REVIEW

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Nucleic acid sensing pattern recognition receptors in the development of colorectal cancer and colitis

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Abstract Colorectal cancer (CRC) is a leading cause of cancer-related deaths that is often associated with inflammation initiated by activation of pattern recognition receptors (PRRs). Nucleic acid sensing PRRs are one of the major subsets of PRRs that sense nucleic acid (DNA and RNA), mainly including some members of Toll-like receptors (TLR3, 7, 8, 9), AIM2-like receptors (AIM2, IFI16), STING, cGAS, RNA polymerase III, and DExD/H box nucleic acid helicases (such as RIG-I like receptors (RIG-I, MDA5, LPG2), DDX1, 3, 5, 7, 17, 21, 41, 60, and DHX9, 36). Activation of these receptors eventually leads to the release of cytokines and activation of immune cells, which are well known to play crucial roles in host defense against intracellular bacterial and virus infection. However, the functions of these nucleic acid sensing PRRs in the other diseases such as CRC and colitis remain largely unknown. Recent studies indicated that nucleic acid sensing PRRs contribute to CRC and/or colitis development, and therapeutic modulation of nucleic acid sensing PRRs may reduce the risk of CRC development. However, until now, a comprehensive review on the role of nucleic acid sensing PRRs in CRC and colitis is still lacking. This review provided an overview of the roles as well as the mechanisms of these nucleic acid sensing PRRs (AIM2, STING, cGAS, RIG-I and its downstream molecules, DDX3, 5, 6,17, and DHX9, 36) in CRC and colitis, which may aid the diagnosis, therapy, and prognostic prediction of CRC and colitis.

Keywords Cell proliferation · Colitis · Colorectal cancer · Nucleic acid sensing pattern recognition receptors · Type I interferon

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Introduction

Colorectal cancer (CRC) is one of the most common malignant digestive tract tumors, which is characterized by abdomen masses, bloody stool, and changes in stool character, etc. The incidence and mortality of CRC are about 1.4 million and 693,900 in worldwide in 2012, respectively [1]. Incidence and mortality rate trends of CRC commonly decreased in more developed countries, while increased in less developed countries [2]. According to data from the National Central Cancer Registry of China, it is estimated that there will be about 0.38 million new cases and about 0.19 million deaths caused by CRC in 2015 [3], indicating that CRC poses a serious threat to the health of Chinese people. Mechanisms of tumorigenesis and progression of CRC are very complicated and may include interactions among environmental exposures, diet, and heredity [4]. Inflammation is a putative risk factor for CRC, and emerging clinical studies show that patients with inflammatory bowel disease (IBD) have higher risk of developing CRC than controls [4].

Inflammation is normally initiated by activation of innate immune cells, which express a broad range of sensors termed pattern recognition receptors (PRRs) [5]. PRRs are localized to the plasma membrane, cytosol, or endosomes [5]. The main types of PRRs include Toll-like receptors

(TLRs), Nod-like receptors (NLRs), RIG-I like receptors (RLRs), AIM2-like receptors (ALRs), and C-type Lectin receptors (CLRs). Upon activation of PRRs by pathogen-associated molecular patterns (PAMPs) and/or danger-associated molecular patterns (DAMPs), innate immune cells undergo a series of signaling cascades that lead to the production of diverse pro-inflammatory cytokines and activation of adaptive immune cells to protect the host from invading pathogens [6]. Additionally, PRRs (especially the TLRs and the NLRs) play crucial roles in maintaining intestinal homeostasis and regulating the development of IBD and CRC [7–9].

Many PRRs are involved in detecting pathogenic DNA and RNA or self-DNA and self-RNA in cells and are thus referred to as nucleic acid sensing PRRs. PRRs responsible for RNA recognition include TLRs (such as TLR3, 7, 8, and 13), RLRs [such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5)], and DExD/H box (the x can be any amino acid) nucleic acid helicases [such as Death Box 1, 3, 21, 41, 61 (DDX1, 3, 5, 6, 17, 21, 41, 60)] [10]. PRRs that recognize DNA include TLR9, stimulator of interferon genes (STING), ALRs [such as absent in melanoma 2 (AIM2), interferon-γ-inducible protein 16 (IFI16)], cGAMP synthase (cGAS), DDX41, RNA polymerase III (Pol III), Z-DNA binding protein 1 (DAI, ZBP1), DEAH-Box Helicase 9, 36(DHX9, 36), and Ku70 [11]. Activation of many of these receptors leads to the production of type I interferons (type I IFNs or IFN-α/β), chemokines, and inflammatory cytokines (Fig. 1) [10]. Some nucleic acid sensing PRRs, such as AIM2 and STING, are critical for host defense against intracellular bacteria and viruses [12, 13]. Although the underlying mechanisms are largely unknown, accumulating evidences suggest that many of these nucleic acid sensing PRRs are involved in the development of cancers such as CRC. In addition to mechanism, questions remain as to the importance of PRRs in the development of colitis and CRC. Here, we review the latest researches on the roles of nucleic acid sensing PRRs (Except TLRs that have been reviewed elsewhere [9, 14–17]) in the development of colitis and CRC, focusing on the intensively studied molecules– AIM2 and STING.

