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Chronic Inflammation and the Development of Malignancy In the GI Tract

Stefan Fichtner-Feigl^{1,2}, Rebecca Kesselring¹, and Warren Strober³

¹Department of Surgery, University Medical Center Regensburg, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany

²Regensburg Center for Interventional Immunology, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany

³Mucosal Immunity Section, Laboratory of Host Defenses, National Institute of Allergy and Infectious, National Institutes of Health, Bethesda, MD 20892

Abstract

The role of immunologic factors in the development of gastrointestinal (GI) neoplasia, made evident from the high degree of association of chronic intestinal or gastric inflammation with the development of cancer, has attracted much attention because it promises new ways of treating disease. Here we develop the idea that immunologic factors influence the appearance of GI cancer on two levels: 1) a basic and initiating level during which the epithelial cell is induced to undergo pre-cancerous molecular changes that render it prone to further cancer progression; and 2) a secondary level that builds on this vulnerability and drives the cell into frank malignancy. This secondary level is uniquely dependent on a single epithelial cell signaling pathway centered on Stat3, and it is this pathway upon which stimulation of mucosal cytokine production and microbiota effects converge.

Introduction

The concept that chronic inflammation is quite often associated with the development of neoplasia, first introduced over a century ago by Virchow, is now very well established (1). This association applies with special force to the GI tract inasmuch as this organ is juxtaposed to pro-inflammatory factors of the gut microbiome that cause a constant state of low level inflammation in the GI mucosa. However, it would be incorrect to assume that tumor development occurring under the influence of inflammation is radically different from that characterizing sporadic disease. A more realistic view is that inflammation-associated neoplastic transformation is essentially similar to that occurring in sporadic disease, but that inflammatory factors can both initiate and accelerate the sequence of oncogenic events that characterize the sporadic disease. This view is compatible with and indeed encourages the

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idea that sporadic disease is also influenced by immunologic factors, albeit in a less intense and more inconspicuous fashion.

Here we discuss these accelerating immunologic factors and put their effects in the context of the mucosal immunity. Whereas the discussion herein is limited mainly to inflammation associated neoplasia occurring in the colon, similar principles apply to *Helicobacter pylori* – associated gastric cancer and esophageal cancer (Barrett's esophagus)

Stages of Inflammation-Associated GI Neoplastic Development

In organizing our discussion of the influence of inflammation on the development of gastrointestinal epithelial cell neoplasia it is useful to assume that such influence is exerted in a discontinuous fashion reflective of two major stages. The first stage is a stealthy stage in which the cells are subjected to largely silent mutational “hits” whereas the second stage is a more obvious stage in which the initial hits are exploited to cause more frankly neoplastic changes, possibly in conjunction with new hits. In the first stage, the initial mutations do not lead to the generation of macroscopic benign and/or invasive tumors in most instances because they initiate cellular processes (such as apoptotic cell death) that, at least for a time, results in the elimination of the potentially cancerous cell. In the second stage, however, this elimination process is undermined by inflammation-associated epithelial cell signaling that, in effect, rescues the epithelial cells bearing a pro-oncogenic change from elimination and thereby renders the cell susceptible to further tumor progression. The development of Intestinal tumors during chronic inflammation *de novo* such those occurring in patients with inflammatory bowel disease or mice with IL-10 deficiency experience both the first and second stages of tumor development since in these instances no oncogenic factor is initially present other than the inflammation itself. In contrast, the development of intestinal tumors in mice exposed to a potentially oncogenic agent such as azoxymethane (AOM) and then to inflammation, in effect, experience only the second stage since in this case the epithelial cells have already sustained initial oncogenic changes that are now allowed to develop into frank tumors by pro-oncogenic signaling. It should be noted, however, that inflammation-dependent second stage events are occurring in and are necessary for all tumors that develop during inflammation and may in fact be operative in human tumors that are not ordinarily thought to be associated with inflammation.

1st Stage Effects of Inflammation on Neoplastic Development

As discussed above, first stage pro-oncogenic effects of inflammation cause pre-neoplastic cellular changes that render the cell vulnerable to further neoplastic transformation. These changes are in fact similar to those that occur during sporadic epithelial cell malignancy with the possible exception of the sequence of the neoplastic mutations (e.g., Apc mutations tend to occur earlier in sporadic colorectal cancer than in inflammation-associated malignancy) and thus can ultimately lead to malignancy in the absence or inflammation or in the presence of very low grade local inflammation usually inapparent in sporadic malignancy (2, 3). In the discussion below they are characterized as frankly pro-oncogenic events, albeit ones that are influenced by the presence of chronic inflammation. However, in the two stage formulation they do not actually cause malignancy until cells are subject to

further signaling during the second stage. In this sense they are also a feature of the second phase.

In some cases 1st stage oncogenic events consist of “up-stream” factors that exert both cytokine independent and dependent effects such as factors associated with the gut microbiome that act on cells via TLR and other innate receptors to induce cytokines and growth factors. In other cases these consist of down-stream factors induced by soluble factors such as reactive oxygen or nitrogen species and eicosanoids. In either case, the net effect of these cytokine-related and unrelated factors is to induce stepwise genetic and epigenetic changes in epithelial cells that have the potential to eventuate in frank malignancy.

Cytokines implicated in inflammation-associated malignant transformation of cells are the same as those mediating the inflammation itself such as IL-1 β , TNF- α and IL-6 (4). In general, these cytokines support oncogenesis by activation of well-known pro-inflammatory mechanisms involving the activation of the NF- κ B and MAPK pathways and by downstream effects of the latter such as the induction of growth factors, the inhibition of apoptosis and the enhancement of ROS production (5). However, as we shall see, IL-6 and other cytokines that activate STAT3 have a particularly important role in malignant transformation of epithelial cells.

Molecular analysis of individual malignant epithelial cells has disclosed that these cells can contain as many as 100 coding region mutations including 10-20 that have known oncogenic properties (6). In addition, these cells exhibit numerous somatic rearrangements and multiple methylated genes that have also been associated with malignancy. Knowledge of the mechanisms by which chronic inflammation creates these genetic changes is only fragmentary. Generally speaking, however, they involve inflammation-associated generation of factors that cause inactivation of tumor-suppressor genes or, conversely, the activation of oncogenes. The most cogent example of tumor-suppressor gene inactivation is that involving *TP53*, a gene that is central to the prevention of malignant transformation as a result of its capacity to bind to sites regulating cell cycle, responses to DNA damage or oxidative stress, i.e., processes that expose cells to the possibility of malignant transformation (7). Evidence that inflammatory factors accompanying chronic inflammation can in fact lead to inactivating mutations in *TP53* comes from studies showing that such mutations can be observed in chronic HCV infection and ulcerative colitis prior to the detection of neoplasia (8).

