

# Inflammation in Sporadic Colorectal Cancer

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## Abstract

Chronic inflammation plays a pivotal role in the development of colorectal cancer (CRC) in patients with inflammatory bowel disease (IBD). An orchestrated interplay of immune cells with numerous inflammatory mediators including reactive oxygen and nitrogen species, cyclooxygenase 2, and several cytokines promotes colitis-associated cancer (CAC). Recent findings have shown that inflammatory pathways not only are important in the development of CAC but are also involved in the pathogenesis of sporadic CRC. Hereby, we review the existing experimental and clinical evidence that suggest a link between inflammation and tumorigenesis in sporadic CRC.

**Keywords:** Inflammation, sporadic colorectal cancer, tumorigenesis

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## Introduction

Colorectal cancer (CRC) is the second most common cancer in women and third most common cancer in men worldwide,<sup>1</sup> with an overall age-adjusted incidence rate of 47.9 per 100,000 per year. Only a small fraction of colon cancer cases belong to the known hereditary syndromes while the majority of cases are sporadic, and develop due to the accumulation of multiple alterations in oncogenes and tumor suppressor genes.<sup>2,3</sup> These mutations occur as a result of genetic instability during the early stage of tumorigenesis, and promote adenoma to carcinoma progression. *Adenomatous polyposis coli (APC)* gene mutations that result in  $\beta$ -catenin activation, is the primary event in adenoma formation. Subsequent genetic alterations, including KRAS activation and P53 mutations, occur later in the adenoma to carcinoma sequence.<sup>3</sup> The mechanism of acquiring and maintaining these genetic alterations in the tumor microenvironment is still under investigation.

The emerging evidence suggests a role for the intestinal microbiota and chronic inflammation in the pathogenesis of CRC. Numbers of inflammatory mediators such as tumor necrosis factor, nuclear factor- $\kappa$ B, and prostaglandin E2 are shown to independently activate the wnt/ $\beta$ -catenin pathway in gastrointestinal cancers.<sup>4-6</sup> In this regard, lessons learned from inflammatory bowel disease (IBD)-associated colon cancer have added to our knowledge. At the cellular level, IBD is characterized by periodic mucosal injury and epithelial regeneration,<sup>7</sup> which eventually affect cell proliferation, differentiation, epithelial migration, and apoptosis. The damage ultimately culminates in ulceration and/or tumorigenesis.<sup>8</sup> Several lines of evidence suggest a strong link between gastrointestinal microbiota and IBD pathogenesis. In fact, IBD is perceived as a dysbiosis of host and microflora as a result of a dysregulated immune response.<sup>9</sup> Nonetheless, it remains unclear whether the immune system is activated as a result of an intrinsic defect (either constitutive activation or the failure of down-regu-

latory mechanisms) or because of continued stimulation resulting from a change in the epithelial mucosal barrier or compositional change of the microbiota. Population studies have identified an increased risk of colon cancer in both types of IBD; ulcerative colitis and Crohn's disease.<sup>10</sup> The risk of acquiring CRC in IBD patients depends on the degree by which colon mucosa is exposed to the inflammation as determined by the duration,<sup>11,12</sup> extent,<sup>11,13</sup> and severity<sup>14</sup> of the disease.

IBD-associated and sporadic CRC share biological similarities: they both develop in a sequential manner and exhibit similar key genetic alterations. These alterations coincide with the pathological development of the tumor in both conditions. Chronic inflammation has been long known to be a major driving force in the development and progression of colitis-associated cancer (CAC). Recent findings over the past years suggest that inflammatory pathways can also contribute to the pathogenesis of sporadic CRC. This review aims to summarize the existing experimental and clinical evidence that suggest chronic subclinical inflammation could contribute to the pathogenesis of sporadic CRC.

