samples**

Colonic normal and tumor tissue pairs were sampled from 29 patients (15 male and 14 female) with a mean age of 68 (45–90 years) suffering from sporadic colorectal cancer upon surgery. The tissue was snap frozen in optimal cutting temperature (OCT; Sakura) and liquid nitrogen promptly after resection and stored at −80°C. For IHC, 5-μm sections were attached to glass slides (Starfrost). For the second set of analyses, paraffin-embedded 41 colonic samples from ulcerative colitis (n = 6), Crohn disease (n = 5), colorectal cancer (n = 14), and CAC (n = 8) and normal mucosa (n = 8) were analyzed by IHC. Samples were selected from either endoscopic biopsies or surgical specimens from IBD and CAC patients. Control specimens representing normal mucosa were taken from normal colon tissue as judged by pathologists. The human samples were obtained from the Department of Pathology at Medical University of Vienna (Vienna, Austria). The study was approved by the local ethics committee.

**Cell culture and reagents**

HCT116, HT29, SW480, DLD1, and Lovo cells (obtained from the ATCC). These cell lines were characterized by the ATCC using DNA profile and cytotgenetic analysis. These cells were cultured in Iscove’s Modified Dulbecco’s Medium ( Gibco) supplemented with 10% fetal bovine serum (Biochrom). The cell lines were maintained in the culture with regular passaging for 2 to 3 months after resuscitation. Primary human colon epithelial cells HCEC-1CT (obtained from Jerry W. Shay and Andres I. Roig, University of Texas, Dallas, TX) were characterized and published and cultured as previously described (26). These cells were obtained from human colon biopsies and immortalized by expression of the catalytic component of human telomerase (hTERT) and the nononcogenic proteins cyclin-dependent kinase 4 (Cdk4) and maintained for more than 1 year in culture in the laboratory of Dr. Shay. Cells were cultured in basal X media (DMEM: M199:4:1; Gibco), supplemented with EGF (20 ng/mL; BD Biosciences), hydrocortisone (1 μg/mL; Sigma), insulin (10 μg/mL; transferrin (2 μg/mL); sodiumselenite (5 nmol/L; all from Gibco, Life Technologies GmbH), 2% cosmic calf serum (HyClone), and gentamicin sulfate (50 μg/mL; Sigma). Cells were cultured in Primaria flasks (Becton Dickinson) at 37°C, 5% CO2, and 100% humidity. These cells are tested for the morphology and expression of epithelial cell markers such as ZO-1 and β-catenin upon resitution. The cells are tested for Mycoplasma every 6 months (Mycoplasma Detection Kit; Lonza). Cytokines used were human recombinant IL10 (R&D), IL22, and IL6 (eBioscience).

**RNA extraction and qRT-PCR**

OCT blocks were cut into 10-μm layers and placed in 1 mL of TRIzol reagent (Invitrogen). For IHC, cells were grown on a 10-cm dish, and RNA was extracted using TRIzol reagent. Quantitative real-time PCR (qRT-PCR) was performed on the ABI Prism 7700 (Applied Biosystems) using specific primers and probes for IL10R1, IL10R2, STAT1, STAT3, SOCS1, SOCS3, IL10, IL22, IL6, IL28A/B, and IL29 and housekeeping genes β-Actin and GAPDH. As a positive control, mRNA levels of c-myc were also analyzed. Primers and probes were designed by Primer Express software (Applied Biosystems) and Vector NTI (Invitrogen) or used from previous published literature (for the sequences, see Supplementary Table S1). mRNA (1 μg) was reverse transcribed using a Thermoscript cDNA kit (Invitrogen) according to the manufacturer's instructions.