Inflammation induced inactivating mutations in *TP53* occurring in relation to inflammation have been attributed to free radicals that are generated during oxidative stress. However, other mutational mechanisms may also be operative. One such mechanism is that involving activation-induced cytidine deaminase (AID), an enzyme that facilitates DNA changes necessary for class-switch differentiation in B cells (9, 10). During its normal function this enzyme deaminates cytidine and thus produces a uracil that generates a U:G mismatch; if DNA replication starts prior to recognition by the repair system this, in turn, gives rise to non-mutational C:G to T:A transitions. However, if replication is delayed there may be generation of an abasic site at the U position or recognition of the U:G mismatch by

enzymes that generate a mutation at the C:G site or a nearby A:T site. The possibility that AID is involved in oncogenic mutations comes from the fact that its expression is up-regulated by NF- κ B and thus cellular exposure to pro-inflammatory cytokines (11). Evidence that such inflammation-associated AID expression can lead to oncogenesis comes from the fact that cells expressing constitutively active AID exhibit mutations in *TP53*, and deficiency in AID reduces *TP53* expression during the development of colitis-associated colorectal cancers (12, 13).

Aberrant epigenetic changes that normally occur during physiologic gene activation or inactivation constitute another possible mechanism of *TP53* inactivation. These epigenetic changes consist of either histone or DNA methylation events that change the accessibility of transcription factors to promoter or enhancer sites. For instance, methylation of CpG islands in gene promoters can influence transcription factor accessibility to promoter sites and thus influence the rate of transcription (14). Inflammation induced IL-1 β or ROS (as well as TNF- α and IL-6) have been implicated in the induction of aberrant epigenetic changes association with malignancy via their capacity to alter the activity of DNA methyl transferases, i.e., the enzymes that mediate methylation (15). In this context, it has been hypothesized that macrophage inflammatory cytokines and oxidative stress can lead to the recruitment of such methylases to CpG islands that are not blocked by RNA polymerase II and that while such methylation is generally a rare event, it can lead to more dense methylation at promoter sites to cause gene inactivation (16).

The above discussion of mechanisms of inflammation-associated inactivation of tumor suppressor genes focused on *TP53* inactivation, but in reality similar mechanisms (or indeed other mechanisms) apply to other tumor suppressor genes. Conversely, similar mechanisms of mutational change apply to activation of oncogenes. A prominent example of the latter relating particularly to epithelial cell malignancy is that involving the *Kras* gene, a gene encoding a membrane-bound protein that is an essential down-stream component in the signaling pathway initiated by EGFR and other receptor tyrosine kinases involved in cellular proliferation and survival (17). Whereas in normal cells *Kras* is activated by various extra-cellular signals, in malignant cells it is locked into an activated state that does not require such signals and thus mediates excessive cellular proliferation and/or survival. Of interest, there is evidence that such abnormal activation of *Kras* may be mediated by inflammation-induced expression of pro-oncogenic miRNA, i.e., small RNA species that normally regulate mRNA survival (18).

Other mutations associated with the activation of oncogenes are those leading to perturbations of the epithelial cell Wnt signaling program that is ordinarily involved in epithelial differentiation and proliferation (19, 20). In the normal operation of this program Wnt signaling may be in an “off” mode, because the cells are not being subjected to stimulation by Wnt ligands. During this period, a key Wnt signaling molecule, β -catenin, complexes with several proteins, such as Apc, GSK3 β and axin and is thus targeted for phosphorylation and proteosomal degradation. However, in the presence of mutations of one of these proteins, β -catenin degradation is impaired and is thus available for the formation of complexes with TCF/Lef proteins that mediate its translocation into the nucleus; at the latter cellular site β -catenin can transactivate proto-oncogens such as c-myc and cyclin D1 that

lead to the development of tumors. The most important of the Wnt mutations with respect to colon cancer are those affecting the Apc gene (adenomatous polyposis coli gene), a gene that is mutated in most cases of sporadic colorectal cancer (especially that occurring in individual with familial adenomatous polyposis) as well as in the Apc^{min} murine model of colon cancer (21). Related mutations in the Wnt signaling pathway leading to tumor formation include those involving the GSK3 β and the axin genes (19-21).

The importance of Apc mutations in colon tumor development is recognized not only by the fact that these mutations are found in the majority of human colon cancers, but also by the fact that mice bearing these mutations on a single chromosome, i.e., mice with loss of heterozygosity (the aforementioned Apc^{min/+} mice) develop spontaneous intestinal microadenomas characterized by increased expression of nuclear β -catenin, as predicted from a disturbance in the Wnt signaling pathway (22). The fact that the development of tumors in these mice is not accompanied by presence of pre-existing intestinal inflammation or even changes in numbers of infiltrating inflammatory cells would suggest that the latter is not a key factor in the development of the tumors. However, this conclusion is not justified because it does not take into consideration the fact that Apc^{min} mice that lack innate signaling due to MyD88 deletion exhibit dramatically reduced tumor development and experience increased survival (23). Since such deletion results in a major reduction in the production of cytokines and other immunologic factors in the tumor bearing intestine one can confidently conclude that tumor development does in fact depend on one or more factors produced by activated hematopoietic cells in the area of the tumor development, even if such factors are not immediately evident.

A related important fact is that mice with MyD88 deletion express an equal number of microadenomas as those without deletion; this indicates that the loss of cytokine production was affecting the progression of the tumor to macroadenoma rather than initial tumor development. As noted below, in further studies of Apc-related tumor formation, a key factor necessary for tumor progression may be IL-23 and/or its down-stream induction of Th17 cytokines. This again emphasizes the relation of immune responses not only to the initial phase (1st Stage) of neoplastic development but also to the second phase (2nd Stage) of such development.

1st Stage Oncogenic Events Induced by a Genotoxic Agent, AOM

Whereas, as noted above, the relation of intestinal inflammation to the development of tumors has been studied with the Apc^{min} model it has more commonly been investigated in studies of the effect of induced inflammation on tumor development in mice administered azoxymethane (AOM), a substance that induces epithelial cells to enter a cancer-prone state that generally stops short of neoplasia (24, 25). This approach is fundamentally different than that in models relying on spontaneous tumors because in the AOM models one does not rely on pre-existing albeit limited inflammation to “awaken” the neoplastic process; instead, Stage 1 is by-passed or accelerated and one focuses on the immunologic events that occur during Stage 2.

AOM is a metabolite of the pro-carcinogen 1,2-dimethylhydrazine (DMH) and requires the down-stream formation of a mutagenic metabolite to induce DNA-reactive products (26,

27). Upon administration, AOM is hydroxylated by the cytochrome p450 isoform CYP2E1 in the liver to form methylazoxymethanol (MAM) that is then transported to the colon where it is converted by alcohol dehydrogenase to methyldiazonium ion, a substance that can alkylate macromolecules. Such alkylation results in methylation of guanine leading to formation of 6-methyl-deoxyguanosine and N7-methyl-deoxyguanosine, DNA adducts with high cytotoxic, apoptotic, mutagenic, recombinogenic, potential (28). Specific DNA damage caused by these adducts include mutations in Apc and K-ras and Ctnnb genes, as well as GSK3 β mutations that effect its phosphorylation; thus AOM creates several of the mutations observed in sporadic CRC (29).