## Inflammation in APC Mouse Model

*APC* is the most commonly mutated gene in sporadic CRC. Since the loss of *APC* is an early event in sporadic CRC, multiple *APC*-deficient mice have been extensively used to study CRC.<sup>15</sup> The *Apc*<sup>min/+</sup> mice have a heterozygous mutation in the *APC* gene and spontaneously develop numerous intestinal polyps, which do not progress into carcinomas. When acute inflammation is induced by adding dextran sodium sulfate (DSS) to drinking water the frequency of intestinal tumors increases, specifically at distal segments of the small intestine. Moreover, DSS-treated *Apc*<sup>min/+</sup> mice tend to develop colon tumors more frequently and at higher multiplicity.<sup>16</sup> This shows that inflammation can exacerbate the *APC*-driven colon tumorigenesis. In a different approach, *Apc*<sup>min/+</sup> mice develop significantly more tumors with higher dysplastic grade when they are inoculated with pro-inflammatory CD4+ effector T cells ( $T_E$ ). In contrast, transferring anti-inflammatory CD4+ regulatory T cells ( $T_R$ ) significantly reduces the number of tumors compared to both *Apc*<sup>min/+</sup> and *Apc*<sup>min/+</sup> with  $T_E$  cells.<sup>17</sup> This data suggests the interplay of the immune system in the intestinal tumorigenesis.<sup>18</sup>

Isolated lymphoid follicles (ILFs) are the focal aggregation

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of immune cells in the colon mucosa and together with Peyer's patches, constitute the gut-associated lymphoid tissue. Although the numbers of ILFs are increased in CRC,<sup>19</sup> they remain unchanged in *Apc*<sup>min/+</sup> mice.<sup>20</sup> However, intestinal lymphocyte infiltration is diffusely increased in the latter.<sup>21</sup>

## Reactive Oxygen and Nitrogen Species

Oxidative and nitrosative stress is a common feature in chronic inflammatory conditions, where reactive oxygen and nitrogen species (RONS) are generated in abundance by immune cells as a defense mechanism against the inflammatory stimuli.<sup>22</sup> Inflammation results in the overexpression of inducible nitric oxide synthase (iNOS) in epithelial and immune cells, which generates nitric oxide (NO) in the inflamed tissue. NO is a physiologically important molecule, yet when produced in excess, it may be involved in cellular pathological pathways.<sup>23</sup> iNOS is highly expressed in colon adenoma and carcinoma, but not in the normal adjacent tissue.<sup>24</sup> *Apc*<sup>min/+</sup> mice which lack one or two iNOS alleles develop significantly fewer colon tumors compared to *Apc*<sup>min/+</sup> *iNOS*<sup>+/+</sup> controls.<sup>25</sup>

ONO-1714 is a selective iNOS inhibitor. *Apc*<sup>min/+</sup> mice treated with DSS in drinking water for one week who received ONO-1714 afterwards had significantly longer large bowels and a lower inflammation score compared to *Apc*<sup>min/+</sup> counterparts, which were not given the inhibitor. iNOS inhibition significantly lowered the multiplicity of large bowel adenocarcinoma in *Apc*<sup>min/+</sup> mice treated with DSS.<sup>26</sup>

## Cyclooxygenase 2

Cyclooxygenase 2 (COX2) is another mediator that is upregulated in the inflammatory milieu. It is responsible for the oxidative conversion of arachidonic acid into prostaglandins and other eicosanoids, which in turn can induce the expression of pro-inflammatory cytokines. COX2 is absent in normal colon epithelium, but is up-regulated in IBD-associated<sup>27</sup> and sporadic CRC.<sup>28,29</sup> *Ptgs-2* (prostaglandin-endoperoxide synthase 2) is the mouse homologue of human COX2. *Apc*<sup>min/+</sup> mice deficient in *Ptgs-2* (*Apc*<sup>min/+</sup> *Ptgs-2*<sup>-/-</sup>) develop significantly fewer adenomatous polyps in the distal small intestine and colon compared to *Apc*<sup>min/+</sup> *Ptgs-2*<sup>+/+</sup>.<sup>30</sup>

