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REVIEW

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Inflammasomes in Colitis and Colorectal Cancer: Mechanism of Action and Therapies

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Colorectal cancer is a multifactorial disease and a leading cause of cancer-related deaths worldwide. Inflammation is a driver across multiple stages in the development of colorectal cancer. The inflammasome is a cytosolic multiprotein complex of the innate immune system central to the regulation of inflammation, pyroptosis, and other cellular processes important for maintaining gut homeostasis. Studies using mouse models of colitis and colitis-associated colorectal cancer have highlighted diverse and sometimes contrasting roles of inflammasomes in maintaining a balance between intestinal barrier function and the gut microbiota. In addition, persistent and/or dysregulated stimulation of inflammasome sensors finetune inflammation and tumorigenesis in the intestine. This review highlights the emerging role of inflammasome signaling in colitis and colitis-associated colorectal cancer. We also review the key mechanisms by which inflammasome signaling modulate inflammation and tumor development. Finally, we speculate the importance of using more tightly regulated experimental approaches to examine the role of gut microbiota in colorectal cancer.

INTRODUCTION

Colorectal cancer (CRC†) is the second and third most commonly diagnosed cancer in females and males, respectively, contributing to 1.7 million newly diagnosed cases in 2015 [1]. CRC causes 832,000 deaths annually, with more than half of the cases occurring in developed countries [1,2]. The geographic differences in the incidence and the risk of developing CRC are influenced by genetic and environmental factors, and dietary habits [1]. Notably, patients with inflammatory bowel disease (IBD) have higher risk of developing CRC as compared to healthy individuals and their prognosis of CRC is worse than that of CRC patients without IBD [3]. The molecular basis of CRC is underpinned by the interaction between host immunity and gut microbiota along with contribut-

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†Abbreviations: AKT, serine/threonine-protein kinase, also known as protein kinase B; AOM, azoxymethane; APC, adenomatous polyposis coli; CRC, colorectal cancer; DAMPs, danger-associated molecular patterns; DNA-PK, DNA-dependent protein kinase; DSS, dextran sulfate sodium; ERK, extracellular signal-regulated kinase; IBD, inflammatory bowel disease; IECs, intestinal epithelial cells; iNOS, inducible nitric oxide synthase; KO mice, knockout mice; LPS, lipopolysaccharide; MPO, myeloperoxidase; mTOR, mechanistic target of rapamycin, originally known as mammalian target of rapamycin; NF-κB, nuclear factor-kappa B; NK cells, natural-killer cells; PAMPs, pathogen-associated molecular patterns; PI3K, phosphoinositide 3-kinase; STAT1, signal transducer and activator of transcription 1; STAT3, signal transducer and activator of transcription 3; TNF, tumor necrosis factor; WT, wild-type.

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ing factors such as intestinal injury, oxidative stress, and chronic inflammation [4]. Inflammatory and cell death responses triggered by innate immune signaling pathways, including the inflammasome, contribute to the pathogenesis of colitis and colitis-associated CRC [5].

Inflammasomes are high-molecular-weight cytoplasmic multi-protein complexes composed of one or more inflammasome sensors, an adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC; also called PYCARD) and the cysteine protease caspase-1 [6]. To date, receptors from NOD-like receptor families (NLRs), including NLRP1, NLRP3, NLRC4, and apoptosis inhibitory proteins (NAIPs) have been confirmed to assemble inflammasomes [5,7]. In addition to NLRs, absent in melanoma 2 (AIM2) from the AIM2-like receptor family (ALRs) and Pyrin from the tripartite motif-containing protein family also trigger the formation of inflammasomes [7,8]. Further, NLRP6, NLRP7, NLRP9, NLRP12, the DNA sensor IFNγ-inducible protein 16 (IFI16), and the RNA sensor retinoic acid-inducible gene I protein (RIG-I, also called DDX58) have been reported to promote caspase-1 activation, but their ability to form an inflammasome complex requires further confirmation [5,7,9]. In this review, we highlight recent development in our understanding of inflammasomes in colitis and CRC and outline the therapeutic potential of modulating inflammasome responses.

INFLAMMASOME BIOLOGY AND BASIC MECHANISMS

An inflammasome complex can be activated via a canonical or non-canonical pathway [5,7]. Activation of the canonical inflammasome pathway does not usually require caspase-4 and caspase-5 in humans or caspase-11 in mice, whereas activation of the non-canonical pathway is defined by its requirement for these inflammatory caspases [5,7]. In the canonical pathway, inflammasome sensors which carry a pyrin domain but lacking a caspase recruitment domain (CARD), including NLRP3, AIM2, and Pyrin, bind to the adaptor protein ASC in response to pathogen-associated molecular patterns (PAMPs) and/or danger-associated molecular patterns (DAMPs) [10]. ASC further promotes the recruitment and activation of caspase-1. However, inflammasome sensors carrying a CARD, such as NLRP1 and NLRC4, can recruit and activate caspase-1 with or without ASC in response to PAMPs and/or DAMPs [8]. ASC and caspase-1 undergo self-oligomerization and nucleation, forming a functional inflammasome complex.

The non-canonical inflammasome refers to a specific activation pathway initiated by caspase-4, caspase-5 or caspase-11 [11]. Intracellular lipopolysaccharide (LPS) directly binds to these caspases, promoting their self-oligomerization leading to pyroptosis and activation of the NLRP3 inflammasome [12-14]. Emerging evidence suggests that NLRP6 can recruit both caspase-1 and caspase-11 via ASC in response to cytosolic lipoteichoic acid or infection with the Gram-positive bacterium *Listeria monocytogenes* [15], blurring the definition of the canonical inflammasome versus the non-canonical inflammasome.

Regardless of the apparent converging roles of inflammatory caspases in the canonical and non-canonical pathways, caspase-1 and caspase-11 mediate cleavage of the pore-forming protein gasdermin D (GSDMD) [12-14], separating the effector N-terminal domain from the inhibitory C-terminal domain of GSDMD [12,14,16]. The N-terminal domain of GSDMD inserts into the cell membrane and forms a pore of up to 14 nm in inner diameter, mediating the release of the mature cytokines IL-1β and IL-18 and other biomolecules [17-21]. The pores also lead to cell swelling, lysis, and pyroptosis [12,14,16]. The functions of cytokine secretion and pyroptosis are numerous, including induction of immune cell recruitment to promote inflammation and antimicrobial functions, all of which are contributing factors in the pathogenesis of colitis and colitis-associated CRC [22].

INFLAMMASOMES IN COLITIS AND CRC

Most of the research into inflammasomes and colitis and colitis-associated CRC have been based on the use of mouse models [23,24]. Mice develop colitis and colitis-associated CRC in response to chemicals, alterations in genetics, or adoptive transfer of cells from certain host to another [23]. In mice, colitis can be induced by chemicals such as dextran sulfate sodium (DSS), oxazolone (4-ethoxylmethylene-2-phenyloxazol-5-one) and trinitrobenzene sulfonic acid (TNBS) [23]. For example, DSS is a water-soluble polymer of sulfated polysaccharide [23,25]. Administration of DSS in drinking water causes hyperosmotic damage to the epithelial monolayer of the colon [23,26]. This damage triggers an immune response to alter the mucosal barrier function in the colonic epithelium, allowing luminal antigens to access the mucosal immune system [23,26]. Short-term treatment of mice with DSS results in body weight loss, bloody diarrhea, intestinal inflammation, ulcerations, and shortening of the colon, all of which are hallmarks of colitis [23,26]. Injection of the DNA damaging agent azoxymethane (AOM) into mice combined with multiple cycles of DSS leads to the development of colitis-associated CRC [23,26]. AOM is metabolized into methylazoxymethanol to induce methylation of the O^6 position of guanine resulting in G → A transitions, the primary pro-mutagenic lesion caused by AOM [23,26].