It is important to mention that AOM-induced tumors are characterized by the enhanced cytosolic and nuclear expression of β -catenin indicating that such tumors have a dysregulated Wnt signaling pathway. In the setting of AOM-induced-tumorigenesis this can be caused by missense β -catenin mutations of β -catenin phosphorylation sites that prevent β -catenin degradation. Alternatively, they can be caused by mutations affecting the “ β -catenin destruction complex” that facilitates the previously discussed mechanism of β -catenin phosphorylation and polyubiquitination/degradation (28). Thus, AOM can lead to neoplasia quite similar to that in the Apc^{min} mouse.

The fact that AOM administration results in the generation of mutagenic products that are in fact carcinogenic but does not actually cause neoplasia in the absence of inflammation suggests the operation of mechanisms that neutralize the AOM carcinogenic effect. One such mechanism involves the activation of the acute apoptotic response to a genotoxic carcinogen (AARGC) whereby stem cells and other proliferative cells in the crypt undergo apoptosis upon acquisition of a increased “mutational load” (28, 30, 31). The apoptotic response occurring in AARGC is dependent at the functionality of *TP53*, since loss of just one allele of *TP53* reduces the apoptotic response to carcinogen by 50% and p53^{+/-} mice are more highly susceptible to AOM-induced neoplasia than are wild-type mice. This combined effect is exploited in the newly described Tp53^{IEC} animal model of invasive colon tumorigenesis with lymph node metastasis (32). The existence of this and possibly other counter-neoplastic mechanisms that come into play after AOM administration suggests that inflammation leads to cellular changes that render AARGC and other pro-apoptotic processes inoperative.

Finally, it is important to mention that AOM is not the only chemical that can cause pre-neoplastic changes in target cells. For instance 7,12-dimethylbenzanthracene (DMBA) has this effect in epidermis cells (33). Thus, the tumor accelerating effect of AOM is not unique, although it is the only agent of this type that has been extensively used in the study of tumors of gut epithelial cells.

2nd Stage Effects of Inflammation on Neoplastic Development

Epithelial cells, having been subjected to neoplastic influences by stealthy hits characteristic of the first stage of pro-oncogenic inflammatory effects or to a pro-neoplastic chemical such as AOM that provides a similar influence in a more rapid fashion, are now cancer prone cells vulnerable to 2nd stage pro-oncogenic events. It is important to note that this transition to the 2nd Stage may depend to some extent on random events affecting cancer prone cells

such as epithelial injury associated with infection or inflammation as well as epithelial self renewal after such injury. Interestingly, this, in turn, may be regulated by inhibitory immunologic pathways, since it has been shown that NLRP6 inflammasome activity in epithelial cells prevents AOM-induced tumor formation in mice subjected to DSS colitis (34).

In any case, extensive evidence now exists that STAT3 stands at the gateway to the signaling pathway that leads to tumor formation in cancer prone cells. This evidence consists first of the fact that in experimental models in which inflammation causes tumor formation (such as the AOM-related models) epithelial cells manifest increased STAT3 activation and that each of the cytokines such as IL-6 or IL-11 and several growth factors such as EGF, HGF and VEGF shown to influence tumor growth have in common the fact that they directly or indirectly cause STAT3 activation (35-38). Secondly, in AOM-related tumor models tumors with increased size and proliferation are seen in the presence of epithelial cells with increased STAT3 activation (i.e., cells with a gp130^{Y757F} mutation that prevents SOCS3 activation or with SOCS3 deficiency) (39). This finding is mirrored in the Apc^{min/+} tumor model in that Apc^{min/+} mice with mutated gp130 that cannot bind STAT3 and thus manifest reduced STAT3 activation, exhibit a decreased number and size of tumors as well as reduced early stage tumor formation; reciprocally, Apc^{min} mice with gp130 that cannot bind SOCS3 and thus manifests increased STAT3 signaling, exhibit increased tumor formation (40). Taken together, these data provide more or less definitive evidence that STAT3 activation plays a necessary and sufficient role in the 2nd Stage of inflammation associated epithelial cell tumor formation. Additional studies showing that Apc^{min/+} mice with epithelial cell-specific decreased STAT3 activity manifest decreased tumor formation indicate that the STAT3 signaling occurring in epithelial cells is the critical locus of tumor-inducing STAT3 activity (39, 40).

With respect to the down-stream oncogenic effects of STAT3 signaling, it was shown that STAT3 mutations causing decreased STAT3 activation do not necessarily impact Wnt signaling and β -catenin nuclear localization(40). This indicates that STAT3 signaling effects do not act via dysregulation of Wnt signaling and the latter's pro-oncogenic effects are in reality 1st Stage proneoplastic effects that are likely facilitated or aggravated by subsequent STAT3 signaling abnormalities. In the as yet limited studies exploring the neoplastic effects of STAT3 signaling, it has been shown that Apc^{min/+} mice with epithelial cell-specific inactivation of STAT3 display decreased abundance of cell cycle inhibitors p16 and p21 and, concomitantly, decreased expression of the polycomb group protein Bim-1, a protein that represses p16 and p21 (40). Moreover, evidence was obtained that STAT3 transactivates the *Bmi-1* gene. Thus, at least one way STAT3 activation leads to neoplastic transformation is by down-stream activation of a gene that ordinarily suppresses cell proliferation and tumor growth.

Studies linking STAT3 to down-stream pro-oncogenic effects immediately suggest that cytokines present in the inflammatory milieu are the chief purveyors of inflammation-related neoplasia. A wide variety of cytokines can induce STAT3 activation, but IL-6 is perhaps the most important activator in relation to colonic cancer since in murine experimental neoplasia induced by azoxymethane in the context of either DSS or oxazolone colitis, anti-IL-6

treatment or other methods of inhibiting IL-6 signaling is sufficient to prevent neoplasia (35, 41, 42). IL-6 is a member of the IL-6 cytokine family, a family defined by its capacity to signal via the gp130 receptor chain (43, 44). However, the ability of IL-6 and other IL-6 family members to signal through this chain is determined by a co-receptor chain that is unique to each family member and that binds to gp130 upon recognition of an IL-6 family member on the cell surface (43). Thus, the ability of various family members to induce STAT3 activation in epithelial cells depends on the ability of the latter to express the second chain dedicated to the family member. Interestingly, the co-receptor for IL-6, IL-6R α , has been shown to be a secreted receptor that can bind to IL-6 and induce gp130 signaling upon insertion into the cell membrane as an IL-6/IL-6R α complex. Such “trans-signaling” thus facilitates IL-6 signaling in cells, such as epithelial cells, which express relatively low amounts of membrane IL-6R α but which secrete IL-6R α (44, 45). The source of IL-6 in the tumor milieu is primarily myeloid cells as well as epithelial cells themselves. With respect to the latter it is possible that activated STAT3 in combination with NF- κ B can transactivate IL-6, suggesting the operation of a positive feedback IL-6 loop (46). Finally, It should be noted that the prooncogenic effect of IL-6 is not necessarily limited to its ability to activate epithelial STAT3. IL-6 signaling of T cells also suppresses apoptosis and thus increases the lifespan of infiltrating cells mediating carcinogenesis (44).

