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The Gastrointestinal Tumor Microenvironment

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Abstract

Over the past decade, the microenvironment of gastrointestinal tumors has gained increasing attention because it is required for tumor initiation, progression, and metastasis. The tumor microenvironment has many components and has been recognized as one of the major “hallmarks” of epithelial cancers. Although therapeutic strategies for gastrointestinal cancer have previously focused on the epithelial cell compartment, there is increasing interest in reagents that alter the microenvironment, based on reported interactions among gastrointestinal epithelial, stromal, and immune cells during gastrointestinal carcinogenesis. We review the different cellular components of the gastrointestinal tumor microenvironment and their functions in carcinogenesis, and discuss how improving our understanding of the complex stromal network could lead to new therapeutic strategies.

Introduction

Digestive cancers are a significant health care burden worldwide¹. In the US in 2008, it was estimated that more than 270,000 patients were diagnosed with, and more than 135,000 died from, cancers of the digestive system¹. Most of the research and treatment strategies for patients with gastrointestinal (GI) cancers have focused on cell-autonomous mechanisms in the epithelial compartment. However, there is accumulating in vivo evidence that epithelial cells respond to their microenvironment, comprising mesenchymal cells and immune cells, the enteric nervous system, and matrix. The luminal content—particularly the microbiome—

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is another important feature of this complex network; its effects on immunity and tumorigenesis are only beginning to be understood².

Many tumors of the digestive system arise under conditions of chronic inflammation (Fig. 1), including esophageal adenocarcinoma (from Barrett's esophagus), gastric cancer (from *Helicobacter pylori*-associated gastritis), hepatocellular cancer (from viral hepatitis), colon cancer (from inflammatory bowel disease), and perhaps even pancreatic cancer (from chronic pancreatitis)³. Furthermore, eradication of infectious agents (such as *H. pylori*) and treatment with anti-inflammatory therapies (such as prostaglandin synthase inhibitors or mesalazine) prevent cancer^{4,5}. Tumorigenesis under conditions of chronic inflammation is likely to be mediated by immune cells and the factors they produce, which alter the microenvironment to support tumor formation and progression⁶. Importantly, sporadic tumors, which do not develop as a direct consequence of chronic inflammation, are also characterized by an inflammatory microenvironment.

Not all tumor-associated inflammation supports tumor formation—the adaptive immune response has effective roles in immune surveillance and tumor suppression⁷. Nevertheless, it appears that chronic overt and/or low-level inflammation more commonly stimulates tumorigenesis rather than prevents it. Cytokines and growth factors can promote tumor cell proliferation, inhibit apoptosis, and suppress anti-tumor immunity; matrix metalloproteases (MMPs) and angiogenic factors contribute to invasion, metastasis, and tumor blood vessel formation. So it appears that an altered epithelium itself alters the surrounding stroma, and with it the mediators that ultimately create a tumorigenic microenvironment. To design better surveillance strategies and therapeutics, it is important to understand the function and contribution of diverse stromal cell types to the tumor microenvironment.

Cell Types in the Tumor Microenvironment

Although it is not clear whether inflammation precedes or is activated by tumorigenesis, the stroma of GI tumors is infiltrated by a range of bone marrow-derived cells that contribute to an expanding stroma (Fig. 2). The inflammatory environment contains cells of the innate immune system such as tumor-associated macrophages (TAMs), neutrophil granulocytes, myeloid-derived suppressor cells (MDSCs) or immature myeloid cells (iMCs), mast cells (MCs), and dendritic cells (DCs). It also contains cells of the adaptive immune system, such as T and B cells⁸ (Fig. 1). The complex network of immune cells in the tumor stroma affects almost every aspect of tumor biology¹⁰.

Innate and adaptive immune cells can promote tumorigenesis. Innate immune cells, such as TAMs, MCs, neutrophils, and MDSCs, promote tumor development, whereas subsets of adaptive immune cells, such as T-helper (Th)17 and Th2 cells, also contribute to inflammation and can promote tumorigenesis. The tumorigenic properties of these cells require their production of cytokines, growth factors, enzymes, and angiogenic mediators. Immune cells recruited to the tumors not only promote proliferation and invasion, but are also required for anti-tumor immune responses.

The density and location of T cells in colon tumors are associated with patient outcomes; they could be better prognostic factors than histology-based TNM classification^{9,10}. High

densities of cytotoxic and memory T cells in the tumor center and at invasion fronts are associated with reduced recurrence of disease and are positive prognostic factors⁹ for patients with colon cancer and even those with liver metastases. Moreover, the composition of the immune infiltrate in metastases has been associated with patients' response to therapy¹¹.

These observations have led to the idea of an 'immune score' that quantifies the intra-tumor location and density of cytotoxic and memory T cells. Because of its powerful prognostic value, it has been proposed that the immune-score could be incorporated into the routine diagnostic and prognostic assessment of patients with tumors¹². Furthermore, immune-based therapies might be developed that disrupt tumor-induced immune suppression and allow induction of anti-tumor immune responses¹³. So far, the precise mechanism of differential accumulation of T-cell subsets is only partially understood. However, elucidating the regulation of the adaptive T-cell response would have great therapeutic potential.

TAMs are a heterogeneous and plastic population of immune cells; large numbers of these cells in tumors indicate a poor prognosis¹⁴. Accordingly, depletion of macrophages from Apc⁷¹⁶ mice resulted reduced intestinal polyposis¹⁵. Analogous to the Th1 and Th2 classification of T cells, macrophages can be categorized as classically activated M1 or alternatively activated M2 macrophages in vitro. M1 macrophages are induced by interferon- γ and microbial products, produce high levels of inflammatory cytokines, express MHC II molecules, and have cytotoxic activity⁸. M2 macrophages express high levels of the immunosuppressive cytokine interleukin (IL)10, and have reduced antigen presentation and tumoricidal activities but increased tissue remodeling capacities than M1 macrophages. Macrophages accumulate in the stroma of solid tumors, are considered to have a tumorigenic M2 phenotype, and are involved in almost every aspect of tumor biology¹⁶. However, tumor infiltration by M1 macrophages has been correlated with better outcomes of patients with colorectal cancer (CRC), in a stage-dependent manner, but is also accompanied by a concomitant increase in M2 macrophages¹⁷. However, TAMs have partially overlapping cytokine profiles in GI tumors, so it is not possible to distinguish between M1 and M2 macrophages in vivo. The classification of M1s as anti-tumorigenic and M2s as tumorigenic is therefore an oversimplification of the real phenotypic diversity of the macrophage population in vivo.