Other than DSS, the haptenating agent oxazolone

also results in hemorrhagic colonic inflammation and severe submucosal edema in mice [27]. The cellular and cytokine responses observed in oxazolone-induced colitis is mediated by IL-4 and IL-13-producing natural-killer (NK) cells [27]. Another haptenating agent, TNBS, induces transmural colitis in mice, which is characterized by severe diarrhea, weight loss, and rectal prolapse [23,27]. The inflammatory responses induced by TNBS is mainly driven by a T_H1-mediated immune response accompanied by infiltration of $CD4^+$ T cells, neutrophils, and macrophages in the lamina propria [23,27].

In addition to chemical-induced colitis, adoptive transfer of naïve $CD4^+$ T cells ($CD4^+CD45RB^{high}$ T cells) from wild-type (WT) mice into immunodeficient (SCID or *Rag1*$^{-/-}$) mice results in colonic intestinal inflammation after 5 to 10 weeks [27]. These naïve $CD4^+$ T cell populations lack *Forkhead box* P3 (*FoxP3*)$^+$ regulatory T cells (Treg), which function to suppress inflammation by the production of anti-inflammatory cytokines, such as IL-10, IL-35, and TGF-β [27]. Notably, earlier studies have highlighted an immunoregulatory role of IL-10 in colitis [28,29]. The administration of anti-IL-10 receptor antibodies or transfer of $CD4^+$ T cells deficient in IL-10 to *Rag1*$^{-/-}$ mice resulted in severe colitis, suggesting a protective role of IL-10 [28,29]. In addition, enteric microbiota has a major role in IL-10-mediated spontaneous colitis [23]. The Gram-negative bacterium *Helicobacter hepaticus* triggers colitis in specific-pathogen-free *Il10*$^{-/-}$ mice through an IL-12- and IFN-γ-dependent mechanism [30].

Inflammation is one of the major risk factors for colitis and colitis-associated CRC, however, CRC can also develop largely independent of inflammation [4,31]. Mutational inactivation of tumor-suppressor genes is primarily responsible for the development of CRC [31]. One of these genes is adenomatous polyposis coli (*Apc*). The *Apc* gene encodes a scaffold protein called APC that functions to sequester oncoprotein β-catenin into the cytoplasm of resting cells and prevents cellular overgrowth [26]. Patients with familial adenomatous polyposis carry a germline mutation in one *APC* allele and are at a higher risk for developing CRC [32]. Similar to humans, mice carrying a heterozygous nonsense mutation at codon 850 of *Apc* (called *Apc*$^{Min/+}$ mice), analogous to that seen in patients with familial adenomatous polyposis, spontaneously develop tumors in the small and large intestine within 6 to 10 weeks from birth [26,32]. Thus, *Apc*$^{Min/+}$ mice are a useful model to study the development of CRC not generally associated with inflammation [26,32]. These animal models have provided important biological tools to more closely examine the molecular mechanisms of colitis and CRC and to test the efficacies of novel and emerging therapeutics.

CASPASE-1 AND CASPASE-11

Research into the biology of caspase-1 and caspase-11 has revealed unique and overlapping functions of these inflammasome executors in colitis and colitis-associated CRC [33]. Previous studies characterizing mice with a targeted deletion of the gene encoding caspase-1 (also known as ICE or Interleukin-1 beta-converting enzyme) were confounded, owing to a germline passenger mutation in the gene encoding caspase-11 that potentially causes rapid decay of its mRNA [13]. The targeted deletion of the *Casp1* gene was made in embryonic stem cells from the 129-mouse strain which naturally carries the passenger caspase-11 mutation [34,35]. The *Casp11* transcript from 129S6 mice lacked sequences encoded by exon 7 [13]. In addition, macrophages from three additional 129 substrains, 129P3, 129S1, and 129X1, also lack detectable expression of caspase-11 [13]. Despite extensive backcrossing to the C57BL/6 background, the close proximity of the caspase-1 and caspase-11 loci prevented their segregation, and hence, the backcrossed strain lacks both caspase-1 and caspase-11 expression [13]. Therefore, results obtained from these previous studies attributing functions to caspase-1 require re-evaluation.

In this review, we will for consistency refer *Casp1*$^{-/-}$ mice or ICE$^{-/-}$ mice in these previous studies as *Casp1*$^{-/-}$ *Casp11*$^{-/-}$ (also known as *Casp1*$^{-/-}$*Casp11*$^{129mt/129mt}$) mice [36]. *Casp1*$^{-/-}$ *Casp11*$^{-/-}$ mice were initially thought to be more resistant to DSS-driven colitis, potentially due to reduced release of IL-1β, IL-18, and IFN-γ in the colon [37]. Subsequent studies revealed that *Casp1*$^{-/-}$ *Casp11*$^{-/-}$ mice are hypersusceptible to colitis and colitis-associated CRC induced by AOM and DSS [38-43]. In addition to increased morbidity, weight loss, and colon inflammation, these mice have reduced levels of IL-1β and IL-18 in the colon [38-43]. *Casp1*$^{-/-}$ *Casp11*$^{-/-}$ mice backcrossed to the C57BL/6J background treated with oxazolone were substantially more prone to weight loss and intestinal damage compared to WT C57BL/6 mice purchased from Charles River [44]. It is important to note that littermate controls were not used in many studies (Table 1). Therefore, results from these studies should be interpreted with care given WT mice and gene-deficient mice bred independently from one another or have a subtle difference in their genetic background may have differences in their gut microbiota composition [45] (discussed further below).

Under certain genetic predisposition, however, caspase-1 and caspase-11 may exacerbate inflammation and have detrimental effects in the colon. For example, a study found that deletion of the genes encoding the mucus layer component, core 1– and core 3–derived mucin-type O-linked oligosaccharides, in mice (*C1galt1*$^{-/-}$*C3Gnt*$^{-/-}$ mice), causes spontaneous colitis and CRC, and deletion

Table 1. Role of inflammasomes and their components in mouse models of colitis.

Gene	Model	Control	Mutant Mouse Phenotype	Suggested Mechanism	References
Aim2	KO mice DSS	Not specified	Sensitive	• Decreased IL-1β and IL-18 • Higher colonic burden of *E. coli* • Reduced expression of AMPs	[43]
	KO mice DSS	Littermates	No difference	• Equal colonic burden of *E. coli* and similar bacterial growth in both WT and *Aim2*$^{-/-}$ mice	[43]
	KO mice DSS	Not specified	Sensitive	• Dysfunctional IL-18/IL-22BP/IL-22 signaling axis • Elevated STAT3 activation • Reduced expression of AMPs	[107]
	KO mice DSS and T-cell transfer colitis	Littermates	Resistant	• Not described	[54]
Asc	KO mice DSS	Littermates; Non-littermates; Co-housed; separate WT and KO colonies	Sensitive	• Decreased IL-1β and IL-18 • Impaired goblet cell functions leading to increased susceptibility to enteric infection	[38-42,53-55]
Caspase-1/11	KO mice DSS	Not specified	Sensitive	• Decreased IL-1β and IL-18 • Enhanced NF-κB signaling • Increased epithelial barrier permeability • Impaired epithelial cell regeneration and tissue repair	[40,41]
	KO mice DSS	Littermates	Sensitive	• Increased growth of *E. coli* • Decreased expression of AMPs	[43]
	KO mice DSS	Not specified	Resistant	• Decreased IL-1β, IL-18 and IFN-γ	[37]
	KO mice DSS	Not specified	No difference	• Not described	[87]
Caspase-1	KO mice DSS	Littermates	Resistant	• Decreased IL-1β and IL-18 • Reduced goblet cell numbers	[45]
Caspase-11	KO mice DSS	Littermates	Sensitive	• Impaired IL-18 production • Decreased cell proliferation and increased cell death	[49]
	KO mice DSS	Not specified	Sensitive	• Increased production of IL-1β, IL-18 and HMGB1 • Decreased IL-1β and IL-18	[47,48]
	KO mice DSS	Separate WT and KO colonies Littermates	Variable phenotype	• Not described	[50]
	KO mice Spontaneous (*Il10*$^{-/-}$ *Casp11*$^{-/-}$)	Littermates	No difference between *Il10*$^{-/-}$ vs. *Il10*$^{-/-}$ *Casp11*$^{-/-}$	• Increased colonic *Caspase-11* mRNA	[50]