IL-11, another member of the IL-6 family of cytokines, is also a potent activator of STAT3 and inducer of GI malignancy, particularly gastric malignancy. Activation of Stat3 in relation to IL-11 depends on both the production of IL-11 by cells in the gastrointestinal inflammatory milieu and the expression of the gp130 co-receptor specific for IL-11, IL-11R α 1. IL-11 production may originate from epithelial cells or hematopoietic cells manifesting STAT3 activation and initiation by one of several cytokines including IL-22; thus, once IL-11 production is initiated it may exert positive feedback on its own production via its ability to induce STAT3 activation (47). Evidence that IL-11 induces tumor formation comes from studies of mice with constitutive gp130 activation that manifest decreased gastric tumors when subjected to genetic or pharmacologic blockade of IL-11 expression (47); in addition, IL-11 deficiency has a negative effect on intestinal tumor growth in the AOM/DSS and the Apc^{min/+} intestinal tumor models and this negative effect, somewhat surprisingly, is more profound than that exerted by IL-6 deficiency (47, 48). The latter may be related to the as yet unproven thesis that IL-11R α 1 rather than IL-6R α is closely associated with neoplastic epithelial stem cells or more simply that IL-6 effects depend on the presence of soluble IL-6R α that may not always be present in sufficient amounts.

Th17 cells have been linked to tumor cell development in the gut both in experimental systems and in humans with colonic neoplasia; in addition, in studies to be described, inhibition of the differentiation of these cells leads to abrogation of tumor growth (49). This has led to the view that IL-17 itself may also be a STAT3 activating cytokine, but there is little or no evidence that IL-17 has this property. On the other hand, Th17 cells also produce several other cytokines including GM-CSF and IL-22. Most relevant here is Th17 production of IL-22, a potent STAT3 activator by virtue of the ability of STAT3 to bind to a tyrosine-less intra-cellular domain of the IL-22R1 receptor (50). It is therefore not surprising that IL-22 has been linked to GI cancer development and that neutralization of IL-22 is more effective in preventing tumor formation than neutralization of IL-17 (51, 52). Overall, then,

it is likely that Th17 cells supporting STAT3-mediated tumor formation indirectly either by IL-17 induction of IL-6 (53) or directly by induction of IL-22. In addition, it is possible that IL-17 has other effects on epithelial cells that favor its malignant transformation.

Finally, it should be noted that not all cytokines that induce STAT3 activation are not necessarily involved in the support of epithelial tumor formation. IL-10 is a good case in point since signaling by this cytokine induces sustained STAT3 activation owing to the fact that the IL-10 receptor cannot activate SOCS3 (54). In fact, IL-10 production tends to reduce tumor induction because this cytokine has anti-inflammatory effects that reduce the levels of cytokines discussed above that enhance tumor formation. The reason STAT3 activation by IL-10 does not also have pro-oncogenic effects is not at all clear. One possibility is that activation of STAT3 must occur in epithelial cells to achieve a pro-oncogenic and IL-10 activation of STAT3 is mainly a feature of hematopoietic cells rather than epithelial cells. In addition, it is possible that the intra-cellular pool of STAT3 activated in the absence of SOCS3 is more diverse by virtue of post-translational modifications and thus contains STAT3 with effects on genes that decrease production of cytokines with pro-oncogenic effects.

The Microbiome and Induction of Epithelial Neoplasia

In recent years it has become increasingly apparent the a major driver of the inflammation-associated cell signaling in both the first and second stage of malignancy induction discussed above, but especially in the second stage signaling is related to innate stimulation initiated by the microbiome. The concept that the microbiome of the gut influences the development of intestinal neoplasia derives strong experimental support from the observation that in a number of different mouse models of inflammation also associated with the development of neoplasia, the institution of a germ-free state prevents both the inflammation and the neoplasia. This was first noted in DSS colitis (55), but later in a variety of colitides associated with cytokine abnormalities such as IL-10 deficient mice and mice that lack both RAG2 and T-bet and thus produce excessive amounts of TNF- α (TRUC mice) (56-58). It could be argued that in this situation, the gut microflora are not acting alone, but are instead taking advantage of (and acting in conjunction with) an abnormal mucosal immune system. However, as already noted, *Apc*^{Min/+} mice exhibit decreased tumor formation in the small intestine (albeit not the colon) when reared in a germ-free environment even though these mice do not manifest gut inflammation (59).

As summarized by Yang and Pei, particular bacteria appear to have a special propensity to induce neoplasia in mice such as *C. rodentium* or *H. hepaticus* but usually if not always in mice with gut inflammation or a cancer-associated genetic abnormality (56). A notable example of this is *Bacteroides fragilis*, a commensal organism that induces tumor formation in *Apc*^{min} mice when it is capable of producing an enterotoxin (36). In this case, the carcinogenesis could be traced to the ability of the enterotoxin to induce T cells to produce IL-17 that, in turn, cause production of IL-6 and epithelial cell expression of STAT3. Another, somewhat less clear example is mice lacking IL-10 as well as epithelial cell PTEN, the latter a phosphatase that regulates susceptibility to TLR stimulation (60). These mice develop a more severe colitis than that observed in mice with IL-10 deficiency alone that is

accompanied by the appearance of colonic neoplasms. Interestingly, these manifestations are ameliorated if the mice are crossed with TLR-deficient mice, suggesting that they have increased TLR responsivity that leads to pro-oncogenic immune responses. More relevant to the present discussion is the fact that a *Bacteroides* organism (*B. acidifaciens*, not *B. fragilis*) is increased in the fecal microflora of these mice so it is possible that this organism is also involved in signaling that causes neoplasia, but this remains to be proven.

