

Review

Mechanistic roles of epithelial and immune cell signaling during the development of colitis-associated cancer

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Abstract

To date, substantial evidence has shown a significant association between inflammatory bowel diseases (IBD) and development of colitis-associated cancer (CAC). The incidence/prevalence of IBD is higher in western countries including the US, Australia, and the UK. Although CAC development is generally characterized by stepwise accumulation of genetic as well as epigenetic changes, precise mechanisms of how chronic inflammation leads to the development of CAC are largely unknown. Preceding intestinal inflammation is one of the major influential factors for CAC tumorigenesis. Mucosal immune responses including activation of aberrant signaling pathways both in innate and adaptive immune cells play a pivotal role in CAC. Tumor progression and metastasis are shaped by a tightly controlled tumor microenvironment which is orchestrated by several immune cells and stromal cells including macrophages, neutrophils, dendritic cells, myeloid derived suppressor cells, T cells, and myofibroblasts. In this article, we will discuss the contributing factors of epithelial as well as immune cell signaling in initiation of CAC tumorigenesis and mucosal immune regulatory factors in the colonic tumor microenvironment. In depth understanding of these factors is necessary to develop novel anti-inflammatory and anti-cancer therapies for CAC in the near future.

Keywords: Colitis-associated cancer, Sporadic colon cancer, Intestinal epithelial cells, Mucosal immunity, Tumor microenvironment, and Signaling molecules.

Introduction:

Colorectal cancer is the second leading cause of mortality in the US and the third most common cancer when men and women are considered separately. The American Cancer Society has estimated approximately 93,090 new colon cancer patients in 2015. The association between inflammatory bowel disease (IBD) and colitis-associated cancer (CAC) has been identified about eight decades ago by Drs. Burrill Crohn and

Herman Rosenberg (1). IBD is a group of inflammatory disorders and primarily includes ulcerative colitis (UC) and Crohn's disease (CD). Approximately 1.4 and 2.2 million people are affected with IBD in the US and Europe, respectively (2, 3). CAC contributes to 10-15% of death cases in IBD patients who have long standing (>10 years) and extensive inflammation in the colon. The risk of getting CAC increases by 2%, 8% and 18% with the duration of UC diagnosis for 10, 20 and 30

years, respectively (4). The effect of pro- and anti-inflammatory cytokines on immune and epithelial cells in combination with environmental and genetic factors results in CAC. Our current understanding of the pathogenesis of CAC, molecular signaling, and treatment strategies have improved the survival of CAC patients considerably. However, much more detailed functional studies are necessary to develop precise treatment strategies for CAC. This review will discuss the characteristics of sporadic colon cancer and CAC, effect of inducible molecules on intestinal epithelial cells (IECs), role of tumor associated immune cells in CAC development, tumor microenvironment, and possible future therapeutics.

1. Sporadic Colon Cancer and Colitis-Associated Cancer:

Sporadic colon cancer and CAC are the two major forms of colon cancer. Tumorigenesis occurs when accumulation of selected genetic mutations leads to clonal expansion of cells by surpassing the balance between cell death and proliferation. Accumulation of mutations in oncogenes and tumor suppressor genes drive the initiation of sporadic colon cancer whereas chronic inflammation and immune dysregulation predispose dysplasia of intestinal epithelial cells and eventually lead to the development of CAC in IBD patients. Various risk factors are involved in the development of sporadic colon cancer and CAC including family history, age, gender, genomic instability, and severity of inflammation. Approximately 5-10% of colon cancers are initiated by heritable mutation. Most common hereditary-related cancer syndromes are hereditary nonpolyposis colorectal cancer (HNPCC), familial adenomatous polyposis (FAP), and *MUTYH* [*mutY* homolog (*E. coli*)]-associated polyposis (MAP). In the case of IBD, only 20% of patients have a family history of the disease. Accumulated evidence has shown that environmental changes play a pivotal role in IBD development, in particular changes in the composition of the intestinal microbiota. Genome-wide association studies (GWAS) have identified more than 180 susceptibility loci in association with CD and UC. A study by Khalili et al, analyzed the association between risk loci of CD and UC, and CAC using logistic regression modeling (5). They found that rs11676348, a susceptibility gene for UC, is inversely associated with colorectal cancer. This gene is located at chromosome 2 and correlated with the expression level of CXCR2. CXCR2 is strongly associated with CAC. However,

direct role of rs11676348 in colorectal cancer is not elucidated yet. Population studies suggest that men are at slightly higher risk than women in developing colorectal cancer. Since mutations in normal IECs or mucosal immune cells occur randomly and at lower rates, development of sporadic colon cancer is slower and occurs at later age between 50 and 80 years old. Growing evidence shows that mean age of sporadic colon cancer development is 62.2 years old. Since the younger generation is highly susceptible to IBD (6), CAC development occurs between 15 and 30 years old. Based on the retrospective cohort study from 1975-2010 by Bailey et al, incidence rates of colon cancer and rectal cancer among the young patients (between 20 to 34 years old) tend to increase by 90% and 124.2%, respectively (7). However, the prognosis of both CAC and sporadic colon cancer is approximately 50% in 5 years period after the initial diagnosis of cancer (8, 9).

Genomic instability and epigenetic changes significantly contribute to the development of sporadic colon cancer. Chromosomal Instability (CIN) and MicroSatellite Instability (MSI) are the two main types of genomic instability factors which contribute 85% and 15% to the development of sporadic colon cancer, respectively (6, 10). CpG island methylation is one of the major epigenetic modifications, which alters the promoter region of the tumor-related genes and plays an important role in the development of sporadic colon cancer. Histone methylation in APC, INK4a, and MLH has been frequently observed in sporadic colon cancer: APC and INK4a are tumor suppressor genes and MLH controls DNA stability (11-13). Cumulative effects of CIN, MSI and CpG methylation result in continuous activation of Wnt/ β -catenin signaling pathway and formation of adenomatous lesions in the colon. CAC pathogenesis is associated with the severity of inflammation. For example, IBD patients who have longer duration of the disease and chronic inflammation are more susceptible to CAC development. In addition, CIN, MSI and CpG methylation contribute to CAC to a certain degree, but the initiation, timing, location, and frequency of alterations in tumor related genes differ from sporadic colon cancer. Some distinct molecular mechanisms are involved in the initiation as well as the promotion of tumor development between CAC and sporadic colon cancer. Loss of adenomatous polyposis coli (APC) which is a tumor suppressor gene occurs due to CIN at the early stage of sporadic colon cancer and it occurs in a much later stage of CAC development with less frequency. APC inhibits β -catenin nuclear localization by sequestering in the cytoplasmic