	KO mice DSS	Not specified	No role or resistant	•	Not described	[45]
Il-1β	KO mice DSS	Not specified	Sensitive	•	Increased influx of neutrophils in the colon	[63]
	KO mice DSS	Not specified	Resistant	•	Not described	[64]
	KO mice DSS	Not specified	No difference	•	Not described	[42]
	Spontaneous (*H. hepaticus* infected 129SvEv *Rag2*$^{-/-}$ mice)	Not specified	Sensitive	•	Accumulation of proinflammatory $CD11b^{+}Gr1^{Hi}$ granulocytes and IL-17A producing lymphocytes	[58]
				•	Increased $CD4^{+}$ T_H17 cell differentiation	
Il-18	KO mice DSS	Littermates	Sensitive	•	Depletion of goblet cells	[69,74]
				•	Increased levels of IFN-γ	
	KO mice DSS	Not specified	Sensitive	•	Increased growth of *E. coli*	[43]
Mefv	KO mice DSS	Not specified	Sensitive	•	Increased levels of IL-6 and STAT3 and decreased IL-18 and IFN-γ	[111]
				•	Loss of the tight junction proteins occludin and claudin-2	
Naip1-6	KO mice DSS	Non-littermates	Resistant	•	Increased expression of *Il-22* and anti-apoptotic genes	[103]
Nlrc3	KO mice DSS	Littermates	Sensitive	•	Increased IL-1β, IL-6, and TNF	[120]
				•	Enhanced PI3K/AKT/mTOR signaling	
Nlrc4	KO mice DSS	Littermates	Sensitive	•	Decreased IL-18	[104]
Nlrp1a and *Nlrp1*	KO mice DSS	Littermates	Resistant	•	Increased IL-18 and IFN-γ production	[79]
				•	Reduced T_H1 cell response	
				•	Decreased butyrate production by *Clostridiales*	
Nlrp1b	KO mice DSS	Littermates; Co-housed WT and KO colonies	Sensitive	•	Decreased IL-1β and IL-18	[53]
Nlrp3	KO mice DSS	Not specified	Sensitive	•	Decreased IL-1β and IL-18	[40]
	KO mice DSS	Not specified	Sensitive	•	Increased mucus production through NLRP3/caspase-1/IL-18 axis by *H. pylori* extract or live bacteria	[121]
	KO mice DSS	Not specified	Resistant	•	Decreased IL-1β, TNF and IFN-γ	[86]
	KO mice DSS or TNBS	Not specified	Sensitive	•	Increased IL-18 maturation	[38]
				•	Decreased cell proliferation	
				•	Increased epithelial barrier permeability	
	KO mice DSS or TNBS	Littermates; Separate WT and KO colonies	Sensitive	•	Decreased IL-1β, IL-10 and TGF-β	[84]

	KO mice Oxazolone	Not specified	Sensitive	• Decreased IL-1β and IL-18	[44]
	Spontaneous (*Nlrp3*^{R258W}*Rag1*$^{-/-}$)	Littermates	Sensitive	• Enhanced IL-1β signaling • Development of local Treg cells	[122]
	Spontaneous (*Il10*$^{-/-}$)	Not specified	Inhibition of NLRP3 and its component confers protection	• Increased IL-1β production	[88,89]
Nlrp6	KO mice DSS	Not specified	Sensitive	• Impaired IL-18 signaling	[42,91]
	Spontaneous (*Il10*$^{-/-}$*Nlrp6*$^{-/-}$)	Littermates; Co-housed WT and KO colonies	Sensitive	• Expansion of mucolytic *A. muciniphila*	[93]
Nlrp12	KO mice DSS	Not specified	Sensitive	• NF-κB and ERK activation in macrophages • Elevated non-canonical NF-κB activity • Increased activation of NF-κB, ERK, and STAT3 in the colon	[123-125]

Abbreviations: AKT: serine/threonine-protein kinase, also known as protein kinase B; AMPs: anti-microbial peptides; *A. muciniphila*: *Akkermansia muciniphila*; *E. coli*: *Escherichia coli*; ERK: extracellular signal-regulated kinase; *H. hepaticus*: *Helicobacter hepaticus*; HMGB1: high-mobility group box 1; *H. pylori*: *Helicobacter pylori*; IL-22BP: interleukin-22 binding protein; IFN-γ: interferon-γ; KO mice: knockout mice; mTOR, mechanistic target of rapamycin, originally known as mammalian target of rapamycin; NF-κB: nuclear factor-kappaB; PI3K: phosphoinositide 3-kinase; STAT3: signal transducer and activator of transcription 3; TGF-β: transforming growth factor beta; TNF: tumor necrosis factor.

of genes encoding caspase-1 and caspase-11 in these mice reduces their susceptibility to colitis and colorectal tumorigenesis [46].

Studies have now further defined the roles of inflammatory caspases in colitis and colitis-associated CRC using *Casp1*$^{-/-}$ mice and *Casp11*$^{-/-}$ mice generated on the C57BL/6N genetic background [45,47-50]. A study using WT and *Casp1*$^{-/-}$ mice with standardized microbiota has found that *Casp1*$^{-/-}$ mice are resistant to colitis and develop fewer colonic tumors upon AOM and DSS administration as compared with WT mice [45]. Further experiments using cell type-specific ablation of caspase-1 demonstrated that the expression of caspase-1 in intestinal epithelial cells (IECs) and not in myeloid cells was responsible for this phenotype [45]. In addition, both *Casp1*$^{-/-}$ mice and *Casp1*$^{\Delta IEC}$ mice (mice lacking *Casp1* in the IECs) had reduced processing of IL-18 and reduced number of goblet cells compared with littermate WT mice [45]. Goblet cells provide mucus layer coating the gastrointestinal tract and function as a front line of innate host defense against invasive micro-organisms and intestinal injury [51].

The functional roles of caspase-11 in colitis and colitis-associated CRC have been contradictory [45,47-49]. Several studies found that caspase-11 deficiency in mice leads to more severe colitis following DSS exposure [47-49] and enhanced colonic tumor development following AOM-DSS exposure [52]. The hypersusceptible phenotype of *Casp11*$^{-/-}$ mice described in these studies was due to reduced IL-1β and IL-18 in the colon [48,49,52], with the susceptibility prevented following administration of both these cytokines [48,49]. Moreover, bone marrow chimera experiments have identified that caspase-11 is essential in both hematopoietic and nonhematopoietic compartments for mediating protection during acute colitis [48]. By contrast, other studies found that caspase-11 does not affect disease severity in IL-10-mediated and DSS-induced colitis models [45,50]. The opposing results observed in these studies may potentially be influenced by the microbiota. It is possible that distinct biogeography of intestinal bacteria between non-littermate mice [45,47,48] and between littermate mice [49,50] might lead to different degrees of inflammation (Table 1 and Table 2). Thus, additional research using tightly regulated experimental variables such as normalized microbiota composition and littermate controls is warranted to clarify the role of these inflammatory caspases in colitis and colitis-associated CRC.