The DNA sensor AIM2 plays a critical role in the development of CRC and colitis

The ALRs family consists of AIM2 and IFI16 (murine p204) and resides in cytoplasm or nucleus. These proteins contain an N-terminal pyrin domain (PYD), which triggers intracellular signaling, and a one/two C-terminal HIN-200 domain, which binds to double-stranded DNA (dsDNA) [18]. After AIM2 binds to dsDNA in cytoplasm, the PYD interacts with apoptosis-associated speck-like protein

containing CARD (ASC) to recruit pro-caspase-1, forming a multi-protein complex called the inflammasome [19]. IFI16 has been reported to induce the production of IFN-β via the adaptor STING upon sensing cytoplasmic DNA, while forming inflammasome in cell nucleus in response to Kaposi Sarcoma-associated herpesvirus (KSHV) infection[20, 21]. Activated caspase-1, on one hand, mediates the maturation of pro-IL1-β and pro-IL18 into IL-1β and IL-18, respectively [22]. On the other hand, activated caspase-1 can cleave the substrate gasdermin D, and promote cell pyroptosis, a new form of programmed cell death [12]. One recent study showed that AIM2 could also form inflammasome in nucleus in response to radiation-induced DNA damage and further promote the pyroptosis of intestinal epithelial cells (IECs)[23]. AIM2 inflammasome and its effector genes provide host immune surveillance to a range of bacteria, viruses, and fungi by detecting the pathogenic DNA components in host cell cytoplasm [12]. In addition to pathogen defense, AIM2 is also involved in tumorigenesis. However, the role of AIM2 in tumors may vary by the types of cancer. It was firstly found to be exclusively expressed in chromosome 1 of melanoma cells by cDNA subtractive hybridization technique [24]. Reduced expression of AIM2 was identified in prostate cancer and CRC patient tissues subsequently [25, 26]. However, increased expression of AIM2 was observed in nasopharyngeal carcinoma [27].

In recent years, accumulating studies have shown that AIM2 may suppress the development of CRC. The microsatellite instability phenotypes in tumors often arise from mutations in microsatellites-encoding genes that are attributed to the defect in mismatch repair [28]. Notably, more than half CRC tissues and cell lines with high level of microsatellite instability showed frameshift mutation in *AIM2*, and further genetic study showed that the mutations were located in the coding regions [29, 30]. Furthermore, the decreased level of AIM2 in CRC was associated with a poorer prognosis [26]. These studies indicated that AIM2 could be a CRC suppressor. However, until recently, the *in vivo* physiological role of AIM2 in CRC development was unknown, though inflammasome effectors induced by other inflammasome-associated PRRs, including NLR members NLRP3,NLRC4, and NLRP6, were shown to protect the host from colitis and CRC [31–35]. Two recent studies by the Kanneganti group from St. Jude Children's Research Hospital, and the Ting group from the University of North Carolina, both located in the USA, revealed the critical role of AIM2 in the development of CRC in vivo [36, 37].

These two studies utilized a well-established colitis-associated CRC animal model to study host susceptibility of WT and *Aim2*$^{-/-}$ mice to CRC. In this model, CRC is induced via the intraperitoneal injection of the carcinogenic chemical Azoxymethane (AOM) into mice
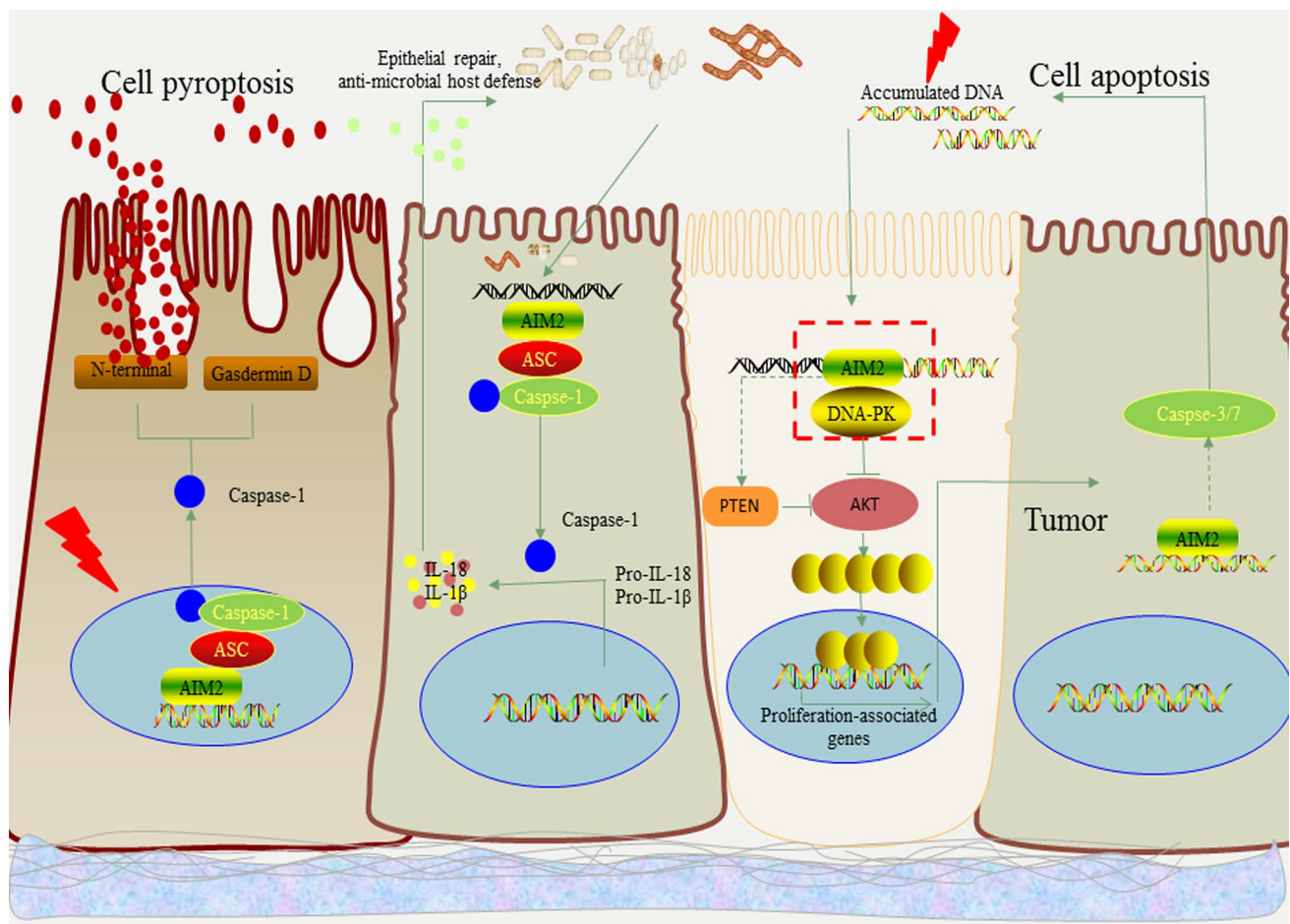
Fig. 1 The model of AIM2 signaling pathway in the development of colitis and colorectal cancer. AIM2 in intestinal epithelial cells (IECs) can be activated by dsDNA in cytoplasm or nucleus. The activation of AIM2 can further assemble inflammasome by recruiting ASC and pro-caspase-1. AIM2 inflammasome maturates caspase-1 in turn. Caspase-1 maturated by cytoplasmic AIM2 inflammasome activated by microbiota and dying cell-derived dsDNA can further cleave pro-IL-1β and pro-IL-18 into IL-1β and IL-18, respectively. IL-18 promotes the repair of damaged IECs and induces an anti-microbial host defense, which potently alleviates the development of colitis. AIM2 can also assemble inflammasome in nucleus where AIM2 encounters damaged dsDNA induced by radiotherapy and chemotherapy. The activated caspase-1 cleaves the substrate gasdermin D and drives the pyroptosis of IECs by its N-terminal fragment. On the other hand, AIM2 binds to DNA-PK directly and inhibits the AKT-induced proliferation of IECs as well as intestinal stem cells to prevent the development of CRC. Moreover, AIM2 inhibits the activity of AKT by enhancing the level of its negative regulator PTEN. Additionally, AIM2 also accelerates the caspase3/7-dependent apoptosis of IECs