MDSCs are CD11b⁺Gr-1⁺ (Ly6G⁺Ly6C^{high}), and are a heterogeneous population of activated iMCs that is expanded in tumor-bearing mice and cancer patients¹⁸. iMCs can be detected in the bone marrow and occasionally in the spleen of cancer-free animals¹⁹, but accumulate in the spleen in animals with chronic inflammation and in response to carcinogens. In the setting of carcinogenesis, iMCs acquire suppressor properties and become MDSCs²⁰. In particular, in models of inflammation-induced gastric and esophageal cancer, MDSCs appear to mediate carcinogenesis^{21,22}. Within the tumor microenvironment, MDSCs suppress anti-tumor immunity by disrupting T-cell functions and promoting differentiation of the immunosuppressive regulatory T (Treg) cells²³.

Beside their well-recognized role in suppressing cytotoxic T cells, MDSCs can also directly promote tumor progression and metastasis. Cis-Apc/Smad4 mice develop invasive colon

tumors that are characterized by strong myeloid cell infiltration specifically at the invasion fronts. iMCs recruited to the cis-Apc/Smad4 tumors produce MMP2 and MMP9, which can degrade the extracellular matrix and collagen IV. These enzymes promote its invasion by tumor cells²⁴. Mice with conditional knockout of p120-catenin (p120ctn) develop esophageal squamous cancer, and cytokines upregulated in the dysplastic epithelium promote mobilization and recruitment of MDSCs—a microenvironment that promotes tumor formation²⁵.

Numbers of MCs are increased in intestinal polyps and colorectal tumors from mice and patients, and are likely to contribute to tumor growth^{26,27}. Depletion of MCs from Apc⁴⁶⁸ mice by bone marrow transplantation reduced the number of polyps that formed and the densities of their blood vessels²⁶. So far, however, functional analyses of MCs have been limited to ablation of this lineage, without more direct evidence such as MC-specific genetic pathways.

In addition to innate immune cells, T-cell subsets can also promote development of CRC. Patients with tumors infiltrated by a high number of T cells that produce IL17 (Th17 cells) have a poor prognosis²⁸. These cells are derived from naïve T cells in response to transforming growth factor- β (TGFB)1, IL6 and IL23, which might all be present at high concentrations in the tumor microenvironment, and induce T-cell production of IL17²⁹. IL17 activates expression of genes that mediate the inflammatory response, which promotes tumor growth and angiogenesis. Inhibiting the function of Th17 cells or blocking IL17 in Apc^{Min/+} mice (or mice with intestinal epithelial cell [IEC]-restricted deletion of *Apc*) reduces intestinal tumorigenesis, probably by preventing establishment of an inflammatory microenvironment^{30–32,39}.

Th17 cells produce not only IL17 but also IL22, a member of the IL10 family that activates the transcription factor STAT3³³. Similar to IL17, production of IL22 is induced in response to bacterial products and has an important role in intestinal homeostasis³⁴. Interestingly, IL22, which is also secreted by innate lymphoid cells, has a dual function in colorectal tumorigenesis. In mice with colitis-associated cancer (CAC), IL22 deficiency accelerated inflammation, probably by impairing colonic repair, but increased tumor cell proliferation after the recovery phase³⁵. Similarly, IL4, produced mainly by Th2 cells, contributes to tumor growth by increasing proliferation of colon tumor cells. Mice that lack the IL4R α chain of the IL4 and IL13 receptor complexes develop fewer and smaller tumors³⁶. Moreover IL4 and IL13 promote differentiation of tumor-promoting macrophages, which contribute to tumor formation and progression³⁷. In contrast, tumor infiltration by cytotoxic T cells, memory T cells, and Th1 cells is associated with longer survival times of patients with CRC²⁸.

Treg cells are required for intestinal mucosal immune homeostasis. They suppress adaptive, and to some extent innate, immunity, mainly by producing the anti-inflammatory cytokine IL10. Detection of Treg cells is associated with poor prognosis for patients with some types of cancer, because these cells suppress anti-tumor immunity and allow the tumor to evade the immune response. Surprisingly, for patients with CRC, the presence of Treg cells in tumors is a good prognostic factor^{28, 38}. Tumor development in the colon is accompanied by

the translocation of bacteria, leading to induction of a strong anti-microbial immune response that involves the activation of Th17 cells. Treg cells in the colonic mucosa can inhibit the Th17 cell responses and therefore reduce the tumorigenic effects of inflammation.³⁵ Adoptive transfer of Treg cells suppresses sporadic and inflammation-induced intestinal tumorigenesis, probably because Treg cells can inhibit the inflammatory response^{39, 40}. On the other hand, in the presence of specific cytokines, Treg cells can differentiate Th17 cells. Treg cells present in the polyps of Apc⁴⁶⁸ mice do not produce IL10 but rather IL17 and are not able to suppress CD4⁺ T cells⁴⁰. Moreover, IL10 activates STAT3, which upregulates several genes involved in proliferation and survival⁴¹. Given the different prognostic value of Treg cells for patients with different types of cancers, and their ability to simultaneously suppress tumor-promoting inflammation and anti-tumor immunity, it is important to develop a better understanding of the roles of Treg cells in CRC pathogenesis.

The immune response determines the progression of gastric atrophy, metaplasia, and dysplasia, because Th1 cells produce specific cytokines in response to infection with *H. pylori*. These include IL1, tumor necrosis factor (TNF)- α , and IL10, which cause chronic inflammation and increase the risk for cancer progression⁴². However, production of individual cytokines could affect predisposition to gastric cancer. During infection with *helicobacter*, the Th1 cytokine interferon- γ can promote or inhibit inflammation-induced gastric cancer, indicating that a more specific immune response mediates cancer surveillance or promotion. Although previous studies have reported that interferon- γ might promote the development of gastric preneoplasia⁵³, overexpression of low levels of interferon- γ in the stomach was recently shown to prevent IL1B- and *Helicobacter felis*-dependent carcinogenesis⁴³. In addition, interferon- γ counteracted the development of Th17 cells. So, different composition of the same cells and cytokines in the tumor microenvironment can promote or inhibit carcinogenesis.