ASC

ASC is a central adaptor protein to many inflammasome sensors [5,7]. Studies have shown that mice lacking ASC are susceptible to colitis and colitis-asso-

Table 2. Role of inflammasomes and their components in mouse models of CRC.

Gene	Model	Control	Mutant mouse phenotype	Suggested mechanism	References
Aim2	KO mice AOM-DSS	Not specified	More tumors	• Enhanced phosphorylation of AKT • Enhanced Myc signaling • Increased proliferation of intestinal stem cells	[54,106]
	KO mice Spontaneous (*Apc*$^{+/min}$)	Not specified	More tumors	• Enhanced phosphorylation of AKT	[54]
Asc	KO mice AOM-DSS	Littermates; Cohoused separate WT and KO colonies	More tumors	• Reduced IL-1β, IL-18, and TNF	[38,40,41,53,54]
Caspase-1/11	KO mice AOM-DSS	Not specified	More tumors	• Reduced IL-1β and IL-18 • Increased infiltration of macrophages in colons • Impaired STAT1 signaling • Enhanced epithelial cell proliferation	[38,40,41,87]
	KO mice Spontaneous (*C1galt1*$^{-/-}$ *C3GnT*$^{-/-}$)	Littermates	Less tumors	• Impaired inflammasome activation • Reduced MPO^{+} neutrophils • Impaired iNOS signaling	[46]
Caspase-1	KO mice AOM-DSS	Littermates	Less tumors	• Decreased IL-1β and IL-18 • Reduced inflammation and tissue damage	[45]
Caspase-11	KO mice AOM-DSS	Littermates	More tumors	• Reduced IL-1β and IL-18 • Impaired phosphorylation of STAT1	[52]
	KO-mice AOM-DSS	Not specified	No difference	• Not described	[48]
Il-1β	KO mice AOM-DSS	Not specified	No difference	• Not described	[54]
Il-18	KO mice AOM-DSS	Littermates; Not specified	More tumors	• Increased expression of • mitogenic/inflammatory cytokines • Enhanced phosphorylation of STAT-3	[39,62]
Mefv	KO mice AOM-DSS	Littermates	More tumors	• Reduced IL-18 • Impaired intestinal barrier integrity • Enhanced phosphorylation of STAT3	[111]
Naip1-6	KO mice AOM-DSS or AOM only	Littermates; Non-littermates	More tumors	• Increased STAT3 phosphorylation in epithelial cells following carcinogen exposure	[103]
Nlrc3	KO mice AOM-DSS	Littermates	More tumors	• Enhanced PI3K/AKT/mTOR signaling • Increased proliferation of intestinal progenitor stem cells	[120]
Nlrc4	KO mice AOM-DSS	Not specified	More tumors	• Enhanced colon epithelial and tumor cell proliferation	[87]

	KO mice AOM-DSS	Not specified	No difference	• Not described	[40]
Nlrp1b	KO mice AOM-DSS	Littermates	More tumors	• Reduced IL-1β and IL-18	[53]
Nlrp3	KO mice AOM-DSS	Not specified	More tumors	• Reduced IL-1β and IL-18 • Increased infiltration of macrophages in the colon	[38,40]
	Hyperactive *Nlrp3*^{R258W} mutant mice AOM-DSS	Littermates	Less tumors	• Remodeling of the gut microbiota by IL-1β	[122]
	KO mice AOM-DSS	Not specified	No difference	• Not described	[87]
Nlrp6	KO mice AOM-DSS	Not specified	More tumors	• Impaired regeneration of the colonic mucosa Enhanced epithelial proliferation and migration	[90]
Nlrp12	KO mice AOM-DSS	Not specified	More tumors	• Enhanced NF-κB, ERK, and STAT3 signaling	[123,124]

Abbreviations: AKT: serine/threonine-protein kinase, also known as protein kinase B; ERK: extracellular signal-regulated kinase; iNOS: inducible nitric oxide synthase; KO mice: knockout mice; MPO: myeloperoxidase; mTOR: mechanistic target of rapamycin, originally known as mammalian target of rapamycin; NF-κB: nuclear factor-kappa B; PI3K: phosphoinositide 3-kinase; STAT1: signal transducer and activator of transcription 1; STAT3: signal transducer and activator of transcription 3; TNF: tumor necrosis factor.

ciated CRC induced by AOM and DSS [38-42,53-55]. The increased susceptibility to colitis in these animals was demonstrated by increased morbidity, weight loss, and colon inflammation, and reduced levels of IL-1β and IL-18 in the colon [38-42,53,54]. In addition, *Asc*$^{-/-}$ mice have impaired goblet cell functions and increased susceptibility to enteric infection, both of which can predispose these mice to colitis [55]. These findings suggest that ASC has a protective role in colitis and colitis-associated CRC potentially by limiting inflammation and promoting epithelial cell repair.

Two independent studies revealed that *Asc*$^{-/-}$ mice harbor a different microbial ecology to that of WT mice [42,55]. The increased sensitivity to DSS in *Asc*$^{-/-}$ mice can be transferred to WT mice by transferring the colitis-prone microbiota from *Asc*$^{-/-}$ mice to WT recipients by means of cohousing [42,55], suggesting that the gut microbial communities and their influence on the outcome of colitis could be transferred. In contrast, prolonged separate housing of *Asc*$^{+/-}$ and *Asc*$^{-/-}$ littermates does not result in a divergent microbiota profile from one another [56], implying that ASC does not shape gut microbiota composition. These results argue against a generalized role for ASC in shaping the gut microbiota composition.

IL-1β

The proinflammatory cytokine IL-1β generates a wide range of local and systemic effects to promote inflammation [57]. In an innate immune colitis model whereby T- and B-cell–deficient 129SvEv *Rag2*$^{-/-}$ mice were infected with *H. hepaticus* to promote colitis, IL-1β promotes granulocyte recruitment and activation of T cells leading to severe intestinal inflammation [58]. Blockade of IL-1β using IL-1β-blocking monoclonal antibody prevents the accumulation of proinflammatory CD11b^{+}Gr1^{Hi} granulocytes and IL-17A-producing innate lymphoid cells in mice [58]. IL-17A is a pro-inflammatory cytokine which stimulates stromal cells to release C-C chemokines and hematopoietic cytokines, such as monocyte chemoattractant protein 1 and granulocyte-colony stimulating factor, respectively, both of which contribute to the development of intestinal inflammation [59]. In addition to IL-17, other effector cytokines, including IFN-γ, TNF, and IL-22, are also secreted by innate lymphoid cells, indicating a potential role of these cells in intestinal inflammation [60]. In a T-cell transfer chronic colitis model, IL-1β promotes differentiation of pathogenic CD4^{+} T cells and synergizes with IL-23 to sustain inflammatory responses in the intestine [58]. These studies identified distinct mechanisms through which IL-1β promotes intestinal pathology.

In the case of DSS-induced colitis, the role of the IL-1 axis is controversial [42,61-64]. A study found that

treatment of *Il1β*$^{-/-}$ mice with DSS did not result in any substantial difference in the susceptibility to colitis compared to WT mice [42], whereas other studies found that *Il1β*$^{-/-}$ mice had more severe colitis as compared to WT mice [61,63,64]. Furthermore, mature IL-1β promotes intestinal inflammation but prevents development of colitis-associated CRC in mice lacking protein tyrosine phosphatase non-receptor type 2 (PTPN2) in myeloid cells (PTPN2-LysMCre mice) [65]. PTPN2 negatively regulates pro-inflammatory signaling cascades in various inflammatory disorders including IBD [65]. The phenotypic discrepancies that have been observed in these studies may be due to the use of non-littermate control mice [42,61,63,64]. Future experiments using littermate control mice would provide a better understanding of how gut microbial ecology of *Il1β*$^{-/-}$ mice may affect the severity of colitis.