The ability of particular organisms to induce tumor formation, noted above, has recently obtained increased salience with the appearance of evidence that carcinogenesis occurring in IL-10-deficient mice may be due to organisms that have special tumor-inducing properties. In the relevant studies it was shown that germ-free IL-10-deficient mice administered AOM and then mono-associated with either adherent-invasive Group B2 *E. coli* developed invasive adenocarcinoma whereas those mono-associated with *Enterococcus faecalis* merely developed benign tumors (61). Since the inflammations induced by infection with both organisms were similar in intensity and cytokine profile, it was apparent that the difference in the tumor response was due to the infecting organism. These findings thus led to a BLAST search of the *E. coli* genome that revealed that the enteroinvasive *E. coli* causing adenocarcinoma but not the *E. faecalis* harbored a polyketide synthases (pks) pathogenicity island that encodes enzymes capable of synthesizing Colibactin, an as yet poorly described peptide-polyketide hybrid previously shown to cause DNA damage and genomic instability and gene mutations in mammalian cells (62). Subsequent studies with *E. coli* with pks deletion revealed that without the pks island the *E. coli* had reduced capacity to induce DNA damage in epithelial cells *in vitro* and, more importantly, attenuation of the ability to induce invasive carcinoma in AOM-treated IL-10-deficient mice. In further control studies it was found that the *E. coli* bearing the pks island does not induce tumors in WT mice and does not induce invasive adenocarcinoma in IL-10-deficient mice not treated with AOM. This indicates that *E. coli* bearing the pks island is not carcinogenic in the absence of immunodeficiency and accompanying inflammation and in the absence of AOM treatment that induces initial epithelial dysplasia. In other words, the Colibactin produced by the pks island acts to move already cancer-prone (1st Stage) cells to cells with a higher level of carcinogenicity (2nd Stage cells). In follow-up of these finding the same group of investigators showed that ex-germ-free IL-10 deficient mice maintain a higher abundance of *Enterobacteriaceae* than WT mice and, moreover, that a small subset of these organisms that express the pks pathogenicity island makes its appearance, probably because such organisms have a survival advantage; thus, the inflamed gut of the mice drives changes in the resident microflora that favor the latter's carcinogenic potential.

It is important to note that the *E. coli* sub-group expressing pks in the studies described above are found quite frequently in normal individuals, particularly those in developed countries; in addition, these organisms are found in neonates and have a tendency to persist in the intestinal microflora for long periods of time. Nevertheless, the rate of carriage of these organisms is far higher than the incidence of intestinal cancer. This is best explained by the fact that, as made clear by the studies described above, they required addition factors to induce neoplasia such as pre-treatment with AOM and the presence of intestinal inflammation. This latter point underscores the fact that the molecular mechanism by which

colibactin actually brings about neoplastic transformation in cells initially exposed to another genotoxin agent, such as AOM is not at all understood.

Previous *in vitro* studies suggest that potentially carcinogenic *E. coli* secreting colibactin cause genetic changes, but only upon direct contact with epithelial cells (63). One mechanism by which such organisms might exploit the environment in an inflamed gut to enhance their survival and to cause invasive carcinoma is by taking advantage of the increased opportunity to bind to the epithelium under these circumstances. This possibility is favored by the observation that inflammation may increase the expression of adhesion molecules such as CEACAM6, an adhesin that has been shown to bind adherin-invasive *E. coli* (so-called AIEC organisms) in Crohn's disease patients that is up-regulated by TNF- α or IFN- γ production (64). Alternatively, the inflammation can conceivably lead to decreased mucin expression and thus degrade the epithelial barrier that ordinarily prevents bacterial adhesion.

In studies addressing this latter possibility and also providing additional mechanistic analysis of the relation of tumor development to mucosal immune responses along the lines initially mentioned above in relation to *Bacteroides fragilis*, Grivennikov et al., analyzed distal colonic tumors occurring in Apc^{F/wt} mice harboring a Cdx2-Cre transgene that were thus lacking Apc expression on one allele of epithelial cells (49). They found that although these mice did not manifest generalized lamina propria inflammation, their tumors and mesenteric nodes harbored dendritic cells (CD11b⁺/Gr1^{low} cells) and macrophages expressing IL-23 as well as T cells producing IL-17. Such IL-23 and IL-17 production appeared to be central to tumor cell development since there was reduced tumor cell proliferation in IL-23^{-/-} mice and in IL-17ra^{-/-} mice. However, the effect of these cytokines on tumors was probably dependent on the induction of "down-stream" cytokines capable of causing STAT3 activation in epithelial cells such as IL-6 and IL-22 that were also elevated in lesional tissue.

In the same studies, the role of luminal commensal microflora in the induction of the pro-neoplastic IL-23-mediated mechanism became apparent from the fact that tumor growth was associated with increased intestinal permeability and penetration of microbes or microbial products into lesional tissue. In addition, tumor growth and the appearance of cytokines inducing STAT3 activation were diminished in mice rendered germ-free by antibiotic treatment. These findings suggested that microflora penetrating into tumor tissue was inducing IL-23 synthesis via TLR signaling. To test for this possibility tumor growth and cytokine synthesis was determined in irradiated Apc^{F/wt} mice reconstituted by transplantation of bone marrow cells incapable of developing into cells that express TLR2,4,9 or MyD88, i.e., cells with defective TLR ligand signaling. Indeed, it was found that such reconstituted mice exhibited reduced tumor growth and intra-tumoral IL-17 or IL-6 synthesis. A final observation bearing on the role of the microflora in IL-23 induction and its effect on tumor growth relates to previous studies showing that Apc deficiency leads to hyper-proliferative epithelial cells and reduced goblet cell differentiation, the latter leading to decreased production of mucus (65). This observation was amplified here with data showing that epithelial cells in tumor tissue of Apc^{F/wt} mice express reduced amounts of mucin associated with diminished tight junction protein production (45). This suggested that

the tumor cells were themselves contributing to tumor development via their loss of epithelial cell barrier function and thus their facilitation of bacterial signaling that further encourages tumor growth. It should be noted that in contrast with the AOM/IL-10^{-/-} tumor model discussed above, in this Apc-deficiency model, the effect of microflora is not limited to bacteria expressing a genotoxic gene such as *pkc*, but rather is a property of a wide range of bacteria capable of expressing TLR ligands. Nevertheless, in both cases the epithelial abnormality essential for tumor development requires input from the host mucosal environment.

Concluding Remarks

In the above discussion we have emphasized that oncogenesis in epithelial cells subject to continuous signaling by pro-inflammatory factors occurs in a cell already prepared for frank malignant change because of prior oncogenic hits. This is the meaning of the observation that experimental induction of malignancy occurs in mice with epithelial cells exposed to a genotoxic agent such as AOM or in mice with cells harboring a genetic defect involving the Wnt pathway such as those in Apc^{min} mice. A new and important observation is that particular types of bacteria (*E.coli* species) are more capable of inducing malignancy than other bacteria. However, in this case malignancy is again induced only in AOM-treated mice so that the oncogenic *E.coli* are not acting *de novo* to produce malignant change. In fact, the most likely mechanism of cancer induction by these pro-oncogenic bacteria is related to their capacity to induce epithelial permeability changes and thereby the entry of non-pathogenic bacteria into the lamina propria that stimulate cytokine production. This last point brings to the fore the idea that inflammation-related gastrointestinal malignancy is in a sense unique because the inflammation recruits microbiome-related stimulation factors that feed into the inflammatory cycle and the STAT3 activation that is the door to further malignant change. Whereas some of the basic factors involved in the pro-neoplastic effect of inflammation have now been delineated, some major questions remain unanswered (Box 1). Insight into these questions should provide inroads into new therapeutic avenues for the treatment of GI disease and cancer.