Apart from cancer cells and infiltrating immune cells, the stromal environment of tumors includes a mixture of mesenchymal cells, comprising mostly cancer-associated fibroblasts (CAFs). CAFs closely resemble normal myofibroblasts in the GI mucosa. However, CAFs behave differently from myofibroblasts and fibroblasts of normal tissues; based on clinical and preclinical studies, these stromal cells contribute to the development and metastasis of GI cancers^{44, 45}. Patients with tumors surrounded by desmoplasia, which contains many CAFs, have a worse prognosis than patients with tumors that do not contain desmoplasia⁴⁶. A number of studies have tried to determine the origin of CAFs, investigating resident fibroblasts⁴⁹, smooth muscle cells, endothelial cells (via endothelial-mesenchymal transition [EndMT]), epithelial cells (via epithelial-mesenchymal transition [EMT]), fibrocytes, and recruited bone marrow-derived cells such as mesenchymal stem cells (MSCs)^{47, 48}. MSCs are a minor population of multi-potent, non-hematopoietic cells in the bone marrow that are actively recruited to the sites of inflammation and injury⁴⁹. The differentiation of MSCs into CAFs or myofibroblasts has been analyzed; a significant portion of CAFs were found to originate from the bone marrow-derived MSCs,⁴⁴ and contribute to the normal bone marrow MSC niche and to MSC self-renewal⁴⁴. These bone marrow niche cells are expanded in a

TGFB-dependent manner and recruited through CXCR4 and CXCL12 signaling, together with MSC, to incipient tumors where they appear as CAFs.

CAFs contribute to tumorigenesis by promoting cancer cell proliferation and invasion. CAFs express a number of tumorigenic factors, including IL11 (which activates STAT3), hepatocyte growth factor (HGF) and fibroblast growth factor (FGF), which activate mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)^{50, 51}. Under normal conditions, HGF release is suppressed by the serine-threonine kinase tumor progression locus-2 (Tpl2). Deletion of Tpl2 specifically from intestinal myofibroblasts leads to increased production of stromal HGF, resulting in phosphorylation of c-Met and activation of AKT in tumor cells. Tpl2-deficient mice given azoxymethane and dextran sodium sulphate to induce CAC develop more and larger tumors than wild-type mice⁵². Increased production of HGF by the tumor microenvironment reduces the sensitivity of melanoma and colon cancer cells to Raf inhibitors by activating c-Met receptors on the tumor cells, supporting the concept that the tumor microenvironment is an important determinant of response to therapy^{53, 54}. Tumor-associated fibroblasts contribute to colorectal tumorigenesis not only by direct signaling to cancer cells, but also indirectly, by recruiting and polarizing cells of the adaptive and innate immune systems to tumor-promoting phenotypes⁵⁵.

The tumor vasculature is another important component of GI tumor microenvironment. Tumors must form new blood vessels to maintain a supply of oxygen and nutrients needed for growth. To achieve sufficient perfusion of the neoplastic tissue, tumor cells and stromal cells produce several factors that induce the formation of new blood vessels⁵⁶. The tumor vasculature is involved in many different processes that promote tumor growth. Activated endothelial cells of the tumor vasculature can recruit inflammatory cells and endothelial progenitors, contributing to tumorigenic inflammation and neoangiogenesis. Furthermore, tumor endothelial cells secrete many paracrine factors that directly foster tumor cell proliferation and maintain cancer stem cells⁵⁷.

The tumor vasculature is also required for metastasis. Newly formed tumor vessels are usually abnormal in structure, which enhances the intravasation of cancer cells⁵⁸. Endothelial cells also produce chemokines that recruit myeloid cells to metastatic sites, which contribute to a hostile microenvironment at the metastases⁵⁹. CCR2 signaling in endothelial cells in response to cancer cell-derived CCL2 alters vascular permeability, promoting extravasation of cancer cells into new tissues and metastasis⁶⁰.

Nerves are an important component of normal GI physiology and could also be involved in malignancies. In addition to regulating GI motility, neural input regulates proliferation in the GI epithelium and there is evidence that the nervous system could regulate stem cell renewal⁶¹. The nervous system was shown to regulate interactions between hematopoietic stem cells and their niche⁶⁶, and there is evidence that intestinal epithelial stem and progenitor cells are controlled by mucosal afferent nerves⁶¹. Preneoplastic and neoplastic tissues induce neurogenesis⁶². However, the exact role and function of nerves in normal GI homeostasis and during carcinogenesis requires further investigation.

Signaling Pathways Activated by Infiltrating Cells

The types and quantities of immune cells recruited to GI sites, along with the cytokines and growth factors they produce, determine whether the tissue environment permits tumor formation (see Fig. 2, Table 1). These factors act in paracrine, autocrine, and juxtacrine manners to activate signaling pathways that converge at 'hubs' in cancer and stromal cells. Several of the central signaling pathways have been extensively studied and their hubs have been determined. This comprehensive knowledge led to the idea of targeting therapies to the hubs, to block multiple pathways required for GI tumor growth and progression. The same signaling pathways that are required for normal biological processes and maintenance of GI homeostasis are also involved in GI carcinogenesis. These include the Wnt, Notch, TGFB, and Hedgehog signaling pathways, which are required for the self renewal of the intestine and stem cell maintenance; the transcription factors NF κ B and STAT3, which are required for tissue repair and immune homeostasis; toll-like receptor (TLR) signaling, which regulates tolerance vs an immunity against the intestinal microbiota; and growth factor signaling pathways, such those that include MAPK or Akt/PKB, which provide mitogenic and survival signals.