Prostaglandin E2 (PGE2) is one of the end products of the COX2 enzyme, which also increases in adenomatous polyps and CRC.<sup>31</sup> Inducible (microsomal) PGE2 synthase (mPGES-1) is the enzyme that converts the final precursor to PGE2. *Apc*<sup>Δ14/+</sup> *mPGES-1*<sup>-/-</sup> mice have a germline heterozygous frameshift mutation in *APC* and are deficient in PGE2 in the small intestine and colon. They develop significantly fewer and smaller tumors in both the small intestine and colon.<sup>32</sup> This can explain the observed association of non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit COX enzymes, and the lower incidence of CRC in humans and rodents.<sup>33-36</sup> Several randomized clinical trials have found that Aspirin decreases the risk of CRC after long-term use.<sup>37-42</sup>

## Cytokines

Distinct cytokine gene expression is a known concomitant feature of many cancers. The role of cytokines in the pathogenesis of IBD and associated dysplasia/neoplasia is well established. On the other hand, the association of several cytokines with sporadic

CRC is being uncovered by rapidly growing data. Different levels of cytokines have been found both in the serum and in the colon biopsy of CRC patients compared to normal controls.<sup>43-45</sup> The tumor microenvironment consists of multiple inflammatory cells and their mediators. However, to address the possibility of the role of cytokines in tumorigenesis, earlier stages of colorectal carcinogenesis still need to be investigated. The distinct pattern of immune cell infiltration and cytokine secretion at early adenoma compared to carcinoma<sup>46</sup> is suggestive of the distinct role of inflammation at various stages of the tumorigenesis process.

In this section, the role of tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-6, IL-10, and transforming growth factor (TGF)- $\beta$  in the sporadic CRC will be discussed. The role of these cytokines has been studied in both CAC and sporadic CRC mouse models. TNF- $\alpha$  and IL-6 are pro-inflammatory, whereas IL-10 and TGF- $\beta$  are anti-inflammatory cytokines. Biochemical neutralization of TNF- $\alpha$  or genetic ablation of its receptor have varied effects on inflammation, but significantly reduce the intestinal tumor incidence and dysplastic changes in the AOM/DSS mouse model of CAC.<sup>47,48</sup> Although the genetic ablation of TNF- $\alpha$  in *Apc*<sup>min/+</sup> mice does not decrease the incidence of colon tumors in the sporadic CRC model<sup>49</sup>, the anti-TNF- $\alpha$  antibody significantly decreases the number of intestinal tumors compared to untreated *Apc*<sup>min/+</sup>.<sup>17</sup> IL-6 deficient (*IL-6*<sup>-/-</sup>) and *Apc*<sup>min/+</sup> *IL-6*<sup>-/-</sup> mice both develop fewer colon tumors compared to wild type and *Apc*<sup>min/+</sup> mice, respectively.<sup>50,51</sup> IL-17A is a downstream effector of IL-6. *Apc*<sup>min/+</sup> *IL-17A*<sup>-/-</sup> mice also develop significantly fewer tumors in both the small and large intestine when compared to *Apc*<sup>min/+</sup> not deficient in IL-17A.<sup>21</sup> IL-10 is a key anti-inflammatory cytokine, and its deficiency in IL-10 knockout mice is associated with spontaneous colitis and CAC.<sup>52</sup> Similarly, loss of IL-10 increases intestinal tumor multiplicity in the *Apc*<sup>min/+</sup> model of sporadic CRC.<sup>53</sup> The TGF- $\beta$  signaling pathway is shown to be frequently inactivated in CRC.<sup>54</sup> In a mouse model of sporadic CRC, the intestinal loss of TGF- $\beta$  signaling increases the rate of high grade dysplasia and adenocarcinoma.<sup>55</sup>

Other cytokines have also been investigated for their role in CRC. However, despite the observed effect of individual cytokines in the pathogenesis of CRC, their effect cannot be fully elucidated unless the cytokine network is systematically analyzed for the spatiotemporal gene expression patterns in the course of tumorigenesis.