IL-1 receptor (IL-1R) signaling is also implicated in the pathology of colitis and CRC [62,64,66]. A study demonstrated that *Il1r*$^{-/-}$ mice (mice lacking the IL-1R, and therefore, cannot respond to either IL-1α and IL-1β) had increased intestinal bleeding after DSS treatment, but developed a similar number of polyps as compared with their WT littermates upon AOM and DSS treatment [62]. A conditional monoallelic APC loss in the mouse colon (*CDX2Cre-Apc*$^{f/wt}$; CPC-APC mice) can induce spontaneous CRC [66]. Genetic ablation of IL-1R1 in the intestinal epithelium of these mice alleviated tumorigenesis [66], suggesting that IL-1R signaling in epithelial cells accelerates the development of CRC. Similarly, deletion of IL-1R1 in T cells of these mice resulted in less inflammation and delayed CRC progression in an IL-17- and IL-22-dependent manner (Figure 1) [66], whereas, deletion of IL-1R1 in myeloid cells leads to dysbiosis, inflammation, and tumor growth [66], suggesting a cell-type specific role of IL-1R in the development and progression of CRC.

IL-18

IL-18 is one of the key mediators in the pathophysiology of IBD [26,67]. During colitis, microbiota and microbial products induce activation of the inflammasome leading to IL-18 production in the colon that supports epithelial cell regeneration, repair and prevent commensal dysbiosis [39,42,68]. Deficiency in inflammasome components leads to reduced production of IL-18, resulting in impaired epithelial remodeling [39,42,68]. In a mouse model of colitis and colitis-associated CRC, IL-18 has a protective role [39,42,43,62,69]. The magnitude of the inflammatory response is increased in *Il18*$^{-/-}$ mice following DSS exposure, characterized by increased body weight loss, intestinal bleeding and diarrhea, as compared to WT mice [62]. Upon AOM-DSS treatment, both *Il18*$^{-/-}$ and *Il18r1*$^{-/-}$ mice (mice lacking the IL-18 receptor, and therefore, cannot respond to IL-18) had an increased number of tumors and decreased expression of genes encoding molecules responsible for DNA damage repair, such as *Atm*, *Atr*, *Msh2*, and *Parp1* [62], suggesting a putative shielding effect of IL-18 in tumorigenesis. Injection of recombinant IL-18 into mice lacking inflammasome components reduces the prevalence of tumors in response to AOM and DSS [39]. Further, injection of recombinant IL-18 into mice lacking IL-18 protects against CRC metastasis [70].

In contrast to its protective role, several studies have highlighted an adverse role of IL-18 in colitis [71-74]. Blockade of bioactive IL-18 in mice with recombinant IL-18 binding protein (IL-18BP) and recombinant human IL-18BP isoform a (rhIL-18BPa) can ameliorate DSS- and TNBS-induced colitis, respectively, by decreasing the production of pro-inflammatory cytokines such as TNF and IFN-γ. [72,73]. Furthermore, a study demonstrated that mice with a conditional deletion of IL-18 or its receptor IL-18R1 in IECs (*Il18r1*$^{\Delta IEC}$ mice) were resistant to DSS-induced colitis compared with cohoused WT mice [74]. The hyperactivated IL-18 signaling following DSS treatment causes depletion and delayed maturation of goblet cells, subsequently leading to enhanced disease activity [74]. In addition, *Il18r1*$^{\Delta IEC}$ mice cohoused with dysbiotic *Il18*$^{-/-}$ mice still had less severe colitis than the *Il18r*$^{fl/fl}$ control mice [74], suggesting that IL-18 can promote the pathology of DSS-induced colitis regardless of the profile of the gut microbiota. Moreover, a transgenic mouse strain overexpressing IL-18 develop severe colitis owing to the colonic invasion of CD11b^{+} macrophages [71]. An explanation for this effect could be that IL-18 can downregulate IL-22-binding protein (IL-22BP) which inhibits the activity of IL-22, a cytokine involved in suppression of early intestinal inflammation (Figure 1) [71,75]. Overall, IL-18 has complex roles in colitis and further experiments would disentangle the contribution of this important cytokine in colitis and colitis-associated CRC.

NLRP1

NLRP1 was the founding member of the inflammasome family [76]. The *Bacillus anthracis* lethal toxin, *Toxoplasma gondii*, and muramyl dipeptide are all known activators of this cytosolic sensor [5,77,78]. The genomic organization of NLRP1 is species-specific and is structurally diverse [77,78]. There are three (*Nlrp1a*, *Nlrp1b*, and *Nlrp1c*) paralogs of the NLRP1 gene in mice [7,67]. A study reported in an AOM-DSS-induced colitis-associated CRC model, *Nlrp1b*$^{-/-}$ mice had an increase in morbidity, inflammation, tumorigenesis and low levels of IL-1β and IL-18 in the colon compared to WT mice [53].