NF κ B regulates genes that control many physiologic and cellular processes, such as survival, apoptosis, immunity, and inflammation, but NF κ B also promotes tumorigenesis⁶³ via distinct mechanisms. In epithelial tissues, inflammation increases survival of initiated epithelial cells by activating NF κ B, which leads to transcription of genes that promote cell survival and prevent apoptosis^{64,65}. Interestingly, constitutive activation of NF κ B in epithelial cells only leads to destructive forms of inflammation when it is accompanied by MAPK activation, induced by microbial stimuli⁶⁶. Furthermore, persistent activation of NF κ B in IECs upregulates inducible nitric oxide synthase, which can lead to DNA damage and loss of heterozygosity to promote transformation⁶⁷. NF κ B contributes to inflammation-induced EMT by inducing the COP9 signalosome, which blocks the ubiquitination and subsequent degradation of the transcription factor Snail⁶⁸. In the myeloid cells, NF κ B controls the production of inflammatory cytokines such as IL6, IL11, and TNF α . These cytokines in turn activate proliferation and survival pathways in epithelial cells, via NF κ B and STAT3^{64, 65}. Blocking the activity of NF κ B in myeloid cells of mice reduces the number and size of colitis-associated tumors⁶⁴

STAT3 regulates genes involved in the process of wound healing, and like NF κ B, controls survival and proliferation of epithelial cells. Inhibition of STAT3 in epithelial tissues prevents azoxymethane-induced formation of adenomas in mice, but accelerates inflammation, due to reduced expression of genes that promote cell survival and prevent cell death^{69, 70}. Conversely, mice that express a mutant form of the gp130 chain (the signal transducing subunit of the IL6-type cytokine receptor), which increases IL11-induced activation of STAT3, spontaneously develop gastric polyps^{71, 72}. Similarly, in mice with CAC, mutations in gp130 increase STAT3 activation by IL6 and IL11, which protects against epithelial damage and inflammation from dextran sodium sulphate but accelerates tumorigenesis⁶⁹. In gastric cancer cells, STAT3 activates expression of TLR2; inhibitors of TLR2 prevent gastric tumorigenesis, but not inflammation, indicating its role in the oncogenic functions of STAT3⁷³.

STAT3 not only affects proliferation and survival of epithelial cells, but also controls expression of inflammatory cytokines and increases production of immunosuppressive factors that can inhibit DC maturation by activating STAT3 in progenitor cells⁷⁴. STAT3 activation in immune cells that infiltrate tumors promotes development of the tumor. In TAMs, STAT3 activates transcription of IL23, but in DCs it inhibits NFκB-dependent expression of the anti-tumor cytokine IL12⁷⁵. IL23 produced by macrophages subsequently activates STAT3 in IL23R-expressing regulatory T cells, leading to upregulation of the immunosuppressive cytokine IL10, which also activates STAT3 and can contribute to immune evasion. IL23 and STAT3 have each been implicated in the generation and expansion of the tumorigenic Th17 cells and production of IL17^{31, 32}. The effects and regulation of STAT3 and NFκB are connected at many levels; they regulate each other in paracrine and cell-autonomous manners. Unphosphorylated STAT3, together with NFκB, promotes transcription of NFκB-regulated genes by mediating acetylation of RelA, which retains NFκB in the nucleus^{76, 77}. Interestingly, STAT3 activation also interferes with TGFβ signaling, by inducing transcription of the TGFβ signaling antagonist Smad7⁷¹.

TGFβ signaling has dual roles in tumor development—it has pro- and anti-carcinogenic activities⁷⁸. TGFβ signaling in the T cells suppresses inflammation and therefore can inhibit tumor formation. Transgenic mice with Smad4 deficiency in the T cells develop spontaneous GI tumors, produce increased levels of inflammatory cytokines, and have higher concentrations of Th17 cells^{79, 80}. On the other hand, TGFβ-mediated immune suppression can lead to immune evasion and reduce anti-tumor immunity. Mice with a transgene that overexpresses Smad7 in T cells develop severe colitis, but have increased anti-tumor immunity and develop fewer tumors⁸¹.

TGFβ signaling is also important in other stromal cells, such as fibroblasts. The loss of responsiveness of fibroblasts to TGFβ leads to development of invasive squamous cell carcinoma of the forestomach, associated with increased production of HGF and epithelial cell proliferation. These findings indicate that under normal conditions, HGF production is negatively regulated by TGFβ in fibroblasts⁸². Beside the important role of TGFβ signaling in the immune cells, TGFβ signaling by epithelial cells regulates tumor progression. Loss of epithelial TGFβ signaling results in increased chemokine production and recruitment of MMP-producing iMCs to the tumor margins, where they promote invasion⁸³. Interestingly, however, platelet-derived TGFβ, which synergistically activates TGFβ signaling to Smad and NFκB in cancer cells, promotes metastasis by inducing an invasive mesenchymal-like phenotype⁸⁴.

Consistent with the observations from mouse models that alterations in TGFβ activity affect tumor development, alterations in TGFβ signaling are often found in human colorectal tumors. Many GI tumors, including colorectal tumors, have decreased expression or loss of the TGFβ receptor (TGFBR). Moreover, mutations in TGFBR2 or in Smad4, and a lesser extent in Smad2, are present in a considerable proportion of human colonic tumors⁷⁸.

Tumor development is associated with tissue rupture and translocation of bacteria, so signaling pathways that are activated in response to microbial stimuli might be involved in GI tumorigenesis. In the tumor microenvironment, bacteria and their products are sensed via

pattern recognition receptors (PRRs), such as TLRs and Nod-like receptors (TLRs and NLRs). The ability of the human body to discriminate between useful commensals and pathogenic bacteria largely depends on the function of the PRRs. TLRs are expressed in almost every cell in the intestine and signal via MAPK and NF κ B, with the help of several adaptor proteins, such as MyD88. Activation of these pathways leads to the production of chemokines, inflammatory cytokines, and anti-microbial peptides⁸⁵.