## Nuclear Factor kappa B

Nuclear factor kappa B (NF- $\kappa$ B) is a transcription factor implicated in bridging inflammation and cancer. NF- $\kappa$ B can be activated in epithelial and immune cells by a diverse range of stimuli from intestinal microbiota, cytokines, and cellular products. Activated NF- $\kappa$ B is translocated to the nucleus where it binds to the sequence consensus in the promoter region of its target genes and induces the expression of multiple inflammatory cytokines in the immune cells and anti-apoptotic, and oncogenic genes in the malignant cells.<sup>56</sup> NF- $\kappa$ B is implicated in intestinal tumorigenesis in the chronic inflammatory setting in IBD. Inhibition of NF- $\kappa$ B activity by small interfering RNAs or chemical inhibitors ameliorates the intestinal inflammation in response to DSS treatment.<sup>57-59</sup> Intestinal epithelial cell-specific lack of canonical NF- $\kappa$ B pathway activity causes spontaneous pancolitis and increases the expression of cytokines in the colon mucosa.<sup>60</sup> In CAC models

where tumorigenesis is chemically induced by AOM/DSS, abrogation of the canonical NF- $\kappa$ B pathway in the colon epithelium reduces the number of tumors. Partial suppression of NF- $\kappa$ B activity in myeloid cells markedly reduces the expression of multiple inflammatory cytokine genes as well as tumor incidence.<sup>61</sup> Likewise, chemical inhibition of NF- $\kappa$ B pathway in AOM/DSS-induced CAC suppresses inflammation and reduces intestinal tumor incidence.<sup>62</sup>

NF- $\kappa$ B is constitutively active in sporadic CRC.<sup>63</sup> Its activity is increased in colon adenoma compared to normal mucosa in *Apc*<sup>min/+</sup> mice and CRC patients<sup>64-68</sup> and is inversely correlated with tumor differentiation.<sup>65,67</sup> In sporadic CRC, NF- $\kappa$ B target genes are differentially expressed in malignant tissue compared to normal mucosa. Moreover, these genes had distinct expression patterns within the tumor tissue. The invasive front of the tumor was found to have a higher expression of multiple NF- $\kappa$ B target inflammatory genes compared to the center of the tumor.<sup>69</sup> It has also been shown that single nucleotide polymorphism in the NF- $\kappa$ B gene is associated with an increased risk of sporadic CRC.<sup>70,71</sup> All together these data suggest a role for the NF- $\kappa$ B pathway in the sporadic CRC tumorigenesis that needs to be further investigated.

## Microbiota

The human intestine is home to a variety of microorganisms that live in a mutually beneficial state with the host. Intestinal epithelium provides a physical barrier against the microbiota. The first attempt to link microbiota to colon carcinogenesis was made decades ago.<sup>72</sup> However, the mechanisms by which microbiota could contribute to sporadic CRC are largely unknown. Commensals can contribute to the tumorigenesis process through multiple mechanisms: they can convert luminal ingredients into carcinogens, generate reactive oxygen species, hydrogen sulfide, and N-nitrosocompounds (which can cause DNA damage), and they are capable of inciting inflammation.<sup>73</sup> Animal studies clearly demonstrate that germfree animals develop relatively fewer intestinal tumors compared to gnotobiotic and conventional counterparts (germfree animals which were fed strains of enteric bacteria or feces).<sup>74,75</sup> In addition, gastrointestinal infection with *Citrobacter rodentium*, *Bacteroides fragilis*, and *Helicobacter pylori* has been found to activate the Wnt/ $\beta$ -catenin pathway,<sup>76-78</sup> which is the principal pathway responsible for normal intestinal homeostasis and tumorigenesis. The composition of intestinal microflora is altered in sporadic CRC patients compared to normal controls,<sup>73,79</sup> however its significance needs to be elucidated.

There is a constant interaction between intestinal mucosa and microbiota. Both intestinal epithelial and immune cells express pattern-recognition receptors (PRRs), which are a group of cell surface or cytoplasmic proteins. They detect the conserved structural units of microorganisms and are activated by bacterial stimuli.<sup>80</sup> Toll-like receptors (TLRs) and NOD-like receptors (NLRs) are two major classes of PRRs implicated in the intestinal inflammation and tumorigenesis and will be discussed in more detail.