Fig1. A

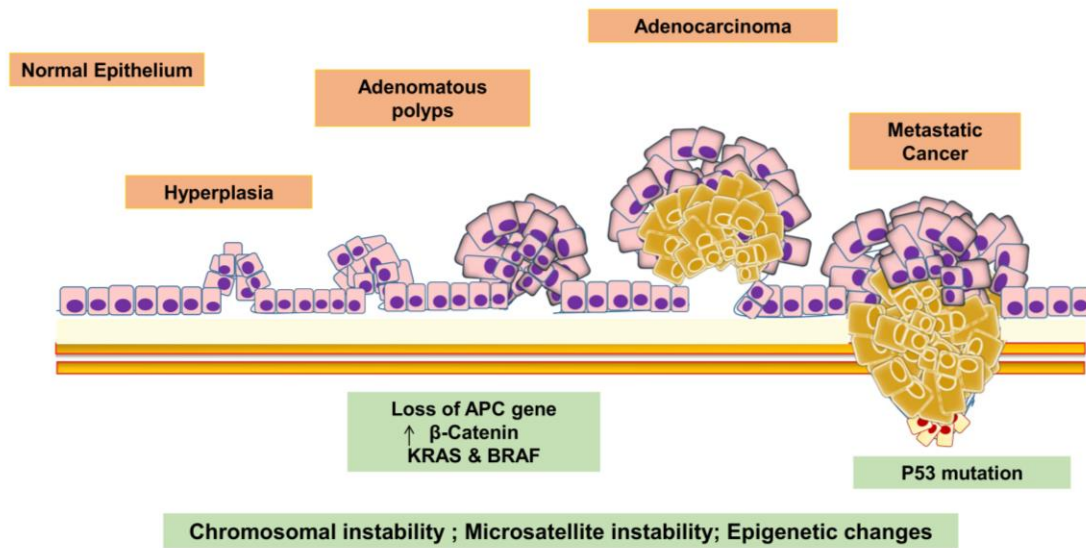


Fig1. B

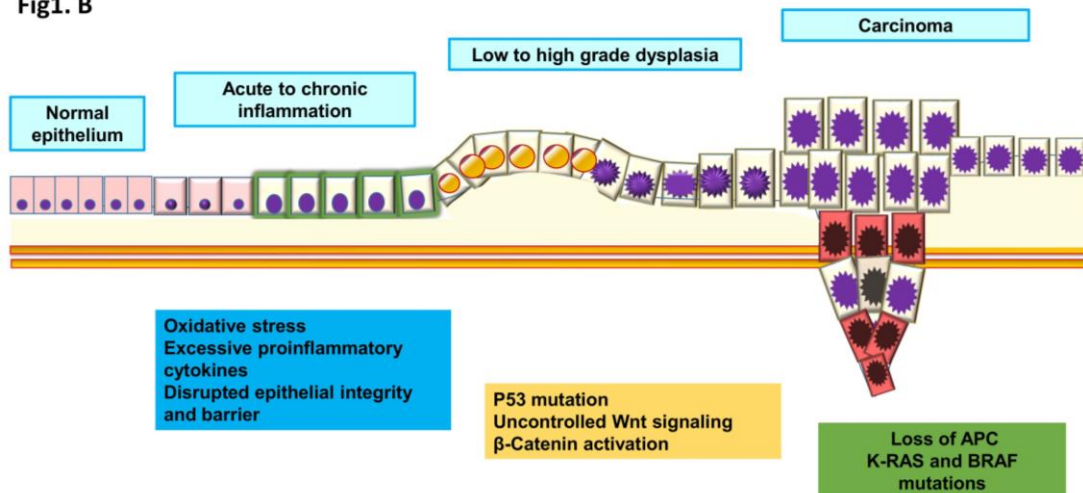


Figure 1: A. Process of sporadic colon cancer development. Cumulative mutations due to chromosomal instability, microsatellite instability, and epigenetic changes result in DNA damage and loss of tumor suppressor genes including APC. These changes lead to clonal expansion of mutated IECs. Sporadic colon cancer development initiates from hyperplasia to adenoma and eventually into adenocarcinoma. **B. Process of colitis associated cancer (CAC) development.** Epithelial regeneration signaling is activated as a repair mechanism in response to chronic inflammation. Increased oxidative stress, aberrant inflammatory and tissue repair signaling lead to p53 mutation, Wnt signaling and β -catenin activation, which initiate dysplasia in IECs. Dysplasia in IECs progress to low to high grade dysplasia and eventually to carcinoma. Sustaining inflammation is the key for the development of CAC. Genetic and epigenetic changes in DNA further contribute to CAC carcinoma at later stage of carcinoma.

compartment (14, 15). Wnt dependent signaling leads to a proteolytic degradation of APC as well as translocation of β -catenin to the nucleus (16). Furthermore, mutations in p53, K-Ras and BRAF are

associated with the neoplastic changes of IECs during the sporadic colon cancer development; BRAF and K-Ras mutations have been considered as prognostic markers for MSI (17). P53 and/or K-Ras mutations

occur later in large adenomas of sporadic colon cancer patients. In contrast, cytokine stimulation and/or NF- κ B activation drive p53 mutation in some inflamed mucosa and most of the non-dysplastic mucosa at early stages of CAC development (18). During acute and chronic inflammation, Tumor Necrosis Factor α (TNF α), Prostaglandin E2 (PGE₂), NF- κ B, and Akt signaling pathways lead to β -catenin activation independently from accumulated mutations (19-21). These signaling favor cell proliferation and initiation of tumorigenesis. Initiation of sporadic colon cancer and CAC occurs with adenomatous polyps and early dysplasia of epithelial cells with abnormal proliferation, respectively. In general, carcinogenic change to sporadic colon cancer is a stepwise progressive process from normal epithelium to the “adenoma-to-carcinoma sequence” (Fig.1A). In contrast, CAC follows the sequence of no dysplasia to indefinite dysplasia, low-grade dysplasia, high-grade dysplasia, and eventually to invasive adenocarcinoma, so-called the “inflammation-dysplasia-carcinoma sequence”(22) (Fig.1B). Although, initiation mechanisms of sporadic colon cancer and CAC are distinct from each other, both tumors show similar characteristics at the later stage. For example, K-Ras and BRAF mutations have been associated with some cases of CAC as well. Accumulated evidence has shown that the sporadic colon cancer tumor progression and metastasis are largely influenced by proinflammatory and anti-inflammatory cytokines as well as the other soluble factors, which are secreted by hematopoietic immune cells and non-hematopoietic cells including mainly IECs (23).

In addition, several studies have also reported the association between dysbiosis of microbiota, and development of sporadic colon cancer and CAC (24). Tjalsma et al proposed a “Driver-Passenger model” for colon cancer (25). Based on their model, bacteria with the pro-carcinogenic potential act as drivers and induce inflammation, which facilitates the growth of pathogenic or pro-biotic passenger bacteria. Most of the studies have characterized the bacterial/viral species/genus associated with tumor tissues (26, 27). In depth analysis of the strains of microbiota and its metabolomics are crucial to identify the involvement of unique microbiota composition in colon cancer. Furthermore, diet also play an important role in microbiota composition (28). However, identifying a specific set of microbiota as an aetiological agent is challenging because of the dynamic changes in the composition of microbiota during the course of disease progression.

2. Role of inducible molecules on intestinal epithelial cells (IECs) during the development of CAC

Numerous factors including tight anatomical barriers between IECs, pattern recognition receptors (PRRs) on IECs, its molecular signaling, cytokines, and growth factors play a significant role in the maintenance of epithelial homeostasis. The intestinal epithelial layer provides a first line of immune defense against luminal exogenous antigens, such as bacterial and food-related antigens. Loss of the epithelial barrier results in penetration of bacteria from lumen to lamina propria (LP) and initiation of the inflammatory cascade. Growth factors and pro-inflammatory cytokines may directly or indirectly stimulate IECs during inflammation and facilitate epithelial cell proliferation and restitution. Toll-like receptors (TLRs) and Nucleotide-binding domain and leucine-rich repeats (NLRs) have recently emerged as critical mediators of gastrointestinal inflammation which contribute to the initiation and progression of CAC. TLR signaling leads to the production of many proinflammatory cytokines including IL6. IL-6-mediated STAT3 activation plays a central role in IEC regeneration and proliferation (29-31). Other proinflammatory cytokines such as IL-17, IL-18, IL-22, and IL23 also contribute to CAC development and progression at different levels.