Preventing TLR signaling by deleting the adaptor protein MyD88 reduces tumor growth and tumor number in Apc^{Min+/-} mice⁸⁶. However, MyD88-deficient mice develop severe inflammation and have accelerated growth of colitis induced tumors⁸⁷. These seemingly contradictory data indicate that MyD88 signaling has distinct roles in inflammation-induced intestinal tumorigenesis and in sporadic or familial cancers that have no obvious link to inflammation. During inflammation, MyD88 signaling is likely to be indispensable for efficient tissue repair; the lack of Myd88 signaling results in accelerated inflammation and increased tumor development. In sporadic or familial CRC, however, MyD88 might contribute to tumor growth by inducing an inappropriate tissue repair response, leading to tumor cell proliferation and increased tumor growth. Interestingly, mice with disruptions in IL18 signaling, which is mediated by the inflammasome, are similar to Myd88-deficient mice in their susceptibility to CAC⁸⁷. In addition, deficiencies in components of the inflammasome, such as Nlrp3, Nlrp6, caspase-1, and ASC, reduce levels of IL18 and accelerate inflammation-associated tumorigenesis, leading to a phenotype similar to that observed in IL18-deficient mice⁸⁸. Moreover, it was recently shown that IL18 is required for downregulation of IL22 binding protein (IL22BP)³⁵. IL22BP is a soluble, high-affinity receptor for IL22 that limits the availability of IL22 under steady-state conditions⁸⁹. However, upon epithelial damage, IL22BP is downregulated³⁵, allowing the binding of IL22 to its transmembrane receptor and induction of the tissue repair process. So lack of IL22, via reduced levels of IL18 and dysregulation of inflammasome signaling, results in more-pronounced inflammation and subsequently to accelerated tumor development.

The Tumor Microenvironment is a Niche for GI Tumor Stem Cells

The homeostasis of the untransformed stem cell niche depends on its microenvironment (Fig. 3). Normal intestinal stem cells (ISC) reside at the bottom of the crypt and depend on adhesive and soluble stroma–epithelial interactions that create a niche⁹⁰. Genetic lineage-tracing studies in mice identified markers of 2 different types of ISC. Crypt base columnar cells (CBC) are fast-cycling stem cells that express Lgr5 and CD133 (Prom-1)^{91, 92}. Slower cycling cells, which are usually found at the +4 position of the crypt, are characterized by high expression of Bmi1 and Tert^{93, 94}. Although these 2 types of cells are functionally interconnected⁹⁵, their exact hierarchical relationship has not been determined.

Regardless of their location (CBC or +4 position) or their function, ISCs depend on signals from stem cell niche cells such as pericryptal myofibroblasts and neighboring differentiated epithelia⁹⁶. Important signaling pathways required for ISC maintenance and proliferation comprise the Wnt, Notch, Bone morphogenetic protein, and Hedgehog pathways⁹⁰. Several markers of CRC stem cells (CSCs) have been proposed, such as CD24, CD44, CD133, and CD166^{97–99}. However, a robust marker of CSCs has not been identified. For example,

CD133 is ubiquitously expressed in differentiated murine and human colonic epithelium¹⁰⁰ and therefore not specific for either multi-potent stem cells or progenitor cells. Interestingly, doublecortin-like kinase 1 (Dclk1) could be a valid marker of CSCs—it is detected in potential cancer stem cells but not normal stem cells¹⁰¹.

Although a specific niche for colon CSCs has not been defined, a microenvironment that maintains tumor-initiating cells is likely to exist. Wnt signaling is required by tumor initiating cells and recently ISC (Lgr5⁺)-specific negative feedback regulators have been identified¹⁰² (Fig. 3). Moreover, secretion of HGF by stromal fibroblasts increases Wnt signaling in tumor-initiating cells,¹⁰³ and a higher proportion of tumor cells within myofibroblast regions (compared with other regions) have nuclear β -catenin¹⁰⁴. Importantly, TNF α -dependent activation of NF κ B can increase β -catenin signaling and transcription of the stem cell transcriptome,¹⁰⁵ resulting in expansion of Lgr5⁺ stem cells¹⁰⁶. More importantly, in post-mitotic epithelial cells, the interacting effects of NF κ B and β -catenin activation induce dedifferentiation, or reprogramming of non-stem cells into tumor-initiating stem cells (Fig. 3).

The capacity to initiate tumorigenesis is therefore no longer an exclusive property of normal stem cells. Instead, an inflammatory microenvironment (such as in the intestines of patients with inflammatory bowel diseases) can lead to the conversion of normal, differentiated epithelia into CSCs. However, this concept also applies to established tumors, and to therapies that aim to eradicate CSCs by depleting, for example, Dclk1⁺ cells¹⁰¹. The inflammatory microenvironment of advanced tumors could allow the dedifferentiation of tumor cells into CSCs, to replenish eliminated stem cells.

Additional evidence for the interaction between the inflammatory microenvironment and CSC comes from a transgenic mouse model of Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC)²¹. Bile acid-induced IL1 β - and IL6-dependent inflammation activated formation of gastric cardia stem cells, which migrated into the distal esophagus and gave rise to columnar-like metaplasia and dysplasia. These findings indicate that BE and EAC can arise from gastric progenitors²¹ (Fig. 3). So, in addition to upregulating reactive oxygen and nitrogen species that can lead to DNA damage and mutations in CSCs, an inflammatory microenvironment is also required for formation and maintenance of a CSC niche. It will be a challenge to further define and characterize this specific niche, but reagents designed to target it could have great therapeutic potential.

Microbiota Contribute to the Tumor Microenvironment

The composition of the human microbiome has been associated with the incidence of a number of human diseases, including cancer¹⁰⁷, and is therefore another important compartment of the tumor microenvironment (Fig. 4). A number of individual bacterial species can initiate and promote intestinal tumorigenesis in animal models. However, it is not known whether one particular species is primarily responsible for inducing GI cancers, or whether a small community of different species contributes to transformation.

H. pylori infection is the best-described bacterial risk factor for gastric cancer. However, dysplasia and cancer tend to develop when *H. pylori* colonization has either been greatly

reduced or, in some cases, disappeared from the stomach altogether. Gastric cancer almost always occurs in the setting of prolonged gastric atrophy and hypochlorhydria, a condition that predisposes to enteric bacterial overgrowth. Although antibiotic therapy to eradicate *H pylori* delays and inhibits development of gastric cancer in mice¹⁰⁸, antibiotics eradicate not only *H pylori* but also other microorganisms that colonize the atrophic, hypochlorhydric stomach. Monoassociation of otherwise germ-free INS-GAS mice with *H pylori* delayed development of gastric cancer, compared to *H pylori*-infected INS-GAS mice colonized with conventional flora¹⁰⁹. *H pylori* could therefore be the initial, or the most prevalent, microbial factor responsible for gastric cancer development.