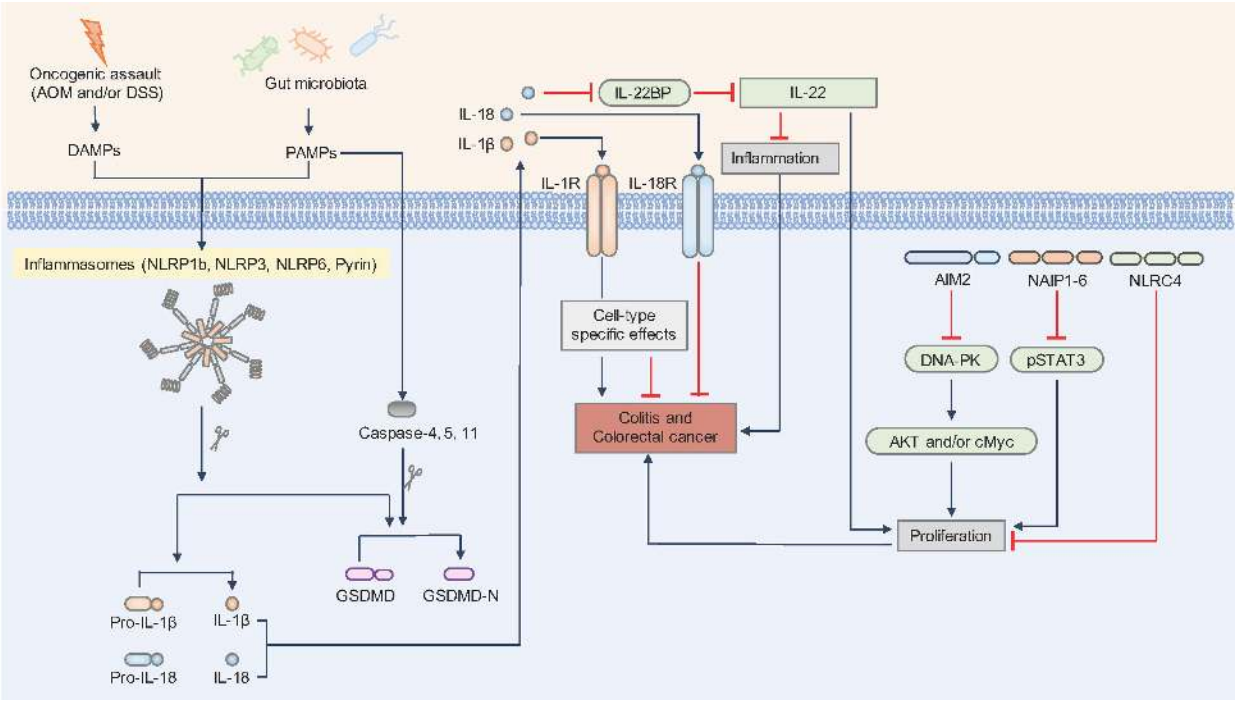

Figure 1. Critical role of inflammasomes and related molecules in colitis and colorectal cancer. The inflammasome sensors NLRP1b, NLRP3, NLRP6, and Pyrin can sense danger-associated molecular patterns (DAMPs) and/or pathogen-associated molecular patterns (PAMPs) leading to the formation of an inflammasome complex containing the adaptor protein ASC and the cysteine protease caspase-1. Activation of caspase-1 mediates the cleavage of pro-IL-1β, pro-IL-18, and the pore-forming protein gasdermin D (GSDMD) into their bioactive form. Human caspase-4, 5 or mouse caspase-11 can sense intracellular lipopolysaccharide (one of the PAMPs derived from Gram-negative bacteria) leading to cleavage of GSDMD and pyroptosis. The pore-forming fragment of gasdermin D (GSDMD-N) induces potassium efflux which activates the NLRP3 inflammasome and caspase-1-dependent maturation of IL-1β and IL-18. IL-1β is linked to the development of colitis and colorectal cancer, however, IL-1R signaling elicits cell-type-specific responses. For instance, IL-1R1 signaling in epithelial and T-cells is pro-tumorigenic, whereas in myeloid cells, particularly neutrophils, prevents inflammation and colorectal cancer progression. IL-18 and IL-18 receptor signaling confer protection against both colitis and colorectal cancer. IL-18 also promotes downregulation of soluble IL-22-binding protein (IL-22BP), which controls the ability of IL-22 to suppress inflammation or induce tumorigenesis in the gut. In addition, NLRP6 promotes the secretion of mucin and anti-microbial peptides (AMPs) in intestinal epithelial cells and provides protection against colitis and colitis-associated colorectal cancer. AIM2 inhibits the phosphorylation of DNA-dependent protein kinase (DNA-PK) which induces colorectal tumorigenesis via activation of AKT and the transcription factor MYC. NAIP1–6 proteins, present in mouse intestinal epithelial cells, can control tumorigenesis by limiting the phosphorylation of signal transducer and activator of transcription 3 (STAT3). Furthermore, NLRC4 can block cellular proliferation and protects against colorectal cancer. In addition to the protective roles, inflammasomes and their components have detrimental roles in colitis and colorectal cancer.

Administration of recombinant IL-1β and IL-18 reduced the colitis severity in *Nlrp1b*$^{-/-}$ mice [53]. Bone marrow reconstitution experiments revealed that NLRP1b functions in the nonhematopoietic cell compartment to attenuate tumorigenesis [53]. Another study revealed that mice lacking all three paralogues of NLRP1 were resistant to DSS-induced colitis [79], suggesting that the protective function of NLRP1b might be dominated by the detrimental effects of NLRP1a and NLRP1c. Mice deficient in NLRP1 had abundant *Clostridiales,* a group of bacteria that produces the short-chain fatty acid butyrate known to alleviate intestinal pathologies, to mediate protection against DSS-induced colitis [79,80]. The protective microbiota from *Nlrp1*$^{-/-}$ mice can be transferred to WT mice by means of cohousing [79]. Moreover, an activating mutation in *Nlrp1a* (*Nlrp1a*$^{Q593P/Q593P}$) can lead to increased IL-18 and IFN-γ production and decreased butyrate production in the colon of mice, all of which can exacerbate colitis [79]. Given that NLRP1 paralogues may have divergent roles in colitis, it would be worthwhile investigating the functionality of each paralogue in greater detail.

NLRP3

NLRP3 can be activated by a broad range of micro-organisms and sterile triggers of inflammation [81-83]. In addition, fluctuations in intracellular potassium or calcium levels, mitochondrial dysfunction leading to the release of oxidized DNA and reactive oxygen species, cytosolic release of lysosomal cathepsin B, and formation of nonspecific pores on the cell membrane are linked to NLRP3 inflammasome activation [5,7,77,78]. Owing to its diverse activity and expression profile in multiple cell types, NLRP3 contributes to the development of IBD and CRC [5]. Studies have shown that *Nlrp3*$^{-/-}$ mice exhibit substantially increased mortality, colonic inflammation, and tumorigenesis following treatment with AOM and DSS [38-40,84]. The phenotype of *Nlrp3*$^{-/-}$ mice was attributed to a decrease in IL-18 production that subsequently leads to a dysfunctional epithelial barrier [38]. The activity of the NLRP3 inflammasome during DSS-induced colitis is controlled by the microRNA molecule miR223 derived from hematopoietic cells [85]. Blockade of NLRP3 and IL-1β activity by MCC950 and anakinra, respectively, abrogates the enhanced pathology of colitis in mice lacking miR223 [85]. Bone marrow chimera experiments identified that NLRP3 in hematopoietic cells, but not in stromal or epithelial cells, was essential for its tumor-suppressing effects [38,40].

In addition to DSS, studies using TNBS have also demonstrated a protective role of NLRP3 in colitis [38,84]. Similarly, the lack of NLRP3 can lead to aggravated colitis in mice treated with oxazolone, a condition which can be reversed after exogenous supplementation of IL-1β and IL-18 [44]. Further, NLRP3 can inhibit CRC metastasis in a transplantable tumor model derived from a murine primary colon carcinoma [70]. NLRP3 enhances the tumoricidal activity of NK cells via IL-18, independently of adaptive immune cells and the intestinal microbiota [70].

In contrast to findings that NLRP3 is protective, a study reported that mice lacking NLRP3 were more resistant to DSS-induced colitis compared with WT mice [86]. A further study demonstrated that *Nlrp3*$^{-/-}$ mice carry a similar tumor burden compared to WT mice following AOM and DSS treatment [87]. The inconsistent observations between studies comparing WT mice and mice lacking NLRP3 in the response to DSS or AOM and DSS could be due to differences in gut microbiota, animal housing conditions and subtle differences between experiment procedures and substrains of the mouse lines used (discussed further below).

In a spontaneous colitis model using *Il10*$^{-/-}$ mice, the protein levels of NLRP3 and IL-1β are upregulated even before the onset of colitis [88,89]. In the colon explant culture from these mice, inhibition of inflammasome activity by IL-1 Receptor Antagonist, IL-1Ra, or the caspase-1 inhibitor, Ac-YVAD-cmk, reduced the release of IL-17 [89]. Further, intraperitoneal injection of Ac-YVAD-cmk into *Il10*$^{-/-}$ mice substantially reduced the severity of spontaneous colitis [89]. Similarly, treatment of *Il10*$^{-/-}$ mice with the NLRP3 inhibitor glyburide (also an anti-diabetic drug) markedly suppressed NLRP3 inflammasome activation, leading to alleviation of ongoing colitis and delayed disease onset [88]. These data seem to suggest that NLRP3 inflammasome activity might be detrimental in colitis under conditions of defective anti-inflammatory IL-10 signaling.

NLRP6

NLRP6, a widely expressed NLR in the gastrointestinal tract, can assemble an inflammasome complex in response to several microbiota-derived metabolites or bacterial lipoproteins [5,7]. Genetic deletion of NLRP6 renders mice more susceptible to DSS-induced colitis [42,90,91]. Epithelial cell repair is attenuated in *Nlrp6*$^{-/-}$ mice owing to decreased IL-18 levels in the colon [42,91], suggesting that the mechanism behind the protective role of NLRP6 in response to acute intestinal injury is linked to the cell renewal process associated with IL-18. In addition to intestinal epithelial cells, another study reported that NLRP6 is highly expressed in infiltrating inflammatory monocytes in the colon following DSS exposure [75]. Adoptive transfer of these cells from WT mice to *Nlrp6*$^{-/-}$ mice leads to IL-18-dependent secretion of tumor necrosis factor, which is important for reducing DSS-induced mortality [75]. These studies collectively suggest that the protective function of NLRP6 might be executed by both intestinal cells and inflammatory monocytes.