TLRs: The TLR family consists of 10 and 13 receptors in humans and mice, respectively. TLRs are uniquely distributed in the gastrointestinal tract (GIT), which indicates how well their function is coordinated to maintain intestinal homeostasis. TLR2 and TLR4 are expressed at low levels in IECs and abundantly present in crypts of the colon. In contrast, TLR3 and TLR5 expression are predominantly present in mature enterocytes of small intestine and colon, and IECs of colon, respectively. IECs are continuously exposed to intestinal luminal antigens, microbes or microbial products. Interaction of TLRs with microbe associated molecular patterns (MAMP) initiates NF- κ B signaling, which regulates intestinal homeostasis and cell repair through IL6/STAT3-dependent pathway. Uncontrolled NF- κ B signaling results in hyper proliferation of IECs. Therefore, in healthy individuals, TLRs are hyporesponsive to prevent aberrant signaling of NF- κ B. Negative regulators such as Toll-interacting protein (TOLLIP) and Single immunoglobulin IL-1R related molecule (SIGIRR/TIR8) inhibits aberrant signaling by TLR4 and maintain intestinal homeostasis in healthy individuals (32). TLR4 mediated inflammation is also attenuated by peroxisome proliferator activated

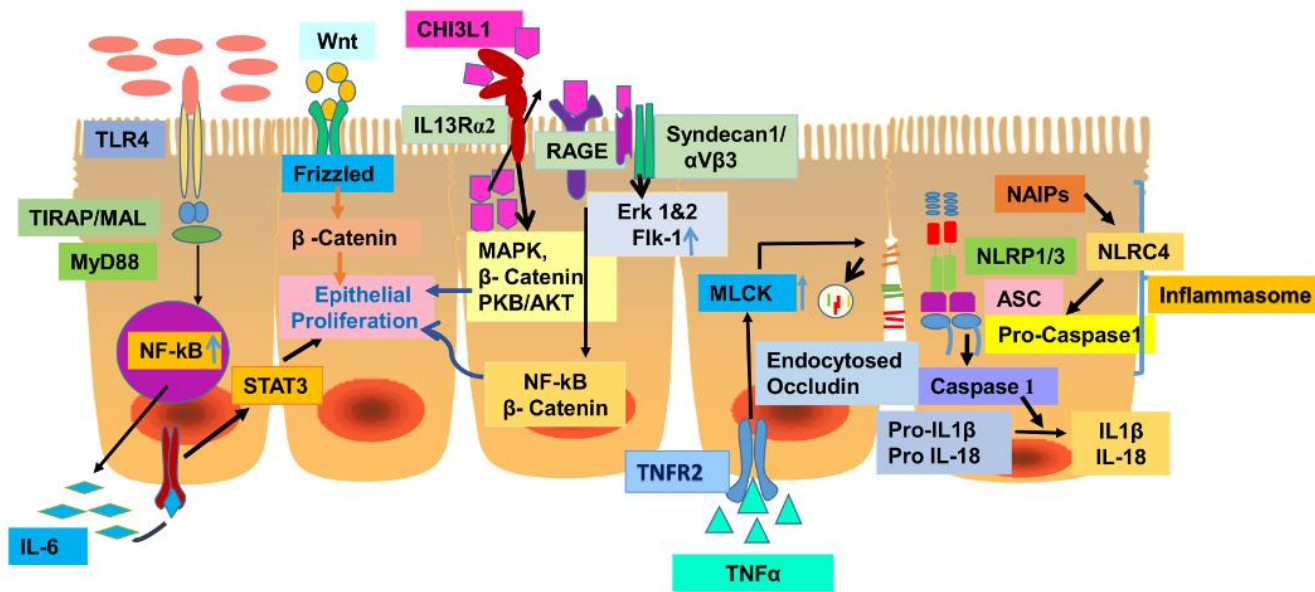


Figure 2: Colonic epithelial inducible signaling in CAC development. TLRs and NLRs bind with microbe associated molecular patterns (MAMP) or microbial products. In particular, binding of LPS with TLR4 results in NF- κ B signaling and induces IL-6 and other pro-inflammatory cytokine production. IL6-IL-6R interaction exerts uncontrolled STAT3 mediated cell proliferation and tumor. Signaling through different NLRs converts pro IL-1 β and IL-18 into biologically active IL-1 β and IL-18 either through forming inflammasome complex or independent signaling. NLRs have been shown to suppress CAC development. However, IL-18 has been reported to play a dual role in CAC. CHI3L1, a secretory molecule, binds with multiple receptors including RAGE, IL-13R α 2 and syndecan-1/ α V β 3, and induces carcinogenic and angiogenic signals, which promote CAC initiation and progression. Wnt- β -catenin signaling is crucial in cancer initiation, progression and metastasis. In addition, epithelial barrier significantly contributes to the pathogenesis of CAC through facilitating chronic inflammation. TNFR2-TNF α interaction upregulates MLCK which results in internalization of occludins and loss of epithelial barrier. Multiple signaling pathways are simultaneously induced in IECs upon chronic inflammation, which initiate the transition from dysplasia to CAC and favor successful progression.

receptor- γ (PPAR γ). Growing evidence shows the close association between TLRs and CAC development. Increased expression of TLR 4, 5, 7 and 8 have been reported in colon cancers and carcinoma cell lines (33). In particular, TLR4 expression has been shown to be upregulated in CAC lesions in humans and mice as well (34, 35). The role of TLRs has been demonstrated in mice which were induced to develop CAC using Azoxymethane (AOM) and dextran sulfate sodium (DSS). A combination of AOM and DSS are widely used to chemically induce tumorigenesis in the colon. AOM activates cyclooxygenase 2 (COX-2) and tyrosine kinase of epidermal growth factor receptor (EGFR), and suppresses TGF- β signaling. DSS is directly toxic to IECs and initiates inflammation (36). TLR4 knockout (KO) mice were resistant to develop CAC when they were induced tumors using AOM and DSS (37).

Adoptive transfer of bone marrow (BM) cells from TLR4 KO mice to WT and, WT BM cells to TLR4 KO mice demonstrated that TLR4 expression in IECs is one of the critical factors for epithelial proliferation and CAC tumorigenesis (35). Myeloid differentiation factor 88 (MyD88) is the adaptor protein which mediates the formation of TLR2/4 signalsome and results in the nuclear translocation of NF- κ B signaling (38). Studies have reported that MyD88 KO mice protect against CAC progression (39, 40). In contrast, some other studies showed that MyD88 KO mice are highly susceptible to colonic inflammation and CAC (41). It's plausible that distinct TLR/other receptor-MyD88 signaling drive opposite outcome of the carcinogenic change of IECs. Although MyD88 can bind with TLR directly, TLR2 and 4 also bind with a second adaptor molecules called TIRAP [TIR (Toll-IL-1 receptor)

domain-containing adapter protein], also known as Mal (MyD88 adaptor-like protein). A recent study by Aviello et al, demonstrated that Mal expression in IECs plays an important protective role against CAC (42) (Fig.2). The role of TLR2 in CAC has been reported in favor as well against CAC development. The function of TLRs in innate immune cells are like a double edged sword exhibiting anti-tumorigenic response as well as a pro-tumorigenic response as reviewed recently (43).

NLRs: NLRs belong to the PRR family and they significantly modulate IBD and CAC pathogenesis. NLRs include NLRP (pyrin domain-containing family), NLRC4 (CARD containing protein), and NAIP (apoptosis inhibitory protein). Single Nucleotide Polymorphisms (SNPs) in a variety of NLRs including NOD1 and NLRP3 are known to predispose IBD. GWAS studies have identified a link between NLRP1 mutation and CD development (44-46). Growing evidence has shown the association between NLRP1, NLRP3, NLRC4, NLRP6, and CAC. NLR, the adaptor protein ASC and caspase1 compose a macromolecular scaffold complex called an inflammasome. Activation of NLR through microbes or damage-associated molecular patterns (MAMP/DAMP) leads to activation of caspase 1, which converts pro-IL-1 β and pro-IL-18 into biologically mature and active IL-1 β and IL-18 (Fig.2). ASC KO and Caspase 1 KO mice treated with DSS and DSS/AOM were highly susceptible to UC and CAC, respectively. A study by William et al, analyzed the expression of 25 NLR and NLR-associated genes in human IECs and monocytes and confirmed NLRP1 as the only NLR highly upregulated in IECs (47). They further demonstrated that NLRP1 is a critical modulator which attenuates IBD and CAC using a DSS/AOM model. Adoptive BM transplantation of Nlrp1b KO to WT mice clearly demonstrated that NLRP1 attenuates CAC through non-hematopoietic cells (mainly IECs). Levels of IL-1 β and IL-18 were low in these KO mice. Contributions of IL-1 β and IL-18 cytokines to CAC pathogenesis remain highly controversial. There is a number of evidence to support that IL-18 is essential to protect from CAC, however there are also reports refuting this that indicate IL-18 promotes CAC: It is likely that higher concentrations of IL-18 promote tumorigenesis and optimum concentration of IL-18 prevents tumorigenesis (48-51). However, the cutoff point remains elusive. Expression analysis of various NLRs in colon cancer tissue from human patients resulted in significant down-regulation of NLRP1, up-regulation of NLRP3 and NLRC4, and unchanged NLRP6 (47). NLRP12 KO mice were highly susceptible

to UC and CAC due to increased production of proinflammatory cytokines, tumorigenesis factors, chemokines, and failure to control NF-kB activation in activated macrophages (52).