It is important to characterize the bacteria associated with CRC, which might help to identify individuals at increased risk of CRC and develop new therapeutic approaches. However, more-sensitive techniques and bigger cohorts will likely be required to precisely identify the species specifically associated with CRC. Furthermore, because the composition of the CRC-associated microbiota might change as the tumor progresses, tumor-stage specific definition of CRC-associated bacteria should be also considered. A recent analysis of frequencies of molecular features (CpG island methylator phenotype [CIMP-high], microsatellite instability [MSI-high], and BRAF mutation) that increase linearly from the rectum to the ascending colon according to a continuum model¹¹⁰, indicate a continuous change of the microbiota in from proximal to distal colon.

With the advent of high-throughput sequencing techniques, the possibility of comprehensively mapping dysbiosis associated with human CRC has become feasible. Studies have revealed considerable changes in the composition of the microbiota in patients with CRC, and have shown that different species of bacterial tend to colonize either the tumor tissue or the adjacent healthy mucosa. One of the most striking findings was the association of *Fusobacterium spp.* with CRC tissue¹¹¹. Interestingly, opportunistic pathogens belonging to the family *Enterobacteriaceae*, such as *Shigella*, *Salmonella*, *Cronobacter*, and *Citrobacter* are more abundant in the adjacent normal tissue than in tumor tissue¹¹¹. Consistent with these findings, the composition of intestinal microbiota of CRC patients is enriched in pathogenic bacteria, compared to healthy controls. Conversely, *Roseburia* and other butyrate-producing members of the *Lachnospiraceae* family are less abundant in the gut microbiome of CRC patients¹¹².

Determining exactly how these bacteria initiate or influence tumorigenesis remains an important objective for future research (Fig. 5). One tumor-promoting property of certain colonic bacteria involves the release of bacterial toxins that induce colitis and formation of colonic tumors by a Th17-cell-mediated response in mice with multiple intestinal neoplasia¹¹³. An alternative mechanism comprises the propagation of specific genotoxic bacterial strains¹¹⁴. In *Il10*-deficient mice, inflammation creates an environment that supports carcinogenesis via its effects on the mice and their microbiota; it promotes expansion of *E coli* strains that contain the polyketide synthase (pks) genomic island, which encodes the bacterial genotoxin colibactin¹¹⁵. These *E coli* adhere to the colonic mucosa, resulting in increased delivery of pks products, which leads to epithelial DNA damage¹¹⁶.

Importantly, in addition to direct genotoxic effects on the intestinal epithelium, an intestinal barrier defect can lead to translocation of non-pathogenic bacteria, which affects intestinal immune system homeostasis by shifting the balance towards tumorigenic immune responses. Bacterial products are sensed via PRRs such as TLRs and NLRs. Activation of these pathways leads to the production of chemokines, inflammatory cytokines, and anti-microbial peptides⁸⁵. The barrier defect is induced by Wnt activation, causing aberrant expression and mislocalization of tight junction proteins, including occludin and various claudins in epithelial cells, as well as concurrent downregulation of protective mucins. During early stages of tumorigenesis, independent of the composition of the microbiome, bacterial invasion induces a complex inflammatory reaction that involves upregulation of IL17 and IL23, which promote tumor development³². During later stages, loss of p53 function in mutagenized epithelia aggravates this barrier defect further and amplifies the generation of an inflammatory microenvironment that involves activation of NF κ B and STAT3 in tumor epithelia and myeloid cells. Collective activation of these signaling pathways in epithelial and myeloid cells supports development of the EMT in p53-deficient tumor cells, which is required for invasion and metastasis¹¹⁷.

Therapeutic Outlook

There is accumulating evidence for the importance of the tumor microenvironment, with its complex network of cells, cytokines, and chemokines, in tumor initiation, formation, growth, and metastasis. The distinct cell types and signaling pathways have important influences on stem cell biology, malignant transformation, and the microbiome. It seems essential to gain a better understanding of the interaction between stromal cells and epithelial cells, and the specific signals of this network that promote or inhibit tumorigenesis, to develop new therapeutic options for GI cancers. The overarching goal should be to identify factors (cytokines, chemokines, bacteria) that help activate an inflammatory and tumor-promoting microenvironment; disrupting these could make cancer cells more susceptible to direct cytotoxic therapies. Although a number of existing treatments already target broadly some stromal components, major advances in stromal-specific therapies are still required.

Considering the strong association of GI cancers with inflammatory conditions, and the remarkable influence of inflammation on tumor development, it is plausible that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) might be used in prevention and treatment⁴¹¹⁸. Aspirin use after cancer diagnosis can affect patient survival, depending on the presence or absence of *PIK3CA* mutations in tumor cells¹¹⁹. Considering a gradual transition of intestinal biogeography, as proposed in the colorectal continuum hypothesis¹¹⁰, aspirin might have different effects on tumors that develop at specific sites or have different molecular features. Nevertheless, the finding that aspirin-induced alteration of the inflammatory response can determine patients' prognoses, along with specific cancer-cell mutations, indicates the promise of reagents designed to target the tumor microenvironment.

Anti-angiogenic therapies such as bevacizumab (a monoclonal antibody against vascular endothelial growth factor [VEGF]) are well-established class of anti-stromal therapies, but a true therapeutic benefit is lacking. Antibodies against VEGF and endothelial growth factor

have not been translated to the adjuvant therapy setting¹²⁰, although a survival benefit was previously observed with bevacizumab in metastatic CRC¹²¹, leading to approval by the FDA in this setting. Anti-angiogenic agents seems to slow tumor growth and progression but not prevent formation of new metastases; this might result from the complicated relationships among blood flow, hypoxia, and tumor growth.

Treating or preventing metastasis or cancer recurrence likely requires targeting of other factors within the tumor microenvironment or stem cell niche. For example, the Dnmt1 inhibitor decitabine and NSAIDs are thought to have therapeutic effects on the stroma. Folate was found to be chemopreventive agent in a mouse model of inflammatory gastric cancer, via its effects on CAF DNA methylation and biology¹²², as well as in human CRC. However, epigenetic modifiers also affect epithelial cells, so it is difficult to attribute their cancer prevention effects entirely to the stroma; their potential for side effects limit their use in the clinic.