**Immunohistochemistry**

For IHC on matched tumor samples, the following primary antibodies were used: rabbit anti-human IL10R2, 1:100 (Lifespan Biosciences) and rabbit anti-human STAT3 1:100 (Cell Signaling Technology). Immunostaining was performed according to the ABC method. Briefly, the slides were fixed in ice-cold acetone for 20 minutes and stored at −20°C. Samples were incubated with the primary antibody for 60 minutes at room temperature. After washing in Tris buffer, sections were incubated in isotype-specific (anti-rabbit or anti-mouse) biotinylated secondary antibody (Vector Inc.) for 30 minutes and washed again in Tris buffer. Slides were incubated with streptavidin horseradish peroxidase (Vector Inc.) for 30 minutes, washed in Tris buffer, and developed with diaminobenzidine (DAB) chromogen (Fluka) for 5 minutes. Sections were counterstained for 30 seconds with Harris Hematoxylin (Merck) and dehydrated through an increasing ethanol series. Specimens were mounted with Histofluid (Marienfeld superior). IHC scoring was performed in the following way: 0, no reactivity; 1+, weak reactivity: faint or light brown reactivity; 2+, moderate reactivity: shades of brown staining of intermediate darkness; 3+, strong reactivity: dark brown to black staining. The percentage of positive cells was scored as: 0 points (0% positive cells); 1 point (<10% positive cells); 2 points (10%–50% positive cells); 3 points (51%–80% positive cells); 4 points (>80% positive cells). Immunoreactive score (IRS) was calculated by multiplying numbers for the percentage of positive cells and the staining intensity, resulting in a value between 0 and 12 maximum. The staining reactivity was analyzed separately for the mucosa and the lamina propria. An IRS was calculated by two investigators (O. Movadat and M. Jambrich). Paraffin tissue sections from IBD, CAC, and colorectal cancer cases were stained with IL10R2 (con n = 8; IBD n = 11; CAC n = 8; colorectal cancer n = 14), IL22 R1 (con n = 11; IBD n = 5; CAC n = 8; colorectal cancer n = 9), and IL22 (con n = 8; IBD n = 10; CAC n = 11; colorectal cancer n = 7). For all antibodies, secondary antibody control for nonspecific reactivity was performed. Staining reactivity was scored using a four-grade system in the following manner: 0, no reactivity; 1, weak reactivity; 2, moderate...
reactivity; 3, strong reactivity. The percentage of positive cells was scored as: 0 points (0% positive cells), 1 point (<10% positive cells), 2 points (10%–50% positive cells), 3 points (51%–80% positive cells), and 4 points (>80% positive cells).

An IRS was calculated by two investigators (A. Frick and G. Paul), multiplying numbers for the percentage of positive cells and the staining intensity (27). IHC results were compared by a paired Student t test. P values were considered as statistically significant if less than 0.05 (*, P < 0.05; **, P < 0.01; ***, P < 0.001).

**Fragment analysis**

Human microsatellite-instability (MSI) markers were amplified using Multiplex PCR (Qiagen). To ensure an equal fluorescence signal, primer concentrations were optimized. PCR conditions were as follows: 15 minutes at 94°C, 40 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, followed by 10 minutes at 72°C. Fragment analysis was performed on a Genetic Analyzer (Applied Biosystems). The PCR product (0.4 µL) was added to 8.6 µL formamide and 0.4 µL GeneScan LIZ 500 size standard (Applied Biosystems, Life Technologies), followed by denaturation at 95°C. MSI from normal and tumor samples was analyzed using GeneMapper software (Applied Biosystems). Specific primer sequences are listed in Supplementary Table S2.

**IL10R2 overexpression**

HT-29 cells expressing IL10R2 were transiently transfected via electroporation with 5 µg of pcDef3 empty vector or pcDef3-IL10R2 plasmid DNA, a kind gift from Sergei Kotenko (New Jersey Medical School, Newark, NJ). One million cells were electroporated using the Amaxa nucleofector device and

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**Figure 1.**

IL10R2 is overexpressed in parallel with STAT3 in colorectal cancer. A, qRT-PCR analysis of the RNA isolated from 29 matched colorectal cancer samples (normal and tumor). Relative expression of IL10R2 and STAT3 was significantly increased in tumor tissue. IL10 was expressed at a higher level in normal tissue. c-myc was analyzed as a control. B, IHC analysis showing overexpression of IL10R2 and STAT3 in colorectal cancer samples compared with normal tissue. STAT3 is mainly localized in the cytoplasm, whereas IL10R2 shows both cytoplasmic and membranous staining. C, IRS performed on IEC and lamina propria showed a significant increase in epithelial IL10R2 in tumor cells. STAT3 exhibited a clear trend toward increased expression in tumor samples. P values were considered as statistically significant if less than 0.05 (*, P < 0.05; ***, P < 0.001).
basic Nucleofector Kit (Lonza) according to the manufacturer's instructions. Protein expression was analyzed 48 hours after transfection.