NLR family apoptosis inhibitor proteins (NAIPs) play a key role in suppressing CAC. NAIPs have four functional copies including 1,2,5,6 and two noncoding copies in mice. NAIPs are highly expressed in colon and innate immune myeloid cells. Since NAIPs have three BIR (Baculovirus Inhibitor of apoptosis protein Repeat) domains, it's likely that NAIPs can function alone or in combination with NLRC4 in an inflammasome complex. Epithelial specific NAIP1-6 KO mice showed significantly increased tumorigenesis in the colon independently from IL-1 β and IL-18, which indicates epithelial NAIPs protect against CAC development (53). In addition, down regulation of NAIPs in colon tumor tissues have also been reported (54).

Chitinase 3-like 1 (CHI3L1/YKL-40): CHI3L1 is a pseudo-chitinase and a secretory glycoprotein with a chitin-binding motif (55). It is expressed by various cell types including IECs, macrophages, neutrophils, chondrocytes, and vascular smooth muscles. Increased serum levels of CHI3L1 have been shown in several inflammatory conditions in human and animal models. In IBD patients, both message and protein levels of CHI3L1 are significantly upregulated in IECs and macrophages during the development of intestinal inflammation (56-58). In response to an injury due to inflammation, CHI3L1 is secreted in the lung as well as in the colon to maintain mucosal homeostasis by promoting epithelial cell restitution (57). Studies have shown elevated levels of CHI3L1 in IBD and CAC patients although the expression is completely absent in the colon of healthy individuals (57, 59). CHI3L1 has been widely reported as a potential diagnostic and prognostic biomarker for solid tumors (60, 61). Our recent AOM/DSS model studies showed that high endogenous CHI3L1 expression enhances epithelial proliferation, which promotes carcinogenic changes of IECs (61). Interestingly, CHI3L1 synergistically activates IL-6-mediated STAT3 signaling in IECs and results in tissue regeneration and proliferation (62). To examine the distinct functional roles of CHI3L1 in hematopoietic and non-hematopoietic lineages, we generated bone marrow chimeras in CHI3L1 KO and WT mice in an AOM/DSS-induced CAC model (63). We found that non-hematopoietic lineage, including IECs, is indeed the major source of CHI3L1, which promotes CAC tumorigenesis and epithelial proliferation. CHI3L1 level proportionately increases

with severity of inflammation. In chronic inflammatory conditions, it competes with S100A9, one of the DAMP molecules, in a concentration dependent manner and binds with cell surface molecule RAGE (Receptor for Advance Glycation End Product) which results in tumorigenesis via enhanced activation of NF- κ B, β -catenin, and MAPK signaling pathways (63). RAGE is a multi-ligand pattern recognition receptor which initiates cellular activation of multiple pathways and is involved in carcinogenesis of various solid tumors. Its ligands are AGE (advanced glycation end products), amyloid β -peptide, HMGB1 (DNA binding protein high mobility group box-1, S100/calgranulins and CHI3L1 (63, 64). RAGE is expressed in IECs and primarily concentrated at lateral membranes of IECs close to the apical cell junction complexes (65). Similarly, CHI3L1 has the ability to bind with multiple receptors (Fig.2) and elicit a variety of cellular responses: Recently, the Elias group elegantly proved that the catalytic domain of CHI3L1 can specifically bind with the extracellular domain of IL13R α 2, which binding is independent from IL13 and induces MAPK p42/p44 (Erk 1/2), AKT, and Wnt/ β -catenin signaling (66). Heparan sulfate chains of Syndecan-1 also interact with CHI3L1 and coordinate Syndecan-1/ α V β 3 signaling. This interaction has been shown to induce Erk1/2 in macrophages and promote angiogenesis in the tumor microenvironment by increasing Flk-1 expression and sensitizing the angiogenic responses to VEGF (67, 68). Overall, CHI3L1 regulates multiple cellular responses including apoptosis, pyroptosis, inflammasome activation, wound healing, tumorigenesis, and angiogenesis due to its ability to bind with different receptors.

Myosin light chain kinase (MLCK): The epithelial barrier is important to maintain mucosal homeostasis between intestinal microbiota and interstitium. *In vitro* studies have demonstrated various cytokine dependent mechanisms of epithelial barrier disruption. The barrier loss occurs either due to downregulation of tight junction protein expression, decreased Na⁺K⁺ATPase production, cytoskeletal contraction, or increased apoptosis/cell death of IECs. Phosphorylation of myosin II regulatory light chain by MLCK leads to cytoskeletal contraction, which in turn results in disruption of the epithelial barrier. In fact, increased MLCK expression has been reported in IECs during the development of IBD and CAC (69). Persistent MLCK over-expression in IECs promotes MLCK-mediated occludin internalization, which results in disruption of tight junctions. Consequently increased permeability of IECs and paracellular influx of luminal contents lead to

immune activation with uncontrolled cytokine production in intestinal LP cells. Studies have shown that TNFR2 signaling in IECs up regulates MLCK and epithelial barrier loss. Blockade of either TNFR2 or MLCK protein expression restored tight junction integrity and attenuated IBD as well as CAC development (70-72).

3. Role of tumor-associated cells in intestinal inflammation

Innate and adaptive immune cells play a pivotal role in the development of CAC. Selected signaling pathways in IECs and myeloid immune cells show opposite function in CAC initiation and progression. For example, IKK β /NF- κ B signaling in IECs plays a pivotal role in CAC tumorigenesis. IEC specific IKK β KO increased apoptosis of tumor cells and decreased colonic tumor incidence, but did not affect inflammation. In contrast, depletion of IKK β in immune cells reduced the colonic tumorigenesis and inflammation but not affected the rate of apoptosis of tumor cells (73).

Macrophages: Tissue-resident macrophages are abundant in the colon and are recruited to the LP by endogenous chemoattractants in non-inflamed mucosa, while circulatory macrophages migrate to LP in response to proinflammatory chemokines during intestinal inflammation. Tissue-resident macrophages exhibit functions of proinflammatory modulation and phagocytic/bactericidal activities (74). During the course of intestinal inflammation, circulatory macrophages mount a typical proinflammatory phenotype and play an indispensable role in IBD and CAC pathogenesis. Polarized macrophages are classified into two types: 1. Classically activated macrophages (M1 phenotype), which secrete proinflammatory cytokines including TNF α , IL1- β , IL-6 and IL-12 and produce reactive oxygen (ROS) and nitrogen species (RON). 2. Alternatively activated macrophages (M2 phenotype) which promote production of anti-inflammatory cytokines including IL-10 and TGF β , and enhanced tissue repair by favoring cell proliferation. Inflammation-dependent oxidative stress induces mutations in IECs and lead to their carcinogenic changes, as seen in UC patients who have highly activated leukocytes which generate excessive ROS and RON productions (75). A study by Wang et al, characterized M1/M2 macrophages to be involved in CAC initiation, promotion and metastasis. They found that M2 macrophages play a significant role in development of CAC and functional changes occur in M1 macrophages which promote CAC without

alteration in polarization (76). Several studies have reported different markers for M1, M2, M1-like-M2 and M2 subtypes, however the phenotypic and functional classifications are ambiguous. Of note, mouse M2 macrophages generally express mammalian chitinases including CHI3L1, CHI3L3 (YM1) and CHI3L4 (YM2). P38/MK2 [mitogen-activated protein kinases (MAPK)-activated protein kinase 2 (MK2)] pathway activation in macrophages has also been shown to partly contribute to the development of CAC (77). TAMs (tumor-associated macrophages), mostly M2 macrophages, play an important role in tumorigenesis, metastasis, immune suppression and angiogenesis by producing various cytokines and growth factors (78, 79). Growth factors and cytokines secreted by tumor cells and other infiltrating cells change the phenotype of recruited circulatory macrophages. There is evidence that peritumoral TAMs exhibit antitumor property and improve survival of cancer patients (80). Based on several studies, it seems that TAMs function and prognosis of cancer patients differ with their localization in tumors (81, 82).