References

1. Heidland A, Klassen A, Rutkowski P, Bahner U. The contribution of Rudolf Virchow to the concept of inflammation: what is still of importance? *Journal of nephrology*. 2006; 19(Suppl 10):S102–109. [PubMed: 16874721]
2. Azer SA. Overview of molecular pathways in inflammatory bowel disease associated with colorectal cancer development. *European journal of gastroenterology & hepatology*. 2013; 25:271–281. [PubMed: 23169309]
3. Myant KB, Cammareri P, McGhee EJ, Ridgway RA, Huels DJ, Cordero JB, Schwitalla S, Kalna G, Ogg EL, Athineos D, Timpson P, Vidal M, Murray GI, Greten FR, Anderson KI, Sansom OJ. ROS production and NF-kappaB activation triggered by RAC1 facilitate WNT-driven intestinal stem cell proliferation and colorectal cancer initiation. *Cell stem cell*. 2013; 12:761–773. [PubMed: 23665120]
4. Klampfer L. Cytokines, inflammation and colon cancer. *Current cancer drug targets*. 2011; 11:451–464. [PubMed: 21247378]
5. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. *Nature reviews. Cancer*. 2003; 3:276–285. [PubMed: 12671666]

6. Stephens PJ, McBride DJ, Lin ML, Varela I, Pleasance ED, Simpson JT, Stebbings LA, Leroy C, Edkins S, Mudie LJ, Greenman CD, Jia M, Latimer C, Teague JW, Lau KW, Burton J, Quail MA, Swerdlow H, Churcher C, Natrajan R, Sieuwerts AM, Martens JW, Silver DP, Langerod A, Russnes HE, Foekens JA, Reis-Filho JS, van 't Veer L, Richardson AL, Borresen-Dale AL, Campbell PJ, Futreal PA, Stratton MR. Complex landscapes of somatic rearrangement in human breast cancer genomes. *Nature*. 2009; 462:1005–1010. [PubMed: 20033038]
7. Fearon ER. Molecular genetics of colorectal cancer. *Annual review of pathology*. 2011; 6:479–507.
8. Hussain SP, Amstad P, Raja K, Ambs S, Nagashima M, Bennett WP, Shields PG, Ham AJ, Swenberg JA, Marrogi AJ, Harris CC. Increased p53 mutation load in noncancerous colon tissue from ulcerative colitis: a cancer-prone chronic inflammatory disease. *Cancer research*. 2000; 60:3333–3337. [PubMed: 10910033]
9. Honjo T, Kinoshita K, Muramatsu M. Molecular mechanism of class switch recombination: linkage with somatic hypermutation. *Annual review of immunology*. 2002; 20:165–196.
10. Conticello SG. The AID/APOBEC family of nucleic acid mutators. *Genome biology*. 2008; 9:229. [PubMed: 18598372]
11. Matsumoto Y, Marusawa H, Kinoshita K, Endo Y, Kou T, Morisawa T, Azuma T, Okazaki IM, Honjo T, Chiba T. *Helicobacter pylori* infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. *Nature medicine*. 2007; 13:470–476.
12. Endo Y, Marusawa H, Kou T, Nakase H, Fujii S, Fujimori T, Kinoshita K, Honjo T, Chiba T. Activation-induced cytidine deaminase links between inflammation and the development of colitis-associated colorectal cancers. *Gastroenterology*. 2008; 135:889–898. 898, e881–883. [PubMed: 18691581]
13. Takai A, Marusawa H, Minaki Y, Watanabe T, Nakase H, Kinoshita K, Tsujimoto G, Chiba T. Targeting activation-induced cytidine deaminase prevents colon cancer development despite persistent colonic inflammation. *Oncogene*. 2012; 31:1733–1742. [PubMed: 21841819]
14. Jones PA, Baylin SB. The epigenomics of cancer. *Cell*. 2007; 128:683–692. [PubMed: 17320506]
15. Hmadcha A, Bedoya FJ, Sobrino F, Pintado E. Methylation-dependent gene silencing induced by interleukin 1beta via nitric oxide production. *The Journal of experimental medicine*. 1999; 190:1595–1604. [PubMed: 10587350]
16. Ushijima T, Watanabe N, Shimizu K, Miyamoto K, Sugimura T, Kaneda A. Decreased fidelity in replicating CpG methylation patterns in cancer cells. *Cancer research*. 2005; 65:11–17. [PubMed: 15665274]
17. Raponi M, Winkler H, Dracopoli NC. KRAS mutations predict response to EGFR inhibitors. *Current opinion in pharmacology*. 2008; 8:413–418. [PubMed: 18619559]
18. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. RAS is regulated by the let-7 microRNA family. *Cell*. 2005; 120:635–647. [PubMed: 15766527]
19. Shitashige M, Satow R, Honda K, Ono M, Hirohashi S, Yamada T. Regulation of Wnt signaling by the nuclear pore complex. *Gastroenterology*. 2008; 134:1961–1971. 1971, e1961–1964. [PubMed: 18439914]
20. Shitashige M, Hirohashi S, Yamada T. Wnt signaling inside the nucleus. *Cancer science*. 2008; 99:631–637. [PubMed: 18177486]
21. Pritchard CC, Grady WM. Colorectal cancer molecular biology moves into clinical practice. *Gut*. 2011; 60:116–129. [PubMed: 20921207]
22. Moser AR, Pitot HC, Dove WF. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science*. 1990; 247:322–324. [PubMed: 2296722]
23. Rakoff-Nahoum S, Medzhitov R. Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. *Science*. 2007; 317:124–127. [PubMed: 17615359]
24. Neufert C, Becker C, Neurath MF. An inducible mouse model of colon carcinogenesis for the analysis of sporadic and inflammation-driven tumor progression. *Nature protocols*. 2007; 2:1998–2004. [PubMed: 17703211]
25. Corpet DE, Pierre F. How good are rodent models of carcinogenesis in predicting efficacy in humans? A systematic review and meta-analysis of colon chemoprevention in rats, mice and men. *Eur J Cancer*. 2005; 41:1911–1922. [PubMed: 16084718]