Cell proliferation
HT-29 cells were transiently transfected with pcDef (empty vector) or pcDefIL10R2 using Effectene (Qiagen). After 24 hours, medium was changed and cells were seeded in a 96-well plate and treated with IL10, IL22, and IL6. Cell proliferation was measured by the CyQUANT NF Cell Proliferation Assay Kit (Life Technologies) using the manufacturer's protocol. Fluorescence intensity was measured using a fluorescence microplate reader (Chameleon, HVD Life Sciences) with excitation at 485 nm and emission detection at 530 nm. P values were considered as statistically significant if less than 0.05.

Western blot analysis and immunoprecipitation
Western blot analysis was performed as described previously (28). Briefly, whole-cell lysates were prepared in RIPA buffer (50 mmol/L Tris–HCl pH 7.4, 150 mmol/L NaCl, 1% NP40, 0.25% Na-deoxycholate, 1× Roche complete mini protease inhibitor cocktail). For membranous fractionation, cells were grown in 15-cm dishes and were collected with 400μL cold hypotonic buffer (10 mmol/L Tris–HCl pH 7.5, 0.2 mmol/L MgCl2, with protease and phosphatase inhibitors (Roche) with a cell scraper. The extract was spun at 15,000g for 45 minutes at 4°C, and the supernatant was discarded. The pellet was washed twice in hypotonic buffer and then resuspended by vortexing in 50 μL lysis buffer (150 mmol/L NaCl, 20 mmol/L Tris–HCl pH 7.5, 1% Triton X-100, and proteinase inhibitors). The extract was vortexed for 10 seconds every 10 minutes and kept on ice for 30 minutes. Afterward, the supernatant was collected as the membrane fraction. Protein concentrations were measured by the Bradford assay (Bio-Rad). Protein sample (20 μg) was incubated with Laemmli sample buffer at 95°C for 10 minutes. Proteins were separated by SDS–PAGE and immunoblotted onto a PVDF membrane. The protein bands were visualized with IRDye-coupled anti-rabbit or anti-mouse antibodies (either or both mouse/rabbit; LI-COR) and scanned on an Odyssey imager (LI-COR Biotechnology). The following antibodies were used: pSTAT3 (Tyr 705), pERK1/2, Na/K-ATPase; Cell Signaling); pAKT1 (Thr 308), IL10 R1, Actin, and Lamin B1 and IL10R2 (Santa Cruz Biotechnology); IL22 R1, α-tubulin (Abcam). Quantification of bands was performed by drawing rectangles in Odyssey software (LI-COR).

For immunoprecipitation, untreated and cytokine-treated RIPA cell lysates were incubated with either IL10R2 or IL22R1 antibody or control IgG, followed by incubation with protein G plus agarose beads (Santa Cruz Biotechnology). The beads were washed and boiled directly in SDS sample buffer (for IP Western blot).

Copy-number variation assay
RT-PCR–based relative gene amplification for IL10RB was performed as described previously (29) on DNA extracted from 25 matched tumor samples and cell lines. Three different primers covering exons 1, 4, and 6 (Supplementary Table S3) were used. The reference gene was GAPDH, and c-MYC was used as a positive control. The assay was first established using colorectal cancer cell lines with validation of c-MYC amplification in the known cell line HT-29 (30).

Statistical analysis
Statistical analysis was performed using SPSS (version 21.0). Metric outcome variables were compared using ANOVA with least significant difference and the Bonferroni test as a post hoc test. The IRS was compared using a two-tailed t test. P values less than 0.05 were considered statistically significant. All data are expressed as mean ± SD. The Wilcoxon rank sum test (Mann–Whitney U test) was used for correlation between MSI status and IL10R2 and STAT3 expression levels.