and then the administration of a inflammation-inducing chemical, Dextran Sodium Sulfate (DSS), into the drinking water for three rounds [38]. Both studies found that *Aim2*^{−/−} mice harbor a dramatically increased colon tumor burden after AOM/DSS treatment compared to wild type (WT) mice despite similar changes in colon inflammatory infiltration, colon length, and the production of inflammatory mediators such as IL-6, tumor necrosis factor (TNF-α), and granulocyte colony stimulating factor (G-CSF) [36, 37]. Interestingly, the colonic expression levels of caspase-1 and the inflammasome effectors, IL-1β and IL-18, were comparable between *Aim2*^{−/−} and WT mice at various time points during CRC development [36, 37]. These results indicate that inflammation or an inflammasome defect in *Aim2*^{−/−} mice is probably not a major contributor to CRC susceptibility.

After excluding inflammation and inflammasome factors, these two studies found that, in comparison with WT mice, *Aim2*^{−/−} mice exhibited more proliferation of IECs at early time points after AOM/DSS treatment [37]. Microarray analysis revealed a number of upregulated proliferation-associated genes, such as *S100a9*, at day 14 post AOM injectionin *Aim2*^{−/−} mice [37]. To better understand the underlining mechanisms by which AIM2 inhibits enterocyte proliferation, the phosphorylation levels of signaling molecules in proliferation or apoptosis pathways, such as mitogen-activated protein kinase (MAPK) and phosphatidyl inositol 3-kinase-serine/threonine kinase (PI3K/AKT)

signaling pathway, were measured in $Aim2^{-/-}$ and WT mice. It was shown that the level of phosphorylated AKT (p-AKT) significantly increased whereas the PI3K-negative regulator tensin homology deleted on chromosome 10 (PTEN) decreased in $Aim2^{-/-}$ mice [36, 37]. Loss- and gain-of-function experiments *in vitro* and *in vivo* confirmed the important role of AIM2 on AKT activation and the inhibition of colon tumorigenesis [36]. However, the negative effect of AIM2 on AKT is in cell type-dependent manner. The level of p-AKT in CRC cell line as well as intestinal stem cell can be suppressed by AIM2, while AKT activation was similar in $Aim2^{-/-}$ and WT bone marrow-derived macrophages (BMDMs) upon stimulation [36]. To further understand which cell type contributes to AIM2-induced CRC restriction, bone marrow transplantation experiments were conducted. Consistently, Ting group identified that IECs were the main cell sources that were associated with AIM2-medicated CRC restriction [36]. However, Kanneganti group found that both AIM2 in bone marrow and IECs might be involved in this process [37]. AKT activation is regulated by a variety of kinases and phosphatases including DNA-dependent protein kinase (DNA-PK), and mTOR phosphorylates AKT at Ser473 [39, 40]. Co-immunoprecipitation experiments revealed that AIM2 interacts with DNA-PK but not with AKT or mTOR [36]. Although both AIM2 and DNA-PK bind DNA, AIM2 still interacts with DNA-PK after Ethidium Bromide (EtBr) treatment, which abolishes their interaction with DNA, indicating that there is a direct interaction between AIM2 and DNA-PK [36]. Further study suggested that AIM2 is a negative regulatory factor for DNA-PK, which is necessary for the increased AKT phosphorylation in $Aim2^{-/-}$ CRC cells [36]. DNA-PK also promotes the stability of c-Myc, so loss of AIM2 and increased DNA-PK may contribute to the increased production of c-Myc in $Aim2^{-/-}$ mice.

Persistent proliferation and differentiation of intestinal stem cells in crypts are essential to restore structure and function after damage to intestinal epithelial cells [41]. However, aberrant self-renewal of intestinal stem cells can be the source of intestinal tumors [42]. Indeed, the growth rates of intestinal stem cells derived from $Aim2^{-/-}$ mice were significantly higher than those of WT mice [37]. The protective role of AIM2 in sporadic CRC was confirmed by higher tumor burden in the $Aim2^{-/-}/Apc^{Min/+}$ mice than $Apc^{Min/+}$ mice [36], which have aberrant activation of the Wnt/β-catenin signaling pathway in IECs, and have colonic neoplasia [43, 44]. However, whether this process is involved in intestinal stem cell proliferation and the exact association between AIM2 and adenomatosis polyposis coli gene (APC) is still unknown, though previous studies showed that aberrant activation of wnt/β-catenin signaling pathway exists in colon cancer stem cells [45]. To investigate the role of AIM2 in Wnt signaling, $Aim2^{-/-}$ mice