26. Papanikolaou A, Wang QS, Delker DA, Rosenberg DW. Azoxymethane-induced colon tumors and aberrant crypt foci in mice of different genetic susceptibility. *Cancer letters*. 1998; 130:29–34. [PubMed: 9751253]
27. Papanikolaou A, Shank RC, Delker DA, Povey A, Cooper DP, Rosenberg DW. Initial levels of azoxymethane-induced DNA methyl adducts are not predictive of tumor susceptibility in inbred mice. *Toxicology and applied pharmacology*. 1998; 150:196–203. [PubMed: 9630469]
28. Nasuno M, Arimura Y, Nagaishi K, Isshiki H, Onodera K, Nakagaki S, Watanabe S, Idogawa M, Yamashita K, Naishiro Y, Adachi Y, Suzuki H, Fujimiya M, Imai K, Shinomura Y. Mesenchymal stem cells cancel azoxymethane-induced tumor initiation. *Stem Cells*. 2014; 32:913–925. [PubMed: 24715689]
29. Takahashi M, Nakatsugi S, Sugimura T, Wakabayashi K. Frequent mutations of the beta-catenin gene in mouse colon tumors induced by azoxymethane. *Carcinogenesis*. 2000; 21:1117–1120. [PubMed: 10836998]
30. Westley CB, McIver CM, Abbott CA, Le Leu RK, Benkendorff K. Enhanced acute apoptotic response to azoxymethane-induced DNA damage in the rodent colonic epithelium by Tyrian purple precursors: a potential colorectal cancer chemopreventative. *Cancer biology & therapy*. 2010; 9:371–379. [PubMed: 20150770]
31. Hu Y, Le Leu RK, Young GP. Absence of acute apoptotic response to genotoxic carcinogens in p53-deficient mice is associated with increased susceptibility to azoxymethane-induced colon tumours. *International journal of cancer. Journal international du cancer*. 2005; 115:561–567. [PubMed: 15700305]
32. Schwitalla S, Ziegler PK, Horst D, Becker V, Kerle I, Begus-Nahrman Y, Lechel A, Rudolph KL, Langer R, Slotta-Huspenina J, Bader FG, Prazeres da Costa O, Neurath MF, Meining A, Kirchner T, Greten FR. Loss of p53 in enterocytes generates an inflammatory microenvironment enabling invasion and lymph node metastasis of carcinogen-induced colorectal tumors. *Cancer cell*. 2013; 23:93–106. [PubMed: 23273920]
33. Schwarz M, Munzel PA, Braeuning A. Non-melanoma skin cancer in mouse and man. *Archives of toxicology*. 2013; 87:783–798. [PubMed: 23266722]
34. Normand S, Delanoye-Crespin A, Bressenot A, Huot L, Grandjean T, Peyrin-Biroulet L, Lemoine Y, Hot D, Chamaillard M. Nod-like receptor pyrin domain-containing protein 6 (NLRP6) controls epithelial self-renewal and colorectal carcinogenesis upon injury. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:9601–9606. [PubMed: 21593405]
35. Grivennikov S, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, Scheller J, Rose-John S, Cheroutre H, Eckmann L, Karin M. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer cell*. 2009; 15:103–113. [PubMed: 19185845]
36. Wu S, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, Yen HR, Huso DL, Brancati FL, Wick E, McAllister F, Housseau F, Pardoll DM, Sears CL. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nature medicine*. 2009; 15:1016–1022.
37. Han W, Lo HW. Landscape of EGFR signaling network in human cancers: biology and therapeutic response in relation to receptor subcellular locations. *Cancer letters*. 2012; 318:124–134. [PubMed: 22261334]
38. Boccaccio C, Ando M, Tamagnone L, Bardelli A, Michieli P, Battistini C, Comoglio PM. Induction of epithelial tubules by growth factor HGF depends on the STAT pathway. *Nature*. 1998; 391:285–288. [PubMed: 9440692]
39. Bollrath J, Pesse TJ, von Burstin VA, Putoczki T, Bennecke M, Bateman T, Nebelsiek T, Lundgren-May T, Canli O, Schwitalla S, Matthews V, Schmid RM, Kirchner T, Arkan MC, Ernst M, Greten FR. gp130-mediated Stat3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumorigenesis. *Cancer cell*. 2009; 15:91–102. [PubMed: 19185844]
40. Pesse TJ, Buchert M, Stuart E, Flanagan DJ, Faux M, Afshar-Sterle S, Walker F, Zhang HH, Nowell CJ, Jorissen R, Tan CW, Hirokawa Y, Eissmann MF, Poh AR, Malaterre J, Pearson HB, Kirsch DG, Provero P, Poli V, Ramsay RG, Sieber O, Burgess AW, Huszar D, Vincan E, Ernst M.

Partial inhibition of gp130-Jak-Stat3 signaling prevents Wnt-beta-catenin-mediated intestinal tumor growth and regeneration. *Science signaling*. 2014; 7:ra92. [PubMed: 25270258]

41. Becker C, Fantini MC, Schramm C, Lehr HA, Wirtz S, Nikolaev A, Burg J, Strand S, Kiesslich R, Huber S, Ito H, Nishimoto N, Yoshizaki K, Kishimoto T, Galle PR, Blessing M, Rose-John S, Neurath MF. TGF-beta suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling. *Immunity*. 2004; 21:491–501. [PubMed: 15485627]
42. Schiechl G, Bauer B, Fuss I, Lang SA, Moser C, Ruemmele P, Rose-John S, Neurath MF, Geissler EK, Schlitt HJ, Strober W, Fichtner-Feigl S. Tumor development in murine ulcerative colitis depends on MyD88 signaling of colonic F4/80+CD11b(high)Gr1(low) macrophages. *The Journal of clinical investigation*. 2011; 121:1692–1708. [PubMed: 21519141]
43. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *The Biochemical journal*. 2003; 374:1–20. [PubMed: 12773095]
44. Waldner MJ, Neurath MF. Master regulator of intestinal disease: IL-6 in chronic inflammation and cancer development. *Seminars in immunology*. 2014; 26:75–79. [PubMed: 24447345]
45. Chalaris A, Schmidt-Arras D, Yamamoto K, Rose-John S. Interleukin-6 trans-signaling and colonic cancer associated with inflammatory bowel disease. *Dig Dis*. 2012; 30:492–499. [PubMed: 23108305]
46. Iliopoulos D, Hirsch HA, Struhl K. An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell*. 2009; 139:693–706. [PubMed: 19878981]
47. Putoczki TL, Thiem S, Loving A, Busuttill RA, Wilson NJ, Ziegler PK, Nguyen PM, Preaudet A, Farid R, Edwards KM, Boglev Y, Luwor RB, Jarnicki A, Horst D, Boussioutas A, Heath JK, Sieber OM, Pleines I, Kile BT, Nash A, Greten FR, McKenzie BS, Ernst M. Interleukin-11 is the dominant IL-6 family cytokine during gastrointestinal tumorigenesis and can be targeted therapeutically. *Cancer cell*. 2013; 24:257–271. [PubMed: 23948300]
48. Ernst M, Najdovska M, Grail D, Lundgren-May T, Buchert M, Tye H, Matthews VB, Armes J, Bhathal PS, Hughes NR, Marcusson EG, Karras JG, Na S, Sedgwick JD, Hertzog PJ, Jenkins BJ. STAT3 and STAT1 mediate IL-11-dependent and inflammation-associated gastric tumorigenesis in gp130 receptor mutant mice. *The Journal of clinical investigation*. 2008; 118:1727–1738. [PubMed: 18431520]
49. Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, Taniguchi K, Yu GY, Osterreicher CH, Hung KE, Datz C, Feng Y, Fearon ER, Oukka M, Tessarollo L, Coppola V, Yarovinsky F, Cheroutre H, Eckmann L, Trinchieri G, Karin M. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature*. 2012; 491:254–258. [PubMed: 23034650]
50. Dumoutier L, de Meester C, Tavernier J, Renauld JC. New activation modus of STAT3: a tyrosine-less region of the interleukin-22 receptor recruits STAT3 by interacting with its coiled-coil domain. *The Journal of biological chemistry*. 2009; 284:26377–26384. [PubMed: 19632985]
51. Lim C, Savan R. The role of the IL-22/IL-22R1 axis in cancer. *Cytokine & growth factor reviews*. 2014; 25:257–271. [PubMed: 24856143]
52. Kirchberger S, Royston DJ, Boulard O, Thornton E, Franchini F, Szabady RL, Harrison O, Powrie F. Innate lymphoid cells sustain colon cancer through production of interleukin-22 in a mouse model. *The Journal of experimental medicine*. 2013; 210:917–931. [PubMed: 23589566]
53. Wang L, Yi T, Kortylewski M, Pardoll DM, Zeng D, Yu H. IL-17 can promote tumor growth through an IL-6-Stat3 signaling pathway. *The Journal of experimental medicine*. 2009; 206:1457–1464. [PubMed: 19564351]
54. Murray PJ. The JAK-STAT signaling pathway: input and output integration. *J Immunol*. 2007; 178:2623–2629. [PubMed: 17312100]
55. Tanaka T, Kohno H, Suzuki R, Hata K, Sugie S, Niho N, Sakano K, Takahashi M, Wakabayashi K. Dextran sodium sulfate strongly promotes colorectal carcinogenesis in Apc(Min/+) mice: inflammatory stimuli by dextran sodium sulfate results in development of multiple colonic neoplasms. *International journal of cancer. Journal international du cancer*. 2006; 118:25–34. [PubMed: 16049979]