Results
IL10R2 is overexpressed in colorectal cancer
Using matched pairs of human tissue samples (n = 29), mRNA of genes corresponding to the IL10/JAK/STAT signaling pathway (IL10, IL10R1, IL10R2, STAT1, STAT3, SOCS1, and SOCS3) was analyzed by qRT-PCR (Fig. 1A). As a positive control, c-MYC was also included. In normal colonic tissue, SOCS1 was strongly expressed, followed by STAT1, c-MYC, STAT3, IL10R2, and SOCS3. IL10 and IL10R1 mRNA was detected at a low level in both normal and tumor tissues. However, a statistically significant increase in mRNA expression in tumor tissue was detected for STAT3 and IL10R2 (3.8- and 3.5-fold, respectively; *, P < 0.05), but not for IL10R1, STAT1, or SOCS. IL10 expression was downregulated in tumor tissue compared with control. IHC in tumor and normal tissues confirmed overexpression of IL10R2 and STAT3 within tumor cells (Fig. 1B). Within the tumor cells, STAT3 expression was cytoplasmic. IL10R2 was localized mostly to the cytoplasm, but membranous staining was also detected. IRSs were compared for two compartments, i.e., the lamina propria and intestinal epithelial cells (IEC) of tumor and normal tissues, respectively. In IEC, IL10R2 levels were significantly higher in tumor samples (1.4-fold), which did not reach statistical significance for STAT3 (Fig. 1C). In the lamina propria, both IL10R2 and STAT3 expression were somewhat higher in the tumor samples also without reaching statistical significance. These data demonstrate that IL10R2 mRNA as well as protein is overexpressed in colorectal cancer.
IL10R2 overexpression is associated with microsatellite-stable tumors

To better characterize the role of IL10R2 overexpression in colorectal cancer, various cell lines were examined for the expression of the same set of IL10/JAK/STAT signaling pathway genes by qRT-PCR. High levels of IL10R2 mRNA were found mainly in SW480 and HT29 and the lowest in HCT116, LoVo, and DLD-1 (Supplementary Fig. S1), pointing to an inverse relation of IL10R2 overexpression and the presence of MSI. Western blot was performed on the membranous fractions that showed higher receptor expression by chromosomal instable (CIN) cells (HCEC-1CT, HT-29, and SW480) compared with MSI cells (HCT116, LoVo, and RKO; Fig. 2; Supplementary Fig. S3; ref. 31).

CIN and MSI are distinct key mutational patterns of genomic instability in colon carcinogenesis. The above data from colorectal cancer cell lines indicated that IL10R2 expression is associated with CIN. To identify the association of IL10R2 overexpression with either of these mechanisms, we further analyzed MSI status in 25 of 29 tumor samples (in 4 cases, the tissue had been used up with RNA extraction). Five of these 25 samples (20%) were MSI-positive for the markers examined (Table 1A). Three patients showed alterations in mononucleotide markers (NR24 and BAT25), which are highly specific for the detection of MSI tumors. Two patients showed shortening of dinucleotide repeats at two markers each. All other samples were considered as microsatellite stable (MSS). Statistical analysis of the data revealed a significant correlation between MSS and IL10R2 (Table 1B). In line with IL10R2, STAT3 expression levels were significantly lower in tumors displaying MSI. No correlation was observed between IL10R1 expression and microsatellite status, consistent with the finding that colorectal cancers do not overexpress this receptor subunit. Although loss of heterozygosity (LOH) was not detected (as microdissection was not performed), it is likely that all MSS samples reflect CIN tumors. The data therefore suggest that IL10R2 and STAT3 might contribute to colorectal carcinogenesis in CIN but not MSI tumors.

Table 1. MSI analysis of colorectal cancer samples. A, patient samples previously examined for differential expression of IL10 pathway genes were analyzed for MSI as described in Materials and Methods. DNA from the matched pair tissues (25 samples; N, normal; T, tumor) showed 20% MSI (5 MSI-positive cases). The rest of the samples without alteration in the markers examined were MSS. B, the Wilcoxon rank sum test (Mann–Whitney U test) revealed statistically significant correlation between microsatellite status and IL10R2 and STAT3 expression level. IL10R2 expression was strongly associated with MSS tumors. No correlation was detected for IL10R1.