were crossed to a $Prom1^{CreERT2-LacZ\ (C-L)/+}$; $Ctnnb1^{lox(ex3)/+}$;$RosaZsGreen$ mouse strain, and it was found that AIM2 is necessary for intestinal stem cell activation following aberrant β-catenin activation [37]. In this model, aberrant β-catenin activation is induced by tamoxifen, and Prom1 is used as a marker for intestinal stem cells. Although no macroscopic colon tumors were found, increased numbers of Ki67^{+} large intestinal cells were observed in $Aim2^{-/-}$ mice [37]. In addition, increased activation of AKT and c-Myc was found in the colon tissues of $Prom1^{C-L/+}$; $Ctnnb1^{lox\ (ex3)/+}$; $RosaZsG;\ Aim2^{-/-}$ mice in comparison with controls [37]. On the other hand, the activation of the apoptosis indexes, such as caspase-3 and caspase-7, significantly decreased in $Aim2^{-/-}$ mice colon tissues [36, 37]. Collectively, AIM2 may suppress colon tumorigenesis by inhibiting the proliferation and increasing their apoptosis of IECs or stem cells, which is consistent with the observation that upregulation of AIM2 expression in CRC cell lines results in cell cycle arrest [46].

The intestinal microbiota was also found to be an important etiological factor for CRC development. The Kanneganti's study showed that AIM2 contributes to the maintenance of intestinal homeostasis by regulating gut microbiota. The gut microbial spectrum was different between $Aim2^{-/-}$ and WT mice, and this difference could be diminished by co-housing [37]. When $Aim2^{-/-}$ mice were co-housed with WT mice, the colonic tumor load decreased in $Aim2^{-/-}$ mice and increased in WT mice [37]. These results suggest that AIM2 suppresses colon tumorigenesis partly due to its regulation of the gut microbiota though the mechanisms are still undefined.

Although the previous two studies showed that AIM2 protects host against CRC, the role of AIM2 or AIM2 inflammasome in intestinal inflammation is still unknown. Several recent studies investigated the role of AIM2 in the development of colitis [47–49]. $Aim2^{-/-}$ mice developed more severe colitis than WT mice in a DSS-induced colitis model. The phenotype is associated with increased intestinal bacterial burden, especially *Escherichia coli*, because of the defective function of inflammasome effectors in $Aim2^{-/-}$ mice [47]. The further results suggested that the inflammasome effectors IL-1β and IL-18 are responsible for regulating the production of antimicrobial peptides that defend against *Escherichia coli* infection during DSS-induced colitis [47]. Consistently, another recent study found that AIM2 inflammasome in IECs prevent against colitis via IL-18/IL-22/STAT3/Reg3 axis. However, this study also found that AIM2 in myeloid cells is detrimental to the development of colitis, indicating that the effect of AIM2 in intestine is cell type dependent [49]. The discrepancy between this study and previous studies on the dependence of the inflammasome may be due to different sources of $Aim2^{-/-}$ mice as discussed in another

review [12]. Another study using a colitis model induced by *Salmonella* infection also showed a protective role for AIM2 in colitis [48]. In this model, there were less cell proliferation and more apoptosis in the intestinal tissues of *Aim2*$^{-/-}$ mice. In addition, AIM2 restricts *Salmonella* diffusion by increasing the expression of tight junction proteins such as Claudin 3 and Occludin, rather than the inflammasome component caspase-1 [48].

In addition, one recent study showed that AIM2 inflammasome promotes the radiation-induced pyroptosis of IECs [23]. Compared to WT mice, *Caspase-1*$^{-/-}$ mice suffered less radiation-induced gastrointestinal syndrome including intestinal damage, diarrhea, and malabsorption. Further results indicated that AIM2 inflammasome rather than Nlrp3 or Nlrc4 inflammasome was the activator of the death of IECs including intestinal stem cells, in a manner dependent on caspase-1 but not on cytokines, apoptosis, or AKT signaling [23]. The observation under immunofluorescence microscopy suggested that AIM2 detects blocked dsDNA in nucleus and form inflammasome [23]. These indicated that AIM2 could sense the damaged DNA in nucleus and form inflammasome to medicate radiation-induced intestinal damage and pyroptosis. These raised the question of that whether AIM2 inflammasome can also sense the damaged DNA in nucleus of tumor cells and further medicate pyroptosis, since tumor cells often contain genome instability [50].

Collectively, AIM2 is critical for the maintenance of intestinal homeostasis. On one hand, AIM2 in IECs directly binds to DNA-PK and negatively regulates the activation of AKT, thus restricting the proliferation of IECs as well as intestinal stem cells. On the other hand, AIM2 inflammasome promotes the apoptosis as well as pyroptosis of IECs. Furthermore, AIM2 in IECs sense the invasive DNA pathogens, and enhance the intestinal integrity via various mechanisms. These mechanisms may include AIM2 inflammasome-induced IL-18/IL-22BP/IL-22/STAT3/Reg3 axis activation and AIM2-induced AKT activation in the early time of colitis to enhance the integrity of intestinal epithelial barrier (Fig. 1; Table 1).

However, works are still needed to understand the upstream mechanisms of decreased expression of AIM2 in human CRC. How and where AIM2 is activated in tumor setting is also unclear. In addition, more understanding of the function of AIM2 in various cell types in CRC microenvironment may potently aid to the regulation of AIM2 in CRC.