Neutrophils: Neutrophils are the most abundant leukocytes in the blood and play an important role in first line defense. These cells respond to chemokine signals and migrate to the site of infection and/or inflammation efficiently. Neutrophils are critical to control infection and inflammation, however if inflammation is uncontrolled, it can lead to severe tissue injury and promote tumorigenesis. Neutrophils secrete both pro- and anti-inflammatory cytokines, and contribute to angiogenesis and immune surveillance. Recent studies indicated that neutrophils also assume pro-tumorigenic phenotype (N2) and promote neoplastic changes of IECs. Based on the inflammatory milieu, they could differentiate either N2 or anti-tumorigenic phenotype (N1), which are similar to macrophage phenotypes (83, 84). Studies have suggested that peripheral neutrophil to lymphocyte ratio can be potentially used as one of the predictive and prognostic factors for CAC (85, 86).

Neutrophils secrete cytokines and chemokines including TNF α , IL1- β , IL-8, as well as other chemokines including CCs and CXCs. CXCR2 on the surface of neutrophils binds with CXCL1, 2, 3 and 5 and recruits neutrophils to the site of inflammation. In addition, myeloperoxidase (MPO) is considered as a commonly used marker for neutrophil infiltration in inflammation and tumors. Methylation status of RUNX3 (Runt-related transcription factor 3), which is a putative tumor suppressor gene, as well as TNF α

polymorphism, and MPO in UC-associated cancer tissues showed a positive correlation with the severity of the tumor development (87). Mice treated with AOM/DSS showed significant increase in CXCR2 expression in neutrophils. Serine proteases including elastase, cathepsin G, proteinase 3, and matrix metalloproteinase (MMP)-8 and -9 are present in the secondary granules of neutrophils and promote tumorigenesis. Depletion of neutrophils with Ly6G antibodies ameliorated CAC development in terms of tumor number and size (88). It is likely that blocking CXCL2-CXCR2 will also reduce the risk of CAC by controlling neutrophil infiltration in chronic colitis.

Dendritic cells (DCs): Several studies have reported an infiltration of DCs in inflamed tissues of CAC patients. DCs act as sentinel cells and directly capture luminal antigens by using their long processes which travel in between epithelial cells to maintain intestinal homeostasis (74). T-bet, a transcription factor which is required for the priming of Th1 cells, is highly expressed in DCs but not in macrophages. Depletion of T-bet exclusively in innate immune cells [T-bet x Rag double KO (DKO), so called TRUC] in mice resulted in uncontrolled chronic inflammation and subsequent CAC development, suggesting the immunoregulatory and anti-tumorigenic roles of T-bet expression in DCs (89). Since T-bet is a TNF α repressor in DCs, over-expression of T-bet selectively in DCs also markedly reduced the severity of chronic colitis and the incidence of CAC development.

T cells: In contrast to innate immune cells, which act as a first line of defense, acquired immune cells aggravates the inflammation and tissue injury in IBD by activating inflammatory signaling pathways in mucosal tissues. These cells may also lead to CAC initiation if the inflammation is chronically perpetuated. Different subsets of T cells play distinct roles in inflammation, tumor initiation, progression and anti-tumor immunity. When CD4⁺ T cells get activated, they differentiate into distinct T helper (Th) subsets including Th1, Th2, Th17 and Tfh (follicular helper CD4 T cells) based on their ability to produce cytokines and transcription factors. For example, cytokines such as TGF β , IL-6, IL-21 and IL-23 and transcription factors including STAT3, ROR γ (retinoic acid receptor- related orphan receptor γ), ROR α , IRF4 (interferon regulatory factors), AhR (aryl hydrocarbon receptor) and Batf (Basic Leucine Zipper Transcription Factor, ATF-Like) influence Th17 differentiation. Each of these subsets secretes different types of cytokines and elicits diverse immune responses. Th1 immunity completely arrests the tumor growth via

TNF α - and IFN γ -mediated signaling pathways. Th2-deficient mice (IL4 KO) treated with AOM and trinitrobenzene sulfonic acid (TNBS), which induces Th1-type colitis, developed significantly less colonic tumor as compared to Th1-deficient mice (IFN γ KO). This result indicates that Th2 cells specifically promote tumorigenic changes of IECs under inflammatory conditions in the colon (90). Rag1 x CD4 DKO mice developed chronic DSS colitis although tumor formation was markedly reduced in AOM/DSS-induced colitis. Human studies have shown higher infiltration of Th17 cells in UC and CAC colonic tissues. A recent study by Martin et al, demonstrated that absence of ROR γ -dependent Th17 cells induces chronic colitis but fewer macroscopic tumor formation in an AOM/DSS mouse model (91). It suggests that tumor initiation itself was not inhibited but the cell proliferation was not accelerated due to the absence of ROR γ -dependent Th17 cells. Furthermore, this study confirmed that tumor cell proliferation is partially regulated by ROR γ expressing hematopoietic cells.

Presence of CD3⁺, CD8⁺, CD45RO⁺, and granzyme B⁺ cells in human colonic tumors is positively associated with disease prognosis (92). CD8⁺ cytotoxic T cells are known to elicit anti-tumor immune response. Presence of higher number of CD8⁺ T cells and lower number of FoxP3⁺ cells in colonic tumors reflect favorable prognosis in CAC patients (93, 94). Successful control of intestinal pathology depends on the balance between Th cells, CD8⁺ T cells and regulatory T cells (Tregs). Based on cellular phenotype, Tregs can be classified as CD4⁺CD25⁺ FoxP3⁺ cells and CD8⁺CD25⁺FoxP3⁺ cells. The number of CD8⁺ Tregs is 100 times less than CD4⁺ Tregs. Adoptive transfer of Foxp3⁺ Tregs improved the IBD outcome in mice and studies suggested that increasing number of Tregs could replace immunosuppressive drugs. In contrast, increased number of Foxp3⁺ Tregs in tumors suppresses anti-tumor immunity elicited by Th1 cells and CD8⁺ cytotoxic T cells, and result in tumor progression. Transient ablation of Tregs in an AOM/DSS mouse model indicates that reduction/absence of Tregs promote effective anti-tumor immune response. Depletion of Tregs resulted in the increase of CD8⁺ effector T cells with elevated level of IFN γ and granzyme B productions, and the ultimate death of neoplastic cells in CAC (95).

A unique subset of T cells called invariant natural killer T (iNKT) cells express NK receptor and invariant T cell receptor (TCR) α chain. A few studies have shed some light on the anti-tumor role of iNKT cells by

inhibiting IL-13 production in NK1.1⁺ T effector cells and mononuclear cells in inflamed colon (96).