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IL10R2 overexpression provides proliferative advantage to colorectal cancer cells

To examine the functional consequence of IL10R2 overexpression in the context of colorectal cancer, HT29 cells were transiently transfected with pcDef3 and pcDefIL10R2 vectors, and cell proliferation was analyzed in the presence and absence of ligands for IL10R2 (IL10 and IL22). IL22 belongs to the IL10 superfamily of cytokines and shares IL10R2 to form a functional receptor complex with IL22R1 and activates the transcription factor STAT3. IL6 was used as a control cytokine that induces STAT3 phosphorylation independent of IL10R2. Overexpression of IL10R2 (Supplementary Fig. S3) itself did not increase cell proliferation (Fig. 3A); however, ligand binding (of both IL10 and IL22) increased proliferation. IL6 did not have differential effects in control and IL10-R2–overexpressing cells, although it increased cell proliferation compared with untreated controls.

To examine the activation of cell proliferation pathways upon IL22 treatment, Western blotting was performed for activation of p-STAT3 (Tyr 705), p-AKT1 (Thr308), and p-ERK1/2 as well as total proteins. Cells overexpressing empty vector or IL10R2 (IL10RB) were briefly (30 minutes) treated with IL10 and IL22, and Western blot was performed in whole-cell lysates (Fig. 3B). IL22 treatment resulted in STAT3 phosphorylation that was further enhanced upon overexpression of IL10R2. IL22 treatment also increased both total and phospho-AKT and ERK1/2 levels. Upon IL10R2 overexpression, ERK levels increased further.

As IL10R2 overexpression was linked to CIN, we hypothesized that this was due to an increase in DNA copy number. The DNA copy number was assessed in the matched tumor samples and in colorectal cancer cell lines. However, no copy-number variation was observed (data not shown). Next, we examined if proinflammatory cytokines have any effect on receptor expression in normal colon epithelial cells HCEC-1CT. After 8 hours of cytokine treatment, cells were harvested, and whole-cell lysates were subjected to Western blotting. Apparently, TNFα...
induced IL22R1 expression, and IL6 and IL22 induced IL10R1 (Fig. 3C). Both IL22R1 and IL10R2 expression was higher upon TNFα treatment, suggesting responsiveness of cells to IL22 signaling. In the context of colorectal cancer, these results also implicate IL22 in taking advantage of IL10R2 overexpression and facilitating cancer cell survival.

IL22R1 is overexpressed in colorectal cancer

Our data indicated that IL22 can potentially take advantage of IL10R2 overexpression, contributing to STAT3 and ERK phosphorylation and increasing proliferation. IL22 is an important component of type-17 response in intestinal inflammation, and its expression is increased in IBD (19, 32). Functional IL22 signaling with heterodimeric receptors (IL22R1 and IL10R2) requires their concomitant expression on epithelial cells. To assess the role of IL22 signaling, IBD and CAC samples were examined and compared with colorectal cancer samples. IHC analysis for IL22R1 expression in IBD showed a weak epithelial staining, whereas stromal cells showed increased receptor expression (Fig. 4). However, in CAC and colorectal cancer, IL22R1 expression significantly increased. Expression of IL10R2 was also examined in these sections, and the immunoreactivity scores revealed a significant increase only in colorectal cancer (Supplementary Fig. S4A). When examined for IL22 expression, CAC and colorectal cancer sections showed an increase in IL22 reactivity in the cytoplasm (Fig. 4). The IRS score was significantly higher in both groups (Supplementary Fig. S4A). These observations suggest that, although IL22R1 expression is increased in both CAC and colorectal cancer, IL22/STAT3 signaling could be active in colorectal cancer due to high expression of IL10R2.

We further performed coimmunoprecipitation experiment in colorectal cancer cells to confirm interaction of IL10R2 and IL22R1. HT-29 cells were treated with IL22, IL10, and IL6, and IL22 treatment resulted in robust expression and pulldown of IL22R1 compared with untreated control, IL10, or IL6 (Supplementary Fig. S4b). IL10R2 was coprecipitated upon IL22R1 pull-down and was detected by IL10R2 antibody. Together, these data support the notion that in the absence of IL10R1, IL10R2 overexpression can be utilized by IL22 instead of IL10 and contribute to colorectal carcinogenesis through activation of STAT3 signaling.