NLRP6 inflammasome signaling is also important in protecting mice against AOM-DSS induced CRC [42,91]. Studies have suggested that NLRP6 can downregulate IL-22BP via the IL-18 pathway, leading to increased levels of circulating and colonic IL-22 [75]. IL-22, in turn, exerts protective properties during early inflammation but promotes tumor development if uncontrolled [75]. The enhanced tumorigenesis in *Nlrp6*$^{-/-}$ mice caused by uncontrolled epithelial cell proliferation is linked to chemokine (C-C motif) ligand 5 (CCL5)-driven inflammation [92]. Indeed, deletion of the gene encoding CCL5 in mice prevents colitis and colitis-associated CRC [42,92].

Nlrp6$^{-/-}$ mice are reported to carry an altered fecal microbiota compared to WT mice, which comprised of TM7 species and those of the *Prevotellaceae* family [42]. The profile of dysbiotic microbiota in *Nlrp6*$^{-/-}$ mice can be transmitted to cohoused WT mice, resulting in enhanced disease in the recipient WT mice [42,92]. In another model of colitis caused by IL-10 deficiency, enhanced inflam-

mation was observed in *Il10*$^{-/-}$*Nlrp6*$^{-/-}$ mice compared to their *Il10*$^{-/-}$ littermates [93]. The *Il10*$^{-/-}$*Nlrp6*$^{-/-}$ mice had increased abundance of *Akkermansia muciniphila* instead of *Prevotella* and TM7, whereas *Il10*$^{-/-}$ mice had relatively low levels of *A. muciniphila* [93]. These results suggest an ability of NLRP6 to shape the microbiota under littermate experimental conditions [93].

The mechanism shaping microbiota by NLRP6 is reported to be in goblet cells. Goblet cells produce and secrete mucins, predominantly mucin 2, into the intestinal lumen, thereby forming a mucus layer in order to prevent entrance of intruding enteric pathogens (Figure 1) [55,94,95]. Further, NLRP6 regulates the secretion mucin 2 in intestinal epithelial cells through the induction of autophagy [55]. Therefore, mice deficient in NLRP6 have defective goblet cell autophagy, leading to imbalanced host-microbial interactions at the mucosal interface [55].

In addition to a role in goblet cell activity, NLRP6 has also been reported to function in the secretion of anti-microbial peptides. Anti-microbial peptides are a group of endogenously expressed peptides which provide protection against colonic inflammation and infection [96]. NLRP6 inflammasome signaling triggered by microbiota-modulated metabolites is required for IL-18 production, leading to the production of anti-microbial peptides [97]. An imbalance in the anti-microbial peptide profile caused by a deficiency in NLRP6 drives dysbiosis development [97].

Other studies challenged the idea that NLRP6 can regulate the composition of the gut microbiota in mice based on the use of littermate controls [56,98]. Under littermate-controlled experimental conditions, no differential susceptibility to DSS-induced colitis and microbiota composition was observed between WT mice and *Nlrp6*$^{-/-}$ mice [56,98]. An explanation of these conflicting results of NLRP6-dependent microbiota modulation could be differences in microbiota structure between different facilities. In standardized specific pathogen-free (SPF) conditions devoid of pathobionts, microbiota composition is indistinguishable between WT and *Nlrp6*$^{-/-}$ mice [99]. However, fecal transplant from conventionally housed dysbiotic *Nlrp6*$^{-/-}$ mice [42] into both WT and *Nlrp6*$^{-/-}$ mice housed in SPF conditions results in different colonic microbiota composition between the transplanted WT and *Nlrp6*$^{-/-}$ mice [99]. These results indicate that the effect of NLRP6 on the microbiota structure requires the presence of certain microbial taxonomic elements [99]. Given NLRP6 may potentially have microbiota-modulating effects, more integrative approaches including littermate breeding strategies and fecal microbiota transplantations are required to further explore the association between NLRP6, colitis and colitis-associated CRC.

NAIPs AND NLRC4

NAIPs are cytosolic inflammasome sensors of bacterial flagellin and components of type III or IV secretion systems [7,78]. The mouse genome encodes seven NAIP paralogs (*Naip1-7*) [100], whereas the human genome encodes one NAIP with two functional isoforms [101,102]. The ligand-bound NAIP protein interacts with and activates NLRC4, leading to the formation of the NAIP-NLRC4 inflammasome [7,78]. Mice lacking the NAIP1–6 proteins had an increased number of tumors in the colon after AOM and DSS or AOM-only treatment [103]. However, these mice developed less severe colitis in response to DSS treatment [103]. Furthermore, the cell-specific deletion of NAIPs revealed that these proteins predominantly function in the intestinal epithelium in the context of tumorigenesis [103]. NAIP proteins can inhibit phosphorylation and activation of the transcription factor STAT3 and the expression of genes encoding anti-apoptotic and proliferation-related proteins, such as BCL-2, MYC, and CCND1 [103]. These functions of NAIP are independent of NLRC4.

The role of NLRC4 in AOM and DSS-induced CRC in mice is not resolved. Two studies revealed that NLRC4 has a protective role against DSS- or AOM-DSS-induced colitis-associated CRC [87,104]. However, a further study observed no role of NLRC4 in colitis and colitis-associated CRC [40]. Both WT and *Nlrc4*$^{-/-}$ mice show similar susceptibility to DSS-induced colitis even when mice in both the groups carried a different microbiota composition [40]. The reason for these inconsistent observations is currently not fully understood. Therefore, the modulatory function of the NAIP or NLRC4-IL-18 signaling axis in colitis and CRC needs further explorations.

AIM2

AIM2, a member of the pyrin and HIN domain-containing family, binds to dsDNA and forms an inflammasome complex [7,105]. In addition to its role in coordinating host defense against infections, emerging evidence suggests that AIM2 can modulate the magnitude of intestinal inflammation and CRC [5,105]. AIM2 contributes to the inhibition of CRC in mice treated with AOM and DSS [54,106]. AIM2 interacts with and inhibits the DNA-dependent protein kinase, DNA-PK, and limits the activation of the serine/threonine-protein kinase AKT that governs cell proliferation and survival (Figure 1) [54]. Indeed, administration of the AKT inhibitor API-2 reduces the tumor burden of *Aim2*$^{-/-}$ mice, but not of WT mice, treated with AOM and DSS [54]. In addition, AIM2 suppresses overt proliferation in enterocytes and prevents the expansion of the intestinal stem cell population [106]. AIM2 also prevents the formation of early lesions in mice

caused by aberrant Wnt signaling owing to activation of a Cre-dependent mutant allele of β-catenin [106]. The anti-tumorigenic role of AIM2 has also been observed in a mouse model of spontaneous CRC. In the absence of AIM2, *Apc*$^{Min/+}$ mice develop more tumors in the colon compared with *Apc*$^{Min/+}$ mice expressing AIM2 [54]. These genetic mouse models collectively demonstrate that AIM2 inhibits colitis-associated and spontaneous CRC.