Table 1 Main nucleic acid sensing pattern recognition receptors (PRRs) in colorectal cancer (CRC) or colitis

PRRs	Location	Ligands	Adaptors	Main functions in CRC or colitis
AIM2	Cytoplasm Nucleus	dsDNA	ASC	In CRC: AIM2 suppresses DNA-PK/AKT signaling-induced proliferation of intestinal epithelial cells or stem cells, increases their apoptosis, and regulates the gut microbiota [36, 37] In colitis: AIM2 inflammasome and the related effectors protect mice from colitis by regulating the production of anti-microbial peptides [47]. AIM2 is required for the maintenance of the intestinal epithelial barrier by increasingAKT-induced production of tight junction proteins [48]
STING	Cytoplasm	CDNs	TBK1	In CRC: STING restricts colon tumorigenesis by reducing inflammation in the colon, increasing inflammasome activity, and increasing type I IFNs-induced anti-tumor response of CD8$^+$ T cells [60, 62, 67, 68]
cGAS	Cytoplasm	dsDNA	STING	In CRC: cGAS is undetectable in most CRC cell lines that partially contain CpG islands in cGAS promoter region. cGAS may be required for tumor-derived DNA recognition and the triggering of CD8$^+$ T cell-induced anti-tumor response in CRC [67, 68, 71]
RIG-I	Cytoplasm	5′PPP RNA, Short dsRNA	MAVS	In colitis: RIG-I plays a crucial role in colitis by regulating the activation of T cells as well as Gαi2 expression [88]
DDX3	Cytoplasm Nuclear	dsRNA	MAVS	In CRC (Controversial): DDX3 serves as a CRC suppressor by regulating Snail and E-cadherin expression [110]. However, DDX3 may also serve as an oncogene for CRC through regulating Wnt/β-Catenin signaling[111–113]
DHX9	Cytoplasm Nucleus	CpG-B DNA, dsRNA	MAVS/ MyD88	In CRC: DHX9 is upregulated in human CRC tissues and cells [122, 123]
DDX5/17	Cytoplasm	RNA	unidentified	In CRC: DDX5 and DDX17 serve as CRC promoter and enhance the proliferation of CRC cells via regulating oncogenes, such as c-Myc[126, 127]
DDX6	Cytoplasm	RNA	unidentified	In CRC: DDX6 may promote the proliferation of CRC cell lines by down-regulating Wnt/β-catenin signaling pathway[128, 129]
DHX36	Cytoplasm	CPG-A DNA	MyD88	In CRC: Overexpression of DHX36 can reduce the migration of CRC cells[132]

STING signaling pathway in colorectal cancer and colitis

STING is localized in the endoplasmic reticulum (ER) with 5 N-terminal transmembrane domains that anchor it to the ER membrane and a globular in C-terminal domain (CTD) that specifically binds to cyclic di-nucleotides (CDNs), such as cyclic-di-GMP, cyclic-di-AMP, and cyclic-GMP-AMP (cGAMP) [51]. CDNs are a class of second messengers from bacteria that are rarely expressed in eukaryotes [52]. A number of studies have shown that STING is required for the induction of type I IFN (IFNβ) in response to intracellular DNA following bacterial and viral infection [53]. However, further studies indicate that STING is also an adaptor for other DNA sensors based on the observation that STING binds to labeled CDNs directly, and unlabeled CDNs rather than DNA can compete with this binding [51]. Furthermore, cell lines with specific point mutations in regions that are involved in recognizing CDNs in STING showed intact response to cytoplasmic DNA [51]. Subsequently, in addition to DAI [54], DDX41 [55], and IFI16 [20], cGAS has been identified as another DNA sensor upstream of STING [56]. Upon cytoplasmic DNA recognition, cGAS catalyzes the synthesis of 2′3′-cGAMP or 2′5′-linked GAMP from ATP and GTP, which in turn act as second messengers to bind and activate STING in a manner different from the other CDNs[57, 58]. After activation, STING binds to TANK-binding kinase 1 (TBK1) in the ER and translocates to the perinuclear compartment where it interacts with Sec5 and induces the production of IFN-β via interferon regulatory factor 3 (IRF3) or nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling [53]. In addition to IFN-β, STING is also required for the signal transducer and activator of transcription 6 (STAT6)-induced production of Ccl2 and Ccl20, which are crucial for antiviral immune responses [59].

Previous studies on STING were mainly focused on its role in anti-viral immunity. Recent evidences suggest that STING also has a critical role in the development of CRC. Compared to WT mice, $Sting^{-/-}$mice have a higher tumor load in colon tissues after AOM/DSS treatment [60]. Furthermore, histological analysis revealed an increased inflammatory cell infiltration and increased dysplasia in the colorectal tissues of $Sting^{-/-}$mice [60]. Levels of pro-inflammatory factors such as IL-6 and KC were elevated in colon tissues and sera of $Sting^{-/-}$mice in different stages of CRC development [60]. Moreover, the level of the phosphorylated signal transducer and activator of transcription 3 (STAT3), a downstream effector of IL-6R that contributes to colon tumorigenesis [61], was upregulated in colon tissues of $Sting^{-/-}$mice [60]. These results suggest that STING restricts colon tumorigenesis by reducing inflammation in the colon. Notably, the main gut bacterial spectrum showed

no significant difference between WT and $Sting^{-/-}$ mice, indicating that STING may suppress susceptibility to CRC independent of gut microbiota.

Interestingly, decreased maturation of pro-caspase1 and expression of IL-18 were observed in the colon tissues of $Sting^{-/-}$mice [60], indicating that STING may upregulate inflammasome activation. Similarly, another study showed decreased production of IL-18 and IL-22 binding protein (IL-22BP) in AOM/DSS-induced CRC tumor tissues of $Sting^{-/-}$mice [62]. However, this result seems contradictory to the previous observation that IL-18 is a negative regulator of IL-22BP in the presence of NLRP3 or NLRP6 inflammasomes [63]. To further understand the relationship between STING, IL-18 and IL-22BP, an in $vitro$ experiment was conducted. The results confirmed that IL-18 upregulation is STING inducible that may further upregulate the expression of IL-22BP [62], a negative regulator of colon tumorigenesis promoter-IL-22 [63, 64]. It seems that moderate expression of IL-22 promotes the repair of injured intestinal cells, while aberrant and chronic activation of IL-22 leads to hyperproliferation of intestinal cells and tumor formation [63]. These data suggest that STING may also inhibit colon tumorigenesis through the activation of inflammasome activity.