56. Yang L, Pei Z. Bacteria, inflammation, and colon cancer. *World journal of gastroenterology* : WJG. 2006; 12:6741–6746. [PubMed: 17106919]
57. Garrett WS, Punit S, Gallini CA, Michaud M, Zhang D, Sigrist KS, Lord GM, Glickman JN, Glimcher LH. Colitis-associated colorectal cancer driven by T-bet deficiency in dendritic cells. *Cancer cell*. 2009; 16:208–219. [PubMed: 19732721]
58. Garrett WS, Glimcher LH. T-bet^{-/-} RAG2^{-/-} ulcerative colitis: the role of T-bet as a peacekeeper of host-commensal relationships. *Cytokine*. 2009; 48:144–147. [PubMed: 19666230]
59. Dove WF, Clipson L, Gould KA, Luongo C, Marshall DJ, Moser AR, Newton MA, Jacoby RF. Intestinal neoplasia in the ApcMin mouse: independence from the microbial and natural killer (beige locus) status. *Cancer research*. 1997; 57:812–814. [PubMed: 9041176]
60. Im E, Jung J, Pothoulakis C, Rhee SH. Disruption of Pten speeds onset and increases severity of spontaneous colitis in Il10^(-/-) mice. *Gastroenterology*. 2014; 147:667–679. e610. [PubMed: 24882466]
61. Arthur JC, Perez-Chanona E, Muhlbauer M, Tomkovich S, Uronis JM, Fan TJ, Campbell BJ, Abujamel T, Dogan B, Rogers AB, Rhodes JM, Stintzi A, Simpson KW, Hansen JJ, Keku TO, Fodor AA, Jobin C. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science*. 2012; 338:120–123. [PubMed: 22903521]
62. Cuevas-Ramos G, Petit CR, Marcq I, Boury M, Oswald E, Nougayrede JP. *Escherichia coli* induces DNA damage in vivo and triggers genomic instability in mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:11537–11542. [PubMed: 20534522]
63. Nougayrede JP, Homburg S, Taieb F, Boury M, Brzuszkiewicz E, Gottschalk G, Buchrieser C, Hacker J, Dobrindt U, Oswald E. *Escherichia coli* induces DNA double-strand breaks in eukaryotic cells. *Science*. 2006; 313:848–851. [PubMed: 16902142]
64. Barnich N, Carvalho FA, Glasser AL, Darcha C, Jantschkeff P, Allez M, Peeters H, Bommelaer G, Desreumaux P, Colombel JF, Darfeuille-Michaud A. CEACAM6 acts as a receptor for adherent-invasive *E. coli*, supporting ileal mucosa colonization in Crohn disease. *The Journal of clinical investigation*. 2007; 117:1566–1574. [PubMed: 17525800]
65. Barker N, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M, Danenberg E, Clarke AR, Sansom OJ, Clevers H. Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature*. 2009; 457:608–611. [PubMed: 19092804]

Box 1: Outstanding Questions

- How do the panoply of molecular changes in epithelial cells that render these cells cancer prone relate to specific immunologic events?
- How does STAT3 activation initiate molecular mechanisms that tips the balance toward full-blown malignancy and what are these mechanisms?
- Do particular members of the gut microbiome act in the 1st Stage of neoplastic development or do these organisms act only in the 2nd Stage?
- If they act only in the 2nd Stage, by what mechanisms do they induce Stat 3 activation?
- What are the dominant cytokines involved in the 2nd Stage of the various types of neoplastic development and do cytokines interact cooperatively to induce malignant change?
- Do epithelial cells undergoing malignant change engage in cross-talk with the immune system and thus influence the immune response?
- How do developing tumors induce immune responses that prevent or restrict tumor growth?
- How do mechanisms of oncogenesis, particularly those in the 2nd Stage, apply to sporadic tumors that are presumably developing in the absence of immunologic influence?

Highlights

- Two stages of inflammation-associated GI neoplastic development
- Genetic lesions underlie the first stage and determine vulnerability to malignancy
- Inflammatory cytokines contribute to the second stage – frank malignancy
- Cytokine activation of the STAT3 pathway is the gateway to the second phase

Figure 1. 1st Stage Oncogenic Events

The development of malignancy in the gastrointestinal epithelium as a result of chronic intestinal inflammation is usually a two stage process. The first stage is a “stealthy” and relatively prolonged process in which factors in the milieu created by chronic inflammation cause oncogenic “hits” i.e., molecular changes involving tumor suppressor genes or pro-oncogenic genes that result cells vulnerable to immunologic stimulation of signaling pathways that drive frank malignant changes. Inflammatory cytokines such as TNF- α induce down-stream factors such as ROS to cause silent mutational changes in oncogenes such *TP53* and *Kras*. These changes render the cell vulnerable to induction of frank neoplastic changes in the second stage of tumor development. The presence of the *Apc* genetic mutation or the administration of a genotoxic agent such as azoxymethane (AOM) “short-circuit” this initial phase and allow the rapid progress of oncogenesis by immunologic factors.

Figure 2. 2nd Stage Oncogenic Events

Cytokine activation of the STAT3 signaling pathway is the gateway to the second phase of immunologically-mediated oncogenesis. A number of cytokines, most notably IL-6, IL-11 and IL-22 are induced by upstream pro-inflammatory factors and activate the STAT3 signaling pathway. Th17 responses, possibly induced by bacteria in the microbiome are important initiators of IL-6 and IL-22 production. However, there is recent evidence that such activation is favored by the presence of particular non-pathogenic members of the microbiome.