Several therapeutic agents that are already in clinical use can increase anti-tumor immune responses and impair tumor-induced immune suppression. Treatment with sunitinib, a multi-target inhibitor of receptor tyrosine kinases, in combination with immunotherapy, decreased the number of MDSCs and Treg cells, increased intra-tumoral infiltration by tumor-specific T cells, reduced tumor volumes, and increased survival of tumor-bearing mice¹²³. Furthermore, a general goal of most therapies is to prevent metastasis. Analyses of signaling events in CRC cells might identify tumors that are most likely to metastasize. Specifically, stromal TGFB expression in the tumor, both at the primary site and at a distant site were associated with metastatic potential. This observation addresses the paradox that there are high levels of TGFB within tumors, yet mutational inactivation of TGFB signaling in tumor cells. Inhibition of TGFB might therefore prevent CRC metastasis¹²⁴.

It could be possible to target other specific proteins or cell types in the tumor microenvironment. Genetic disruption or pharmacological inhibition of the fibroblast activation protein, which is exclusively expressed on tumor-associated fibroblasts and pericytes in most human epithelial cancers, impairs the growth of subcutaneously transplanted tumors and reduces tumor cell proliferation in mice¹²⁵. However, this approach is likely to cause side effects, because there are few specific markers of these cells. It is therefore necessary to identify new targets in the tumor microenvironment.

The effect of the host immune system on the outcome and response to therapy has led to the idea of an immune score that could be incorporated (by an international task force) into the routine diagnostic and prognostic assessment of patients with cancer¹². The goal should be to characterize the distinct populations of immune cells (beyond T cells) and other stromal cells, to analyze their associations with prognosis and response to therapy, and to incorporate this information with TNM classification. Such an immune score still needs to be proven feasible and then independently validated, to determine its true prognostic value for patients with GI cancers. Nonetheless, the host immune response, as well as increased numbers of lymph nodes, are associated with patient prognosis. Comprehensive assessments of patients' immune response, disease stage, lymph node count and molecular features of tumors are necessary for complete evaluation¹²⁶.

To improve surveillance of patients with intestinal polyps, intestinal metaplasia in the stomach, or BE, it will be important to define the bacterial factors that contribute to tumor formation and progression of preneoplastic lesions to cancer. It is not clear why only a small percentage of patients with inflammatory disorders such as BE or *H pylori* infection ultimately develop EAC or gastric cancer. Improving our understanding of the microbiome could provide entirely new therapeutic strategies for GI inflammation and cancer.

Many efforts are being made to develop new chemotherapeutic drugs that can specifically target CSCs. In this regard the discovery of the druggable kinase Dclk1 as a marker of CSCs might allow us to prevent tumor formation or progression without affecting normal tissue stem cells¹⁰¹. It remains to be determined if targeting only CSC is sufficient to inhibit tumor recurrence or metastasis, or if CSCs develop from normal stem cells. Therefore, it will be important to develop strategies to disrupt stromal pathways important for normal stem cell and CSC maintenance and homeostasis, such as the Wnt, Notch, and Hedgehog pathways¹²⁷. These could induce terminal differentiation, deprive CSCs of their self-renew capacity, or inhibit dedifferentiation.

We are likely to see many new approaches to alter tumor microenvironment and the inflammatory niche that supports tumor growth. It should be a priority to translate our accumulating knowledge of the tumor microenvironment into clinical trials, such as those that combine drugs that target tumor stroma with conventional chemotherapies.

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Figure 1.

- a) Different cellular mechanisms of inflammation-induced carcinogenesis. External and host factors can create an inflammatory environment in the intestine that induces DNA damage and leads to genetic alterations over chronic injury. During carcinogenesis, different myeloid cells, T cells, and CAFs (see Figure 2) are recruited to sites of inflammation and create a microenvironment that promotes epithelial proliferation and prevents apoptosis, due to an imbalance of pro- and anti-tumorigenic factors. A network of multiple cytokines and chemokines generates a niche that enables the appearance and growth of tumor-initiating cells.
- b) Types of cells that are recruited (myeloid cells, T cells, CAFs) or resident (CAF, endothelial cells, nerves) in the tumor microenvironment, and their tumor-promoting or -inhibiting functions. The complex network of signaling among these cell types allows progression from normal to metaplastic to dysplastic gastrointestinal epithelium (carcinogenesis).

Figure 2.

The niche for tumor-initiating cells, or CSCs, is likely formed by different types of cells in the tumor microenvironment that produce tumorigenic cytokines and chemokines. These create a proliferative environment that allows tumor-initiating cells to escape apoptosis and proliferate despite acquired genetic alterations. It is likely that the epigenetic regulation of epithelial cells, and their signaling pathways, is altered by the niche cells. Moreover, the microbiota on the luminal side of the epithelium also contributes to the niche and is responsible for barrier defects and maintenance of chronic inflammation.

Figure 3.

Potential mechanisms of tumor initiation. A) the normal intestinal stem cell niche consists of epithelial stem cells that are surrounded by mesenchymal cells and matrix. A tightly regulated gradient of different signaling molecules determines a stem cell zone, a proliferative zone, and a differentiation zone. The epithelial cells are replaced regularly through renewal of stem and progenitor cells. Three different mechanisms of tumor initiation have been described in the gut: B) Tumor initiation through expansion of stem and progenitor cells at the base of the crypts in the stem cell zone. Deregulation of the signaling network induced by chronic inflammation and the recruitment of different mesenchymal and immune cells allows an expansion of the proliferative zone and provides a niche for tumor initiating cells that derive from tissue stem cells. C) Dedifferentiation of

differentiated epithelial cells at the top of the crypts/villi is induced by chronic inflammation, NF κ B up regulation and genetic alterations of β -catenin or KRas. Normal enterocytes are reprogrammed to tissue stem cells with the ability to proliferate and escape apoptosis despite new genetic mutations. D) In the cardia and esophagus, migration of stem cells towards the inflammatory niche increases proliferation of stem cells that acquire genetic alteration and escape apoptosis in the inflammatory niche the cells migrate to.

Figure 4.