In addition to suppressing inflammation and enhancing inflammasome activity, other studies suggest that STING may induce the production of type I IFNs and further activate anti-tumor CD8$^+$ T cells. Increased CD8$^+$T cells infiltration in CRC tissue is associated with a better prognosis and type I IFN is required for CD8$^+$T cell priming [65, 66]. Two recent studies reported that the STING-IRF3-type I IFN signaling pathway, rather than other type I IFN-inducible signaling pathways, in dendritic cells (DCs) is required for tumor-specific CD8$^+$T cellspriming [67, 68]. In addition, this process is mainly mediated in DCs by cGAS that specifically detects tumor-derived DNA [67–69]. Apart from DCs, recent study suggested that tumor cells themselves, such as CRC cells, could also induce the production of type I IFNs [70].

In summary, STING may suppress colon tumorigenesis through multiple mechanisms, including (but not limited to) inhibiting inflammation, activating the inflammasome, and/or inducing type I IFNs.

Due to the indispensible role of STING signaling in tumorigenesis, their expression profile and functional status in human colorectal cancer cell lines have been investigated. The expression of IFN-β and IL-1β was significantly reduced in most human CRC cell lines with a defect in STING compared to WT cell lines in the presence of dsDNA [71]. As previously discussed, upon activation, STING interacts with TBK1 and translocates from the ER to the perinuclear compartment to phosphorylate IRF3 or NF-κB. However, this process was defective to

different extents in the human CRC cell lines in the presence of dsDNA [71]. To understand if mutations in *STING* were responsible for these observations, the genomes of the human CRC cell lines were sequenced. The results indicate that the STING signaling defects may be attributed to upstream DNA sensors [71]. Consistently, the expression of *cGAS* is undetectable in some CRC cell lines, including LS174T, SW480, SW1417, SW48, HT116, and colo205. However, gene sequence results showed there was no mutation in genome encoding *cGAS* and the suppression of cGAS may be due to epigenetic modification, such as hypermethylation that often leads to gene silencing [72]. Indeed, bisulfite sequencing analysis showed that there were CpG islands in *cGAS* promoter regions of these cell lines, and demethylation could rescue the defective STING signaling in these cells [71]. Additionally, the expression of STING and cGAS was detected in human CRC formalin-fixed paraffin-embedded tissues. Those samples exhibited low expression levels of STING and cGAS starting from stage II, although more studies are needed to understand the correlation between STING and CRC patients' prognosis.

As a central adaptor for DNA sensing, STING mediates the production of IFN-β and plays an important role in host defense against viruses such as herpes simplex virus (HSV) [73]. However, HSV can be utilized to treat cancers [74] and thus STING signaling deficiency in tumor cells facilitates the killing of tumor cells by oncolytic viruses in turn. *In vitro* experiments showed that human CRC cell lines defective in STING exhibited less type I IFN production and underwent more cell death when receiving HSV treatment, partially due to massive HSV replication [71]. This indicates that HSV has greater efficacy against CRC in the absence of STING signaling. This notion was supported by *in vivo* nude mouse experiments that CRC cells with defective STING signaling were more sensitive to HSV therapy [71]. Consistently, it was shown that STING signaling was defective in melanoma cells, and these cells are highly susceptible to HSV treatment [75]. So the effective STING signaling in tumor cells seems a double-edged sword, and evaluation of STING signaling in CRC patients may be used to predict the potential antitumor efficacy of oncolytic viruses. On the other hand, many pre-clinical experiments tried to enhance STING signaling in CD8+ T cells to promote anti-tumor immunity. The results showed that STING agonists, along with traditional therapy managements, could effectively reduce tumor loads in mice, such as CRC, melanoma, and glioma [76–78]. Encouragingly, some agonists have been shown to activate human STING signaling, though more clinical trials are needed to confirm.

All of these depict the peculiar charm of STING signaling pathway in inhibiting the development of CRC. Tumor-derived DNA captured by cGAS in DCs as well as tumor cells themselves in tumor microenvironment (TEM) is the primary driving force to activate STING signaling pathway to induce the production of type I IFNs and the tumor-specific CD8+ T cell priming. Apart from reducing inflammation response in early stage of CRC formation, STING also interacts with inflammasome to activate the caspase1/IL-18/IL-22BP axis and control aberrant proliferation of IECs (Fig. 2; Table 1). Although STING silencing did not influence the gut microbiota, whether gut microbiota changes in colitis and CRC can signal via intestinal STING and influence the diseases progression in turn is still unknown. In addition, despite that STING signaling is defective in most CRC cell lines, more clinical studies are needed to understand the relationship between STING expression and CRC prognosis, and whether it can be more precisely to predict the CRC prognosis in combination of STING level and tumor stages. On the other hand, STING activation in TME may incite both antitumor responses and tolerogenic responses. Tolerogenic responses can reduce the risk of autoimmune disease, while it increases the risk of tumor immune escape somehow. Several studies have shown that STING activation in TME can induce the infiltration of immunosuppressive cells, such as regulatory T cells (Treg), myeloid derived suppressor cells (MDSC) via type I IFNs induced IDO [79, 80]. Therefore, more detailed studies of the role of STING in CRC TME are needed to optimize its application in clinic.