The susceptibility of WT mice and *Aim2*$^{-/-}$ mice to DSS-induced colitis is not always apparent [43,54,106,107]. Studies have reported no difference between WT mice and *Aim2*$^{-/-}$ mice in response to DSS, including hallmarks such as the length of the colon, the production of pro-inflammatory cytokines and the activation of the inflammasome [54,106]. Others have reported increased susceptibility of *Aim2*$^{-/-}$ mice to DSS owing to impaired inflammasome responses [43,107]. A further study using littermate-controlled mice has shown that both WT mice and *Aim2*$^{-/-}$ mice were similarly susceptible to DSS-induced colitis [43]. This finding suggests that the microbiota and/or genetic background of non-littermate WT and *Aim2*$^{-/-}$ mice might have contributed to their differential susceptibility to DSS-induced colitis. Indeed, administration of antibiotics to non-littermate WT and *Aim2*$^{-/-}$ mice abolishes differences in the susceptibility between the two groups to DSS-induced colitis, and that cohousing of non-littermate WT and *Aim2*$^{-/-}$ mice reduces the susceptibility of *Aim2*$^{-/-}$ mice to DSS-induced colitis [43,106,107]. Further, the transfer of microbiota from non-littermate *Aim2*$^{-/-}$ mice to germ-free WT mice heightens the susceptibility of germ-free WT mice to DSS-induced colitis, suggesting *Aim2*$^{-/-}$ mice harbor a colitis-prone microbiota [43]. These studies indicate that carriage of colitis-prone microbiota in non-littermate *Aim2*$^{-/-}$ mice could increase the susceptibility of these mice to DSS.

Further studies have revealed that the *Aim2*$^{-/-}$ mouse line generated from the 129 background, available from The Jackson Laboratory (Stock Number: 013144), expresses the 129-associated *Ifi202* gene [108]. The *Ifi202* gene encodes the p202 protein containing two HIN domains, which binds to dsDNA [109]. Owing to the close proximity between the *Aim2*$^{-/-}$ alleles and the *Ifi202* gene in the 129 background, both genes were inherited as a haplotype, essentially introducing the *Ifi202* gene from the 129 background into the *Aim2*$^{-/-}$ gene trap line during backcrossing to C57BL/6 mice [110]. The *Ifi202* gene of the 129 background is expressed and the *Ifi202* gene from C57BL/6 background is not expressed at an appreciable level [110]. Studies reporting an increased susceptibility of *Aim2*$^{-/-}$ mice owing to impaired inflammasome responses to DSS used *Aim2*$^{-/-}$ mice from The Jackson Laboratory [43,107], whereas studies reporting a lack of difference in the susceptibility between WT and *Aim2*$^{-/-}$ mice used mice generated on the C57BL/6 background [54,106]. Therefore, the effect, if any, of introducing the *Ifi202* gene from the 129 background into the *Aim2*$^{-/-}$ mouse line used in colitis studies remains to be clarified [43,107].

PYRIN

Pyrin belongs to the tripartite motif-containing protein family and is encoded by the gene *Mefv* [5,7]. Pyrin responds to inactivation of the host protein Rho by bacterial toxins [5,7]; its role has also emerged in the context of colitis and colitis-associated CRC. The level of Mefv is substantially up-regulated in mouse colon following AOM-DSS treatment [111]. *Mefv*$^{-/-}$ mice develop more severe colitis, epithelial hyperplasia and increased tumor burden in the colon in response to AOM-DSS treatment compared to WT mice [111]. In addition, *Mefv*$^{-/-}$ mice fail to produce IL-18 and are susceptible to increased epithelial permeability as indicated by the loss of occludin and claudin-2 from intercellular tight junctions [111]. Administration of recombinant IL-18 to *Mefv*$^{-/-}$ mice ameliorates colitis and CRC [111], suggesting a critical role of the Pyrin-IL-18 axis in promoting epithelial integrity and restricting inflammation and tumorigenesis.

IMPORTANCE OF LITTERMATE CONTROLS AND THE GUT MICROBIOTA

Although multiple studies have elucidated the mechanisms of inflammasome signaling pathways in response to pathogens and sterile triggers in cell culture studies, the roles of inflammasomes in colitis and colitis-associated tumor progression are far more complex. Inflammasome components are either protective or detrimental in response to experimental colitis and colitis-associated CRC (Table 1 and Table 2). These seemingly inconsistent findings could be attributed to multiple factors, including differences between experimental procedures, substrains of the mouse lines used, and differences in gut microiota and/or housing conditions. The use of littermate-controls is becoming essential to reveal true biological effects of a specific gene product in mouse models. Subtle genetic differences might arise between independent mouse lines [45]. In addition, independently housed WT mice and mice lacking a specific inflammasome component invariably harbor a different microbiota profile, possibly due to microbiota drift, that substantially influences the susceptibility to colitis and CRC (Table 1 and Table 2). Indeed, alterations in gut microbiota affect the progression of IBD and cancer in humans and mice [112-114]. The landscape of the microbiota is also influenced by lifestyle, diet, metabolism, prevalence of infection, and

host genetics [115].

The inflammasome–gut microbiota axis has been described and multiple studies have suggested a role of inflammasomes in regulating gut microbiota composition [5]. In response, the gut microbiota releases immunomodulatory factors which influence inflammation and immune responses during the progression of disease [5]. Non-littermate-controlled experiments examining WT mice and mice lacking inflammasome components may be insufficient in elucidating a role for inflammasomes at the complex organismal level [115,116]. Therefore, further studies assessing the contribution of inflammasomes in mouse models of colitis and CRC should use littermate-controlled animals to minimize confounding genetic and microbiota factors.

FUTURE PERSPECTIVES

The contrasting roles of inflammasomes in colitis and colorectal cancer elucidated in mouse models might be influenced by certain approaches being used. Pharmacological inhibitors might not affect the desired target in every cell of the body and that the inhibitory effect may not be sustained depending on the dose and length of treatment. Genetic knockout approaches ensure that the effect obtained is due to the lack of the desired target, but genetically-engineered mice might carry subtle background differences, off-target effects introduced during the genetic editing process, and/or differing housing procedures from the wild type strain [117].

Another consideration for future studies is that inflammasome signaling might operate differently in different cell types [5]. Therefore, the use of conditional, time-controlled, and tissue-specific genetic knockout approaches in mice might uncover unexpected cell-type specific roles of inflammasomes and related molecules. The use of human intestinal organoids or "mini-gut" combined with CRISPR-Cas9 or gene silencing techniques might reveal novel insights into inflammasome biology in the human intestinal tract. Further, inflammasome signaling is tightly regulated by other cell intrinsic, inflammatory and cell death pathways, such as apoptosis, autophagy, and metabolism [118]. For example, the autophagy-related kinase GCN2, which senses amino acid depletion, dampens gut inflammation by reducing inflammasome activation [119]. A holistic understanding of the crosstalk between inflammasomes and the broader regulatory network for which inflammasomes are part of in the context of intestinal inflammation and tumorigenesis are required. Therefore, the global signaling network controlling inflammasome activation should be explored in future studies.

CONCLUSIONS

Inflammatory responses triggered by the inflammasome affect the development and progression of colitis and CRC. Inflammasome sensors regulate multiple signaling pathways and the resultant immune responses are largely beneficial to the host. Bioactive cytokines processed by the inflammasome can control intestinal inflammation and tumorigenesis by regulating cellular proliferation, cell maturation, and cell death. In addition, studies using genetically modified mouse strains have revealed that multiple inflammasome sensors have the potential to modulate host microbiota and intestinal pathologies. However, aberrant inflammasome signaling might foster the development of a dysbiotic microbiota, which, in combination with a trigger such as intestinal barrier damage caused by DSS, can aggravate inflammation and promote tumorigenesis. Evidence from mouse models suggests that targeting inflammasome signaling represents a promising and novel therapeutic strategy against colitis and CRC. However, additional effort is required to transform the current understanding of inflammasome biology in colitis and CRC into effective therapies for use in humans.

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