Myeloid-derived suppressor cells (MDSCs) and innate lymphoid cells (ILCs): MDSCs are heterogeneous myeloid progenitor cells including immature granulocytes, macrophages and DCs. MDSCs highly contribute to tumor immune evasion by suppressing T cell-mediated anti-tumor effects and other innate immune cell signaling (97). During the course of chronic inflammation, proinflammatory cytokines such as IL-6, IL-1 β and PGE2 recruit MDSCs which suppress anti-tumor immunity and immune surveillance (98). MDSCs suppress the activities of T cells and other innate immune cells through distinct mechanisms including production of arginase, ROS and nitration of TCR. Increased level of CCL2 and MDSCs infiltration have been reported in patients with CACs, colonic adenoma and sporadic colon cancer. Chun et al, showed that CCL2 promotes CAC tumorigenesis by recruiting monocytic MDSCs and polymorpho nuclear (PMN)-MDSCs to suppress effector T cell function via iNOS and STAT3 dependent ROS production, respectively (99). Depletion of CCL2 prevented the progression of CAC and accumulation of MDSCs around the tumor.

ILCs, predominantly found in gut associated lymphoid tissue (GALT), are an emerging new set of innate immune cells which contribute to initiation and regulation of inflammation. ILCs can be classified into three different types, ILC1, 2 and 3, based on the functional criteria. Different studies have shown increased or decreased number of proinflammatory ILC3s in IBD patients as well as in mouse models of colitis. These discrepancies could be due to the differences in characterization of ILCs by different groups and heterogeneity in the unitized mouse models. A study by Kirchberger et al, concluded that ILCs mediate bacteria-induced CAC through IL-22 production using a *Helicobacter hepaticus*-infected AOM model (100). Depletion of ILCs with anti-Thy1 antibody reduced the severity of colonic inflammation as well as the incidence of CAC development in this model. The role of ILCs at different stages of inflammation in various organs has been extensively reviewed recently (101), which suggest that ILCs also contribute to anti-tumor immunity by limiting inflammation in contrast to their pro-tumorigenic characteristics.

Myofibroblasts (MFs): Intestinal MFs in the LP contribute to tissue repair, inflammation, angiogenesis and fibrosis. In particular, MFs play an important role in

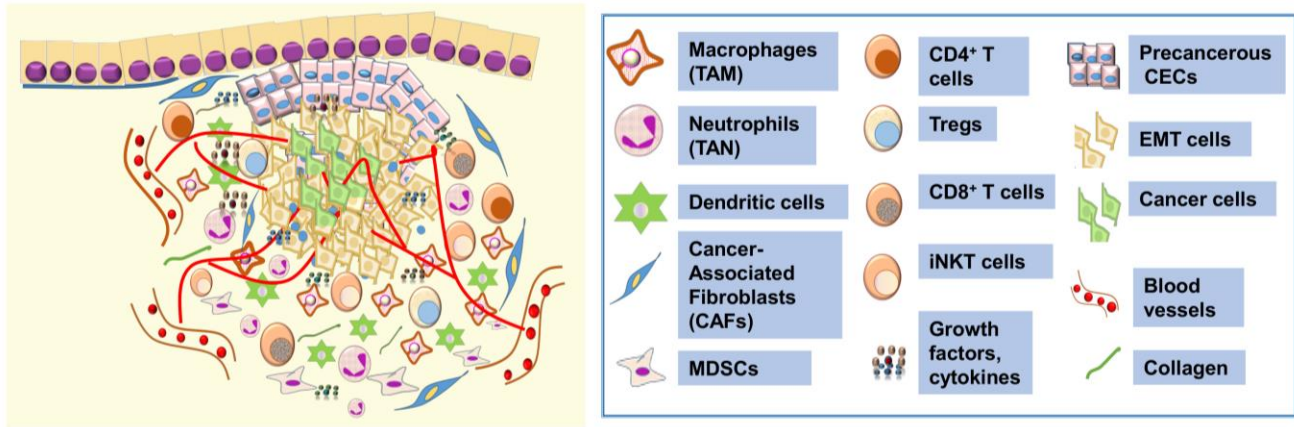


Figure 3: Components of tumor microenvironment (TME). After initiation of tumor, progression and metastasis depend on tightly controlled TME. TME is infiltrated with immune cells including TAMs, TANs, DCs, MDSCs, CD4⁺ T cells, CD8⁺ T cells, Tregs and CAFs, pro and anti-inflammatory cytokines, and growth factors. New blood vessel networks around the tumor provide nutrients and oxygen for the survival of tumor cells and tumor associated cells. Signaling from stromal cells contribute to the stemness of the cancer cells.

maintaining IEC homeostasis by providing a niche rich in non-canonical Wnts, extracellular matrix and growth factors. Similarly, MFs also contribute to carcinogenesis and play a pivotal role in tumor microenvironment (TME). A study by Vermeulen et al, demonstrated that stemness of colon cancer cells is maintained by hepatocyte growth factor (HGF)-secreted by MFs in TME (102). Wnt gene expression profiling analysis of MFs of normal and UC patients revealed down regulation of secreted frizzled-related protein (SFRP), a natural Wnt inhibitor, in MFs of UC patients (103), which shows that UC patients with down-regulation of SFRP are at higher risk of CAC development. Tumor progression locus 2 (Tpl2) is a serine-threonine kinase which belongs to the MAPK family and is involved in colonic inflammation and oncogenesis. MF specific ablation of Tpl2 in mice resulted in increased number and size of tumors, increased epithelial proliferation and reduced apoptosis in an AOM/DSS mouse model (104). Furthermore, Tpl2 deficient mice showed increased expression of HGF in colon. Furthermore, Tpl2 suppresses the colonic tumorigenesis by regulating TGF β and HGF/c-met pathways in IECs. Based on a result of inhibition experiment, CCL3-CCR5 axis seems to be involved in the recruitment and accumulation of fibroblast in TME as well as the CAC development via suppressing the expression of heparin binding epidermal growth factor-like-growth factor (HB-EGF) (105). In

human primary MFs, TNF α induces epidermal growth factor receptor (EGFR) expression and binding of EGF-EGFR induces ERK signaling activation, which results in increased Cox2 expression (106, 107). Whole genome profiling has shown that in both CAC patients and a murine AOM/DSS cancer model, there is an increased epiregulin expression which is mainly produced by MFs. A study by Neufert et al, showed that epiregulin producing MFs in TME promotes tumor-growth via ERK-induced IEC proliferation (108). MFs also secrete fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) which contribute to angiogenesis in TME.

4. Tumor microenvironment (TME):

Successful initiation, progression and metastasis of colorectal cancer depends on the micro environment surrounding the tumor. Inflammation and inflammatory mediators are the major contributors of TME. In general, IBD patients with chronic inflammation tend to have frequent flare up and disease history for more than 10 years. Due to chronic inflammation, loss of epithelial integrity and increased intestinal permeability are observed in these patients over time. Disruption of epithelial barrier allows luminal microbiota to enter freely into the LP and will further activate immune cells which result in secretion of proinflammatory mediators (23). Selected microbes are likely to be involved in

tumor initiation in addition to inducing UC. For example, Enterotoxigenic *Bacteroides fragilis* is known to cause UC and also promote CAC by cleaving E-cadherin and inducing β -catenin signaling activation (109, 110). Studies have shown the enrichment of microbes in tumor tissues (26) which provoke the question whether these microbes promote tumorigenesis, progression and metastasis.