The role of RLRs signaling pathway in colitis or CRC

RNA helicases are a superfamily of proteins expressed by almost all living organisms and are characterized by harboring 7–8 conserved DExD/H domains [81]. They are involved in all aspects of genetic expression by unwinding folded RNAs and modifying the RNA–RNA interactions in an energy-dependent manner via hydrolysis of NTP in cytoplasm [81]. RLRs are a subfamily of DExD/H box RNA helicases composed of RIG-I, MDA5 and laboratory of genetics and physiology 2 (LGP2), which are widely expressed in most tissues and cells [82]. RIG-I and MDA5 share similar structures: a repressor domain (RD) embedded within the CTD, a central DEAD box involved in hydrolysis of NTP and unwinding of RNAs, and an N-terminal CARD [82]. LGP2 lacks the CARD modification and may serve as a positive regulator for RIG-I and a negative regulator for MDA5 [82]. Studies show that RIG-I can be activated by various RNA substrates, including double-stranded RNA (dsRNA) and single-stranded RNA (ssRNA) that contain an uncapped 5′-triphosphate (5′ppp) modification, as well as blunt-ended short dsRNA. The 5′ppp modification in RNA is often specifically present in virus genomes, which are quite different from human RNA structures [83]. Compared to RIG-I, MDA-5 is preferentially activated by long dsRNA with blunt end
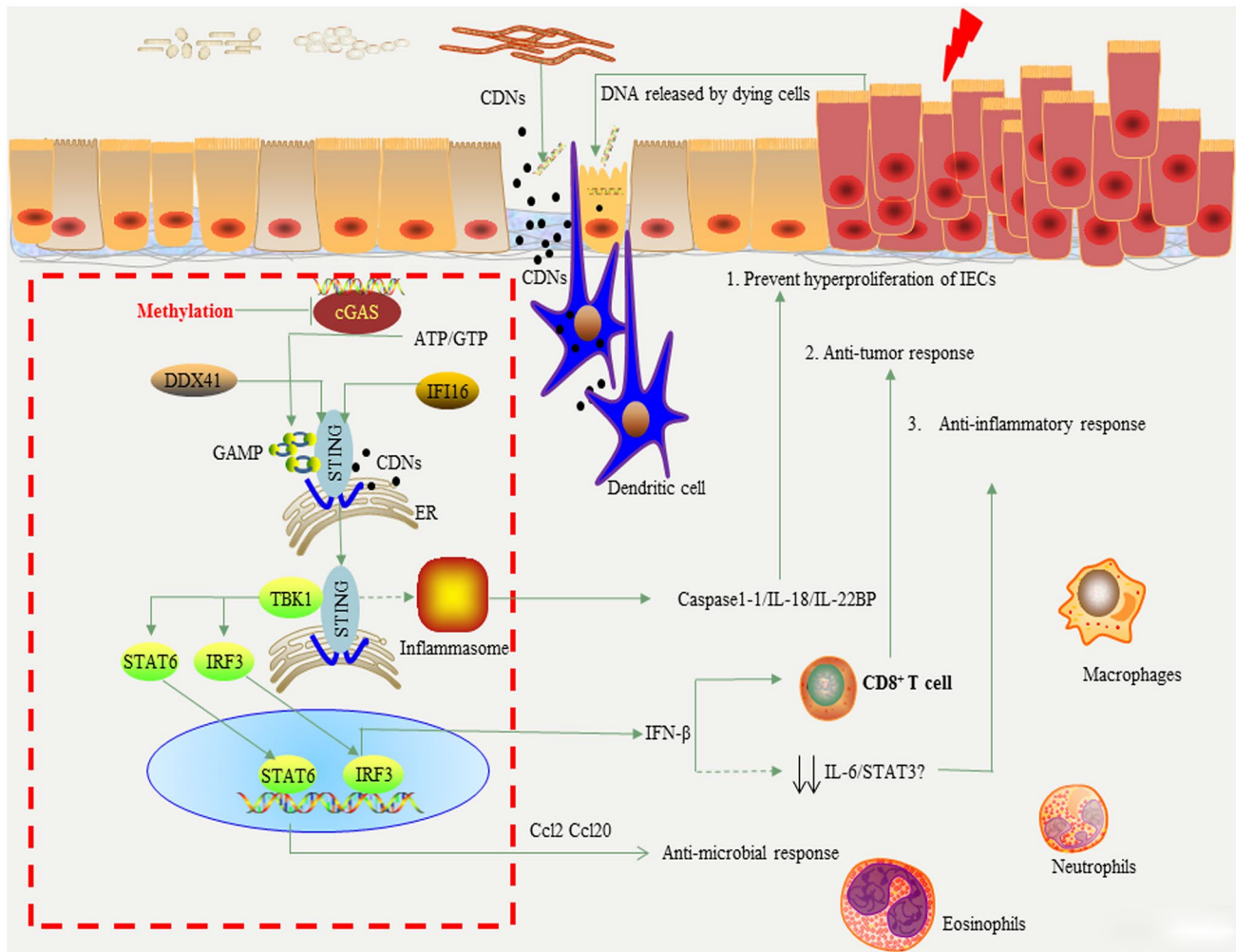
Fig. 2 The model of cGAS-STING signaling pathway in the development of colorectal cancer. The ER-anchoring protein STING is an important adaptor for immune responses to DNA stimulation. STING in antigen presenting cells, especially in dendritic cells, directly binds to cyclic di-nucleotides (CDNs) that are specifically presented in bacteria or generated from ATP/GTP by cGAMP synthase (cGAS). The upstream candidate DNA receptors of STING include DDX41 and IFI16. STING is activated and shuttled to perinuclear compartment where it binds to and phosphorylates TBK1 when tumor-derived DNA is captured by cGAS. The phosphorylation of TBK1 leads to the activation of IRF3 and STAT6, which in turn leads to the production of type I IFN (IFN-β) and Ccl2/20, respectively. IFN-β is required for priming of tumor-specific CD8+T cell and can induce the tumor regression. On the other hand, STING may reduce the inflammatory response in intestine by inhibiting the expression of IL-6 and STAT3. Notably, STING may also induce the activation of inflammasome and suppress the proliferation of intestinal epithelial cells via activating inflammasome-derived IL-18

[82]. Upon activation, both RIG-I and MDA5 recruit the adaptor-mitochondrial antiviral signaling protein (MAVS) via their CARD domains and form a platform to activate TRAF. This in turn leads to the phosphorylation of IRF3 and NF-κB signaling and the production of pro-inflammatory genes and IFN-β, respectively [84]. Accumulating evidences showed that RLRs family plays an important role in anti-virus immune response, and mice lacking either RIG-I or MDA-5 were highly susceptible to virus infection [85]. In addition, different types of virus showed difference fashion on RIG-I and MDA-5 activation and some viruses, such as West Nile virus and rotavirus, can activate both of them

[86, 87]. The mechanisms of RLRs in limiting the diffusion of virus may be partially attributed to the following factors: firstly, the activation of RLRs in different cells may lead to the production of IFN-β, which affects via the IFN receptors and induces the production of interferon-stimulated genes (ISG), vital molecules to initiate anti-virus immunity. Secondly, the RLRs themselves can directly hydrolyze the interaction among RNA–proteins, which are important for virus survival.