Dysbiosis of the microbiota in the intestine can be induced by 1) hereditary or acquired epithelial mutations that cause barrier defects and impair epithelial cell function, 2) external factors such as dietary changes, toxic agents, environmental conditions that all influence the microbiota and select for a certain type of bacteria, and 3) chronic inflammation, which induces barrier defects and inflammatory responses that select for a specific microbiome.

Figure 5.

The intestinal microbiota contributes to tumorigenesis. In the normal intestine, interactions between bacteria and the epithelial cells, protected by a mucus layer and functioning tight junctions, enable necessary homeostasis. During cancer initiation, dysbiosis and/or a barrier defect contribute to a microenvironment that allows for DNA damage, genetic alterations, and a tumor-promoting signaling network. Under conditions of dysbiosis, some intestinal bacteria release toxins that affect the barrier function (reduced mucus layer, defect tight junctions) of the epithelial cells and might also induce genetic alterations. This induces inflammation and enables migration of bacteria in the stroma, leading to recruitment of more inflammatory cells that finally form a tumorigenic environment that leads to carcinogenesis. The barrier defect can also be caused by inherited or acquired genetic alterations that impair epithelial function and cause dysbiosis. The 2 pathways to an epithelial barrier defect likely account for an inflammatory condition that leads to the formation of a tumor microenvironment, and could function independently or synergistically.

Table 1

Factors that define the tumor microenvironment (related to Fig. 3)

Chemokines	Tumorigenic	Antitumorigenic
Ccl9, Ccl15	Recruitment of iMC ^{83,128}	Modulation of T cell responses against leukemic cells (CML) ¹²⁹
Cxcl1, Cxcl2, Cxcl5	Recruitment of granulocytes ¹³⁰	
Ccl2	Recruitment of macrophages ¹³¹ endothelial cell activation and increased endothelial permeability ⁶⁰	
Cxcl12	Recruitment of MSCs/CAFs ¹³²	
Cx3cl1, Cxcl9, Cxcl10		Recruitment of memory and cytotoxic T cells ¹³³
Ccl5	Recruitment of Th ₁₇ cells ⁵⁵	Recruitment of cytotoxic T cells ¹³⁴
Ccl22	Recruitment of regulatory T cells ¹³⁴	Recruitment of regulatory T cells ¹³⁴
Ccl25		Suppression of invasion and metastasis ¹³⁵
Cytokines		
IL1 β	NF κ B activation, modulation of the Wnt pathway ¹³⁶	
IL4	Tumor cell proliferation ³⁶ ; alternative activation of TAMs ³⁷	
IL6	STAT3 activation, proliferation and survival of colonocytes ⁷⁰ ; induction of Th ₁₇ differentiation	Enhancement of CD8 ⁺ T cell homing by modifying the tumor vasculature ¹³⁷
IL8	MDSC recruitment, myofibroblast expansion, tumor angiogenesis ^{138,139}	
IL10	STAT3 activation, immunosuppression ^{41, 74}	Limiting Th ₁₇ responses ¹⁴⁰
IL17	Inducing expression of inflammatory genes	Mediation of antitumor immunity ¹⁴¹
IL18	Downregulation of IL22BP and therefore increasing tumor cell proliferation ³⁵	Regulation of intestinal microbiota ¹⁴² ; downregulation of IL22BP and therefore reducing inflammation ³⁵ induction of interferon- γ ⁸⁸
IL23	Induction of Th ₁₇ cells, STAT3 activation ^{31, 32}	
IL22	STAT3 activation and tumor cell proliferation ³⁵	Tissue repair upon epithelial injury resulting in the reduction of inflammation, protective in CAC ³⁵
TNF α	NF κ B activation, production of proinflammatory cytokines ^{143, 144} induction of EMT ⁶⁸ ; activation of Notch signaling ¹⁴⁵	
TGFB	Induction of EMT ⁸⁴ ; immunosuppression ⁸¹ ; myofibroblast differentiation and expansion of CAFs ⁴⁴ ; polarization of N2 TANs ¹⁴⁶ and Th ₁₇ differentiation	Negative regulation of HGF production ⁸² ; suppression of chemokine production by epithelial cells ⁸³ and cytokine production by T cells ⁷⁹
Growth factors		
HGF/MET	Modulation of Wnt pathway in CSCs	Promotion of cell death induced by
	¹⁴⁷ ; induction of an migratory phenotype ¹⁴⁸	TRIAL (medulloblastoma) ¹⁴⁹
VEGF/VEGFR	Tumor angiogenesis; recruitment of mesenchymal cells that augment the proliferation of colonocytes ¹⁵⁰ ; tumor cell proliferation ¹⁵¹	
PDGF/PDGFR	Tumor angiogenesis ¹⁵² ; enhancement of lymphangiogenesis and lymphatic metastasis ¹⁵³	Increased pericyte coverage of the tumor vessels, decreased tumor growth ¹⁵⁴

Chemokines	Tumorigenic	Antitumorigenic
EGF/EGFR	Control of proliferation, survival ¹⁵⁵ and invasion ¹⁵⁶	Control of epithelial regeneration and inflammation, protective in CAC ¹⁵⁷
Bacterial products		
Colibactin (<i>E coli</i>)	DNA double strand breaks, mutations, chromosomal aberrations ¹⁵⁸	
BFT (<i>B fragilis</i>)	Cleavage of epithelial E cadherin resulting in increased epithelial barrier permeability and wnt activation ¹⁵⁹	
Superoxid (<i>E feacalis</i>)	DNA double strand breaks ¹⁶⁰	
Butyrate (<i>F prausnitzii</i> , <i>Roseburia spp.</i>)		Induction of apoptosis in colon cancer cell lines ¹⁶¹
ECM components and ECM modifying enzymes		
MMP2, MMP9	Degradation of the basal membrane enhancing invasion ⁸³ , cleavage of ECM bound VEGF	Protective in CAC by modulating Notch activation (MMP9) ¹⁶²
MMP7	Tumor initiation and growth ¹⁶³	
MMP10		Resolution of colonic epithelial damage ²³
TNC	Tumor cell invasion ¹⁴⁸	
LOX	Induction of an more proliferative and invasive phenotype through increasing ECM stiffness ¹⁶⁴ tumor angiogenesis ¹⁶⁵	