## Pattern-Recognition Receptors

TLRs and NLRs are the first line of defense in the intestinal epithelium, and are responsible for distinguishing pathogenic and commensal bacteria. Any alteration in their function can result in dysregulated immune response and inflammation. The mechanism

by which they may play a role in tumorigenesis is under rigorous investigation.

TLRs have a specialized function and build a complex network of bacteria recognition properties with distinct roles. Under normal circumstances, only a subset of TLRs is detectably expressed in the intestinal epithelium. However, TLRs are differentially expressed in the intestinal epithelium of IBD patients<sup>81-83</sup> and IBD-associated CRC.<sup>84</sup> Several TLRs are constitutively expressed in the mouse MC26 colon cancer cell line and are upregulated in inflamed mucosa and tumors in the mouse model of CAC. TLR4 is the most highly expressed TLR in both colon cancer cell lines and CAC.<sup>84,85</sup> TLR4 activity can induce COX2 expression. Mice deficient in TLR4 (*TLR4*<sup>-/-</sup>) demonstrate lower epithelial proliferation and higher apoptosis rates upon DSS-induced injury, which is rescued by oral PGE2.<sup>86</sup> These mice show a decreased level of inflammatory cell recruitment to the colon and an increased bacterial translocation to the mesenteric lymph node compared to the wild type.<sup>87</sup> A distinct microflora composition has been observed in *TLR2*<sup>-/-</sup> *TLR4*<sup>-/-</sup> vs. wildtype mice in DSS-induced colitis that suggests an association between TLRs and the composition of microflora.<sup>88</sup> *TLR4*<sup>-/-</sup> mice develop significantly fewer visible tumors and microscopic dysplasia compared to the wildtype when treated with AOM/DSS.<sup>84</sup>

When TLR4 is inactivated in the CRC xenograft in wildtype mice, tumor growth is suppressed and survival increased.<sup>85</sup> On the other hand, deficiency of TLR5 or MyD88 (the main downstream adaptor of the TLR signalling) in CRC xenograft in nude mice confers a growth advantage to the tumor, while the early activation of TLR5 in this model suppresses CRC tumor growth.<sup>89</sup> A higher expression of TLR4 and MyD88 is observed in CRC tumor tissue compared to normal mucosa and adenoma. Disruption of TLR signalling in *Apc*<sup>min/+</sup> *MyD88*<sup>-/-</sup> mice does not alter the number of microadenomas while reducing the incidence of visible tumors. In accordance with the lack of histological evidence of inflammation, *Apc*<sup>min/+</sup> *MyD88*<sup>-/-</sup> have significantly lower levels of COX2, IL-6, and TNF- $\alpha$  compared to *Apc*<sup>min/+</sup> controls.<sup>90</sup>

NLRs are cytoplasmic PRRs that also detect luminal bacterial signals. NOD1 and NOD2 activate NF- $\kappa$ B, whereas NLPR3, 4, and 6 are involved in the inflammasome-mediated caspase-1 activation. The role of various NLRs in the intestinal tumorigenesis has been studied mainly in mouse models of CAC. *Nod1*<sup>-/-</sup>, *Nlrp3*<sup>-/-</sup>, *Nlrc4*<sup>-/-</sup>, and *Nlrp6*<sup>-/-</sup> mice have a higher tumor rate compared to wildtype when challenged with AOM/DSS.<sup>91-94</sup>

TLRs and NLRs regulate the expression of pro-inflammatory cytokines in response to microbial or damage-induced signals. However, their role in the context of sporadic CRC is yet to be further explored.

## Conclusion

The inflammatory response involves an intricate complex of mediators that are secreted from epithelial, stromal, and immune cells in response to external and internal stimuli. The role of chronic inflammation in the development of dysplasia in IBD is well established, while our knowledge of inflammatory components in sporadic CRC is rapidly growing. Future studies are required to shed light on the unknown aspects of inflammation in sporadic colon cancer, and address the important questions in the field from the potential role of microbiota in the pathogenesis to the use of anti-inflammatory drugs in the management of the dis-

ease. Better understanding of these pathways would help improve the preventive, predictive, and therapeutic interventions in CRC.

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