TME consists of various interplayers including tumor cells, stromal cells, infiltrating immune cells, vasculature, extracellular matrix (ECM) and ECM-associated molecules. A variety of cells infiltrate tumor-environment including TAMs, neutrophils, DCs, CD4⁺ T cells, CD8⁺ T cells, MDSCs, NK cells, mast cells, endothelial cells, endothelial progenitor cells (EPCs), platelets, fibroblasts and mesenchymal stem cells (MSCs) (Fig.3). M1 macrophages are part of Th1 response and M2 macrophages are part of Th2 response: M1 contributes to chronic inflammation by producing TNF α , IL-12, IL-23, ROS and RON, while M2 macrophages secrete low levels of IL-12 and high levels of IL-10, decoy R, IL-1RA, CCL17, and CCL22 chemokines. Change in the M1/M2 macrophage phenotype promotes tumor initiation, progression and metastasis. In the study by Wang et al, although M1 phenotype did not change in CAC tissues in an AOM/DSS mouse model, functional changes were similar to M2 phenotype (76). Infiltration of significant M2 polarized TAMs in TME have been reported as a poor prognostic indicator. TNF α , IL-6 and IL-8 secreted by macrophages and neutrophils promote infiltration of T cells and MDSCs. Macrophage and neutrophil products of oxidative stress (e.g., ROS and RON) result in DNA damage and p53 mutation in IECs. Pro-myelopoietic factors such as GM-CSF (granulocyte macrophage colony stimulating factor), IL-6, VEGF and M-CSF (macrophage colony stimulating factor) produced in the TME lead to the expansion of MDSCs. Increased expression of arginase1 and inducible nitric oxide synthase by MDSCs deplete anti-tumor effective T cells and promote FoxP3⁺ Tregs in the environment. MDSCs and Tregs suppress the antitumor immunity and favor tumor progression. The NF- κ B and IL-6–STAT3-mediated signaling pathways are critical for cell proliferation and tumor metastasis. IFN γ is responsible for early inflammation and is involved in anti-tumor property at later stage.

Epithelial mesenchymal transition (EMT) is an important process in inflammation, wound healing and cancer. Epithelial cells obtain the characteristics of fibroblast, and loss of tight junction, desmosomes, and

gap junction results in the failure of cell-cell interaction. Expression of fibroblast markers such as fibronectin, α -SMA (α -smooth muscle actin) and matrix metalloproteinases increase during EMT process (111). EMT is largely controlled by cytokines and particularly TGF- β signaling controls the EMT by inducing SMAD (contraction of SMA and Mad)/Snail/EMT pathway (112). TNF α stabilizes Snail, a key transcription factor, involved in the last step of EMT. In addition, NF- κ B and STAT3 also play a role in EMT process (113-115). Accumulating evidence suggests that the proinflammatory cytokines such as TNF α , IL6 and IL8 substantiate the EMT process. Since cytokines drive the EMT process, mesenchymal cells differentiate back to epithelial cells in metastasized organ due to low gradient /absence of cytokines in that environment.

The increased distance between the tumor cell and blood vessels reduces the chance of getting oxygen and decreases the survival of tumor cells. Therefore, neoangiogenesis in TME is vital to provide sufficient oxygen and nutrients to tumor cell survival (Fig. 3). VEGF contributes to the new blood vessel network. Inhibition of VEGF dramatically reduced the tumor development, angiogenesis and cell proliferation (116). In addition to angiogenesis, VEGF signaling through VEGF receptor 2 promotes tumor cell proliferation in a STAT3 dependent manner. TAMs over-express VEGF and also other pro-angiogenic factors including MMP7, MMP12, and COX2, which contribute to angiogenesis in TME. Prothrombin, a clotting factor for the normal coagulation of blood, is another factor which contributes to initiation and metastasis of CAC (117), and its deficiency reduces the precancerous aberrant crypt foci.

During tumor initiation, surrounding stromal cells mount suppressive response to tumor development. However, subsequent dynamic interaction between tumor cells and stromal cells changes in favor of tumor progression and metastasis with reversible or irreversible genetic mutations/modifications in stromal cells. Cancer-associated fibroblasts (CAF) promote the tumor growth by producing several growth factors including EGF, hepatocyte HGF and FGF. Increased levels of stromal fibroblast activated protein (FAP) have been correlated with an increased risk of tumor metastasis and progression (118). Aberrant activation of Wnt signaling is considered as one of the major pathways in cancer biology. It is a notion that all IECs have tumorigenic capacity. Regardless of this notion, immunohistochemical studies on colorectal carcinoma (CRC) cells showed non-homogenous distribution of nucleus β -catenin. This finding has shed some light that

only a subset of CRC cells have the stem cell-like property and others lose the tumorigenicity after differentiating into neoplastic cells. A study by Vermuelen et al, demonstrated that CRC stemness is closely regulated by TME and tumor-initiating cells (TIC) (102). Wnt/ β -catenin signaling in TICs is induced by HGF secreted by MFs. TME is distinct from the normal tissue environment and consists of unique phenotype of TAMs, neutrophils, MDSCs, stromal cells, cytokines and secretory molecules. Therefore, targeting TME components for therapeutic purposes will benefit prevention of tumor progression and metastasis.

5. Signaling molecules as therapeutic targets:

CAC seems to be preventable if inflammation is controlled effectively and properly. Even after developing CAC, there are several treatment options including surgery, chemotherapy, radiation therapy, cancer-targeted therapy and immunotherapy. The treatment strategy with the greatest chance of cure is surgery, which resects localized cancers, specifically in early-stage tumors. Chemotherapy is controlling cancer by drugs and is often paired with radiation therapy, which uses high energy particles or waves to destroy cancer cells, which result in reduction of tumor size. General drugs used in chemotherapy also cause damage in normal cells to certain extent, to remedy this; cancer-targeted therapy may be used by specifically targeting unique properties of cancer cells, which reduces the risk of destroying normal cells. Another strategy is immunotherapy, which stimulates one's own immune system to fight against cancer. To effectively treat cancers, a combination of these therapies is often used. Cancer treatment should be targeted against cause, molecules-involved in promoting cancer signaling pathways, and immune/stromal cells involved in tumorigenesis, progression, and metastasis. In addition, accumulated evidence has clearly indicated the importance of targeting tumor microenvironment.

As we discussed above, chronic inflammation is one of the major contributing factors for CAC development in IBD patients. Therefore, anti-inflammatory drugs are given to patients to control inflammation, which reduces the risk of neoplastic changes in IECs. Corticosteroids have been used for a long time to control IBD and effectively reduce the CAC risk but also suppress the general immune system which makes the patients susceptible to infections and other complications (119, 120). CAC has also been associated with primary sclerosing cholangitis in IBD patients (121). Studies

have suggested that using Ursodeoxycholic acid (UDCA) reduces bile acids and lowers the risk of CAC in a dose dependent manner (121-123). Studies have shown that excess bile acids particularly secondary bile acids in the colonic lumen increase the risk of IBD as well as CAC development using animal models (124). Also correlated with IBD is folate deficiency, (125-127) and folate supplements will likely reduce the risk of CAC. Statins, a class of drugs that can help lower cholesterol levels in the blood, have also been shown to reduce the risk by 47% in patients who were diagnosed with colorectal tumors (128).

5-amino salicylic acid (5-ASA) or mesalamine is widely used as maintenance therapy for UC. 5-ASA inhibits the NF- κ B pathway, which promotes proliferation of IECs and chronic inflammation. Velayos et al, performed a meta-analysis of 9 studies that included a total of 1932 patients and demonstrated a protective effect of 5-ASA to CAC (129). In contrast, a study by Terdiman et al, concluded that 5-ASA is not effective in patients who was treated with this compound for about 12 months, which indicates that the period of treatment plays a significant role in the outcome (risk/protection) of CAC (130). Other promising chemical compounds like sesquiterpene, lactones-like parthenolide, and micheliolide have been shown to exhibit anti-inflammatory effects by inhibiting the NF- κ B signaling pathway which results in the attenuation of IBD and CAC in AOM/DSS mouse model (131).

Histone deacetylase (HDAC) inhibitors are another therapeutic strategy for CAC treatment and have been also utilized in other cancer clinical trials currently. HDAC modulates the acetylation state of different transcription pathways including NF- κ B signaling pathway. The pro-apoptotic characteristic of HDACs makes them good candidates for anti-cancer drugs. Glauben et al, reported that HDAC inhibitor ITF2357 attenuates CAC by inhibiting IFN γ production, NF- κ B activation, and increasing apoptosis of mononuclear cells in the LP (132, 133). Pro-biotic bacterial metabolites such as butyrate and acetate are considered as a class of HDAC inhibitors. These short chain fatty acids have the anti cancerous, anti inflammatory and anti proliferative properties. Few clinical trials on probiotic effect of *Lactobacillus* species against colon cancer showed suppression of the diseases (134, 135). However, the sample size of these studies is not sufficient to draw a definitive conclusion.