Discussion

It is widely accepted that IL10/STAT3 signaling is central to gut homeostasis. In the context of intestinal pathophysiology, impaired IL10 signaling predisposes to colitis, and STAT3 activity contributes to colorectal carcinogenesis. This study highlights that overexpression of IL10R2, which is shared by IL10 superfamily...
members, such as IL22, can be exploited for STAT3 activation by cancer cells for survival and proliferation. IL10R2 overexpression was specifically found to MSS sporadic colorectal cancer as it was increased neither in chronic inflammatory diseases (IBD) nor in CAC. In vitro studies demonstrated that IL22 (which engages IL10R2 along with IL22R1) activated STAT3 and growth-promoting pathways such as ERK in colorectal cancer cells. IL22R1 was also found to be overexpressed in an independent set of MSS colorectal cancers, substantiating these observations. The data indicated an association of IL10R2/IL22R1 overexpression and MSS phenotype in colorectal cancer. Together, the data from this study provide a novel insight on IL22/STAT3 signaling contributing to colorectal carcinogenesis (Fig. 5).

It was interesting to find that IL10R2 expression was elevated in sporadic cancer (colorectal cancer) without MSI and not in CAC or microsatellite-unstable colorectal cancer. In chronic inflammatory diseases, such as IBD, IL10 signaling is commonly impaired due to either receptor mutations or IL10 deficiency (3, 33). On the other hand, sporadic cancers such as colorectal cancer elicit an inflammatory environment. Cytokines elevated in such inflammation facilitate tumor growth. On that note, anti-inflammatory drugs such as aspirin are effective in impeding carcinogenesis (34). Our data suggest that proinflammatory cytokines, such as TNFα, can induce cytokine receptor expression, in this case IL22R1 on epithelial cells, and hence rendering the cells responsive to IL22. Recently, IL22 has been shown to promote colorectal cancer by activation of STAT3 and maintenance of cancer stem cells (35). Besides, IL22 is also important for protecting gut epithelium through production of antimicrobial peptides and restoration of epithelial barrier functions (36). On the other hand, IL22 can be exploited by pathogens and can cause gastroenteritis (37) or induce proinflammatory response in colonic myofibroblasts (21). We observed an increase in IL22 expression in colorectal cancer tissue as well as in IBD and CAC samples, indicating a key role of IL22 during stress conditions.

The proportion of colorectal cancers with MSS/CIN phenotype is by far larger than that of colorectal cancers with the MSI phenotype (about 15% ref. 38). The likelihood of IL10R2 overexpression as part of the MSI phenotype is low, as we found IL10R2 overexpression only in CIN cell lines and colorectal cancer samples without MSI. Nonetheless, the association of the CIN phenotype and IL10R2 overexpression should be validated in a larger cohort with the potential to perform microdissection.

IL10R1 is expressed mostly by immune cells, whereas IL10R2 is ubiquitously expressed; however, IL22R1 is expressed only by nonhematopoietic cells. Interestingly, simultaneous presence of both receptor subunits (IL22R1 and IL10R2) was not observed in CAC (displaying IL10R2 similar as in IBD). This suggests that IL22 signaling is restricted in CAC. Although a higher level of IL22 was found in IBD samples, which has also been reported previously (23, 32), such downregulation of IL10R2 may curtail the IL22–STAT3 axis.

Our in vitro data substantially support the role of IL22/STAT3 signaling and increased activity of MAPK/ERK pathways in colorectal cancer cells. IL10R2 overexpression as such was not sufficient for cell proliferation and ligand binding stimulated cell growth. With regard to ligands for IL10R2, besides IL10 and IL22, IL26 and IFNβ also share this receptor subunit. Knowledge about
IL-26 and IFN-α in gut homeostasis and colorectal carcinogenesis is limited and warrants further investigation.

Collectively, the findings from this study demonstrate the potential of shared cytokine receptors to be exploited by cancer cells for their growth and survival and opens up an alternative approach to target oncogenic IL22/STAT3 signaling in colorectal cancer. Moreover, overexpression of IL10R2 in colorectal cancer provides a mechanistic link in the understanding of sporadic versus inflammation-driven colorectal carcinogenesis.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: V. Khare, G. Paul, C. Gasche
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): V. Khare, G. Paul, O. Movadat, A. Frick, M. Jambrich, A. Krnjic
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