Modulating inflammation is a prerequisite for CAC prevention. Blocking TLRs and MLCK using antibodies

may attenuate the disease development. However, TLRs intervention should be done without altering the beneficial effect of TLR. CHI3L1 is highly involved in promoting inflammation-associated tumorigenesis and angiogenesis, and therefore neutralizing CHI3L1 by anti-CHI3L1 antibodies will potentially reduce the tumor development and progression. Since CHI3L1 binds with multiple receptors (**Figure 2**), inhibiting one receptor will not completely abrogate CHI3L1-mediated signaling pathways.

Experimental and clinical data have shown that anti-inflammatory cytokines/receptors can be used as therapeutic targets for CAC prevention. Anti-TNF α antibodies are extensively used in IBD patients, in particular CD patients (**136**). As we discussed above, IL-6–STAT3 signaling plays a central role in CAC pathogenesis. Increased level of IL-6 trans signaling through soluble IL-6 receptor has been reported in IBD and CAC. Glycoprotein 130 (gp130) is a natural inhibitor of IL-6 trans signaling but not membrane bound IL-6R signaling, which is why gp130 fusion antibodies have been proposed for therapeutic use in CAC. IL-6 stimulates the differentiation of Th1 to Th17 cells, which is the major source of IL-17. An increased level of IL17 has been associated in IBD as well as CAC pathogenesis (**137**). Blocking of IL-17A and/or IL-17F has attenuated the chronic inflammation and CAC tumorigenesis as well (**138**). IL-22 has been shown to induce CAC and inhibition of IL-22 significantly attenuated CAC development compared to IL-17 inhibition (**100**). IL-10 is an anti-inflammatory cytokine which suppresses IBD and CAC. In fact, IL-10 KO mice are highly susceptible to UC and CAC due to uncontrolled inflammation in the colon (**139, 140**). IL-15 suppresses CAC by promoting anti-tumor immunity by CD8⁺ T cells and NK cells (**141**). Several studies have shown that either inhibition of a combination of proinflammatory cytokines, cytokine receptors or addition of recombinant anti-inflammatory cytokines will likely reduce the CAC in IBD patients.

VEGF is playing a major role in forming new blood vessel networks around tumors. Targeting VEGF or VEGFR directly inhibits the angiogenesis and survival of tumor cells. Several clinical trials are ongoing, targeting VEGF (by anti-VEGF antibodies) and VEGFR (by adoptive cell therapy).

COX-2 and prostaglandin E2 (PGE₂) genes are one of the first responsive genes to be expressed by IECs and inflammatory cells in response to growth factors and proinflammatory cytokines during inflammation (**142**). Nuclear hormone receptor peroxisome proliferator-

activated receptor δ (PPAR δ) is a ligand dependent transcription factor which plays an important role in CAC. Downstream signaling of PPAR δ -mediated COX-2/PGE₂ signaling significantly contributes to chronic inflammation and CAC by maintaining the cross talk between neoplastic IECs and TAMs (**143**). In contrast, Ishikawa and Herschman reported expression of COX-1 and COX-2 is not necessary for CAC using an AOM/DSS mouse model (**144**). PGE₂ also recruits more MDSCs to the tumor site. Non steroidal anti-inflammatory drugs (NSAIDs), which specifically inhibit the inducible isoform of COX-2, are used as a general therapy to reduce inflammation and cancer. Growing evidence shows that COX-2 selective inhibitors including CG100649 and celecoxib are likely to be used in treatment with other therapies (**145-147**).

In addition to the above therapies, several clinical trials have been conducted on targeting inhibitory T cell pathways to unleash antitumor immune responses. CTLA-4 (cytotoxic T lymphocyte-associated antigen 4), one of the inhibitory molecules of T cells, binds with B7 on antigen presenting cells (APCs) which inhibits T cell activation and suppresses the antitumor immunity. Anti-CTLA-4 antibodies are extensively studied and approved for the treatment of melanoma. Clinical trials are ongoing to determine the efficacy of anti-CTLA-4 antibodies against colon and other cancers (**148, 149**). In addition to CTLA4, PD-1 (programmed cell death protein 1) is another T cell inhibitory molecule, which is present in activated T cells. PD-1 ligands (PD-L1 and PD-L2) are present in APCs and tumor cells. Clinical trials on antibodies against PD-1, PD-L1, and PD-L1 positive T cells to treat various cancers including colon cancer are currently in progress (**150**).

Conclusion:

Accumulated evidence on CAC has clearly shown the close contribution of several factors including diet, microbiota, secretory molecules, pro-/anti-inflammatory cytokines, transcription factors, growth factors, and other components, which are produced during the development of CAC. Since CAC development involves a complex network, different treatment strategies are continuously explored in this field. However, blocking one signaling-associated molecule will only partially ameliorate CAC development. Therefore, effective combined therapies should be tested in clinical trials. Furthermore, in depth studies are necessary to delineate the critical underlying mucosal and immune cell mediated mechanism

involved in the transition from IBD to CAC to optimize the current therapeutic regime.

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Abbreviations:

AGE, advanced glycation end product
 AOM, azoxymethane
 BIR, baculovirus inhibitor of apoptosis protein repeat
 BM, bone marrow
 CAC, colitis-associated cancer
 CAF, cancer-associated fibroblast
 CD, Crohn's disease
 CHI3L1, chitinase 3-like 1
 CIN, chromosome instability
 COX-2, cyclooxygenase2
 CRC, colorectal cancer
 CTLA-4, cytotoxic T lymphocyte-associated antigen 4
 DC, dendritic cell
 DSS, dextran sulfate sodium
 ECM, extracellular matrix
 EGFR, epidermal growth factor receptor
 EMT, epithelial mesenchymal transition
 FAP, familial adenomatous polyposis
 FGF, fibroblast growth factor
 FAP, fibroblast activation protein
 GALT, gut-associated lymphoid tissue
 GIT, gastrointestinal tract
 GWAS, genome-wide association study
 HDAC, histone deacetylase
 HGF, hepatocyte growth factor
 HMGB-1, DNA binding protein high mobility group box-1

HNPCC, hereditary non-polyposis colorectal cancer
 MAP, MUTYH [mutY homolog (E. coli)]-associated polyposis
 IBD, inflammatory bowel disease
 IRF4, interferon regulatory factors
 IECs, intestinal epithelial cells
 iNKT, invariant natural killer T
 ILCs, innate lymphoid cells
 MAMP, microbe-associated molecular pattern
 MDSCs, myeloid-derived suppressor cells
 MSI, microsatellite instability
 MSC, mesenchymal stem cell
 MLCK, myosin light chain kinase
 MyD88, myeloid differentiation factor 88
 MFs, myofibroblasts
 NAIP, NLR family apoptosis inhibitor protein
 NLR, nucleotide-binding domain and leucine-rich repeats
 NSAIDs, nonsteroidal anti-inflammatory drugs
 PD-1, programmed cell death protein-1
 PGE2, prostaglandin E2
 PPAR γ , peroxisome proliferator activated receptor- γ
 RAGE, receptor for advanced glycation end product
 ROR γ , retinoic acid receptor-related orphan receptor γ
 ROS, reactive oxygen species
 RON, reactive nitrogen species
 SFRP, secreted frizzled-related protein
 SIGIRR, single immunoglobulin IL-1R-related molecule
 SNP, single nucleotide polymorphism
 TAM, tumor-associated macrophage
 TME, tumor microenvironment
 TIR, Toll-IL-1 receptor
 TIRRAP, TIR domain containing adaptor
 TLR, toll-like receptor
 TOLLIP, toll-interacting protein
 Tpl2, tumor progression locus 2
 TIL, tumor-initiating cells
 TNBS, trinitrobenzene sulfonic acid
 UC, ulcerative colitis
 VEGF, vascular endothelial growth factor
 UDCA, ursodeoxycholic acid

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