In addition to the roles of both RIG-I and MDA5 in the host defense against viruses, they are implicated in intestinal inflammation and cancer. Compared to WT mice,

Rig-I$^{-/-}$ mice exhibit more intestinal inflammation in DSS-induced colitis [88]. Interestingly, it was found that both the number and size of Payer's patches, the first line of immune defense in the gut, significantly decreased in *Rig-I*$^{-/-}$ mice with increased number of apoptotic B220$^+$ cells. The deregulation of T cells is associated with the development of colitis, and the *Rig-I*$^{-/-}$ mice also exhibited increased number of naïve T cells and decreased number of effector T cells in spleen [87, 88]. Further study found that associations may exist between RIG-I and Gαi2, a subunit of G protein which is involved in a range of biochemical activities since previous studies showed that *Gαi2*$^{-/-}$ mice were also highly susceptible to colitis that was characterized by regression of Payer's patches and deregulation of T cells subsets [89]. Indeed, further results confirmed that the expression of Gαi2 was significantly reduced in the colon tissues and lymphocytes of *Rig-I*$^{-/-}$ mice, and RIG-I promoted the activity of Gαi2 promoter [88], which may be incited by NF-κB [90]. One more recent study showed that RIG-I was mainly expressed in apical surface of the IECs, and RIG-I mRNA and protein levels were dramatically reduced in intestinal tissues of patients with Crohn's disease (CD) [91]. All of these indicated that RIG-I can prevent the development of colitis, though the underlying mechanisms remain to be defined.

As downstream adaptors in RLR signaling pathways, MAVS and IRF3 also play roles in the development of colitis. Compared to WT mice, *Mavs*$^{-/-}$ and *Irf3*$^{-/-}$ mice developed more severe colitis [92, 93]. Notably, *Mavs*$^{-/-}$/*MyD88*$^{-/-}$ mice died earlier than the *Mavs*$^{-/-}$ mice, indicating that RLR and TLR signaling may work cooperatively to protect mice from DSS-induced colitis [92]. The bone marrow transplantation experiments confirmed that MAVS in non-hematopoietic cells is crucial for the prevention of colitis [92]. In addition, apart from virus RNA, commensal bacteria-derived RNA can specifically activate RIG-I-MAVS signaling pathway in IECs and BMDMs and induce the production of IFN-β, inflammatory cytokines, and RegIIIγ. Both IFN-β and RegIIIγ are crucial molecules in anti-bacterial response [92]. As the downstream molecule of RLRs signaling, IRF3 can directly bind to and activate the promoter of thymic stromal lymphopoietin (TSLP), which is expressed both in hematopoietic cells and IECs and plays a preventive role against colitis [93]. All of these results suggest that the RIG-I-MAVS-IRF3 signaling pathway in IECs may protect mice from colitis. However, the underlying mechanisms by which RIG-I inhibits the development of colitis, and whether RIG-I is involved in CRC development are still unknown.

In summary, experiments in animals and IBD patients indicated that deregulation of RIG-I and MDA-5 signaling pathway may contribute to IBD development, despite that the specific contribution of RLRs in colitis is still poorly understood. It seems that RIG-I enhances the activation of Gαi2 and, thus, regulates peripheral T cells activation, while MDA5 senses invasive RNA microbiota in gut to maintain intestinal homeostasis by mediating the expression of type I IFNs and antimicrobial peptides. Apart from inducing the expression of type I IFNs, the downstream molecules of RIG-I/MDA5 may also interact with TSLP to prevent the progression of IBD (Fig. 3; Table 1). More animal and clinic experiments are needed to improve the limited understanding of the role of RLRs in colitis as well as CRC. Especially, issues about whether RLRs-induced DCs and NKs activation are involved in CRC development are worthy to be studied. Also, we should not ignore the relationship between RLRs and gut microbiota homeostasis. Moreover, whether the RIG-I and MDA5 induce similar phenotype remains to be unfolded in light of that the existence of RLRs member LGP2 leads to tumor cells including CRC cell lines insensitive to ionizing radiation-induced cytotoxic effects [94].

Others nucleic acid sensing PRRs in colitis or CRC

The TLRs family is the first identified and the most studied PRRs. Nucleic acid sensing TLRs, including TLR3, TLR7, TLR8, and TLR9, are involved in the development of colitis and CRC, which have been reviewed elsewhere [9, 14–17]. Recent studies have showed that, in addition to TLRs, AIM2, STING, and RLRs, some other DExD/H box helicases, including DDX1, DDX3, DDX5, DDX6, DDX17, DDX21, DDX41, DHX9, DHX36, and DDX60, are also involved in pathogen recognition, immune responses, and cancers (including CRC) progression besides their functions on regulation of nucleic acid metabolism (Table 1) [95–98]. Among these receptors, DDX3, DDX5, DDX6, DHX9, DDX17, and DHX36 have been shown to be involved in the development of CRC. Furthermore, inherited and acquired mutation of DDX41, the DNA sensor upstream of STING that can activated by both cytoplasmic DNA and CDNs [55, 99], was frequently identified in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), indicating that DDX41 may serve as a tumor suppressor [100–104]. In this part, we will discuss the role of DDX3, DDX5, DDX6 and DHX9 in CRC development.

DDX3

DDX3, a member of DEAD-box proteins, is involved in diverse biological activities including binding RNA and acting as a transcriptional cofactor. DDX3 has two homologues, DDX3X and DDX3Y. The gene encoding DDX3X is located in X-chromosome in most tissues, while the gene encoding DDX3Y is located in the Y-chromosome in testis [105]. DDX3 is involved in regulation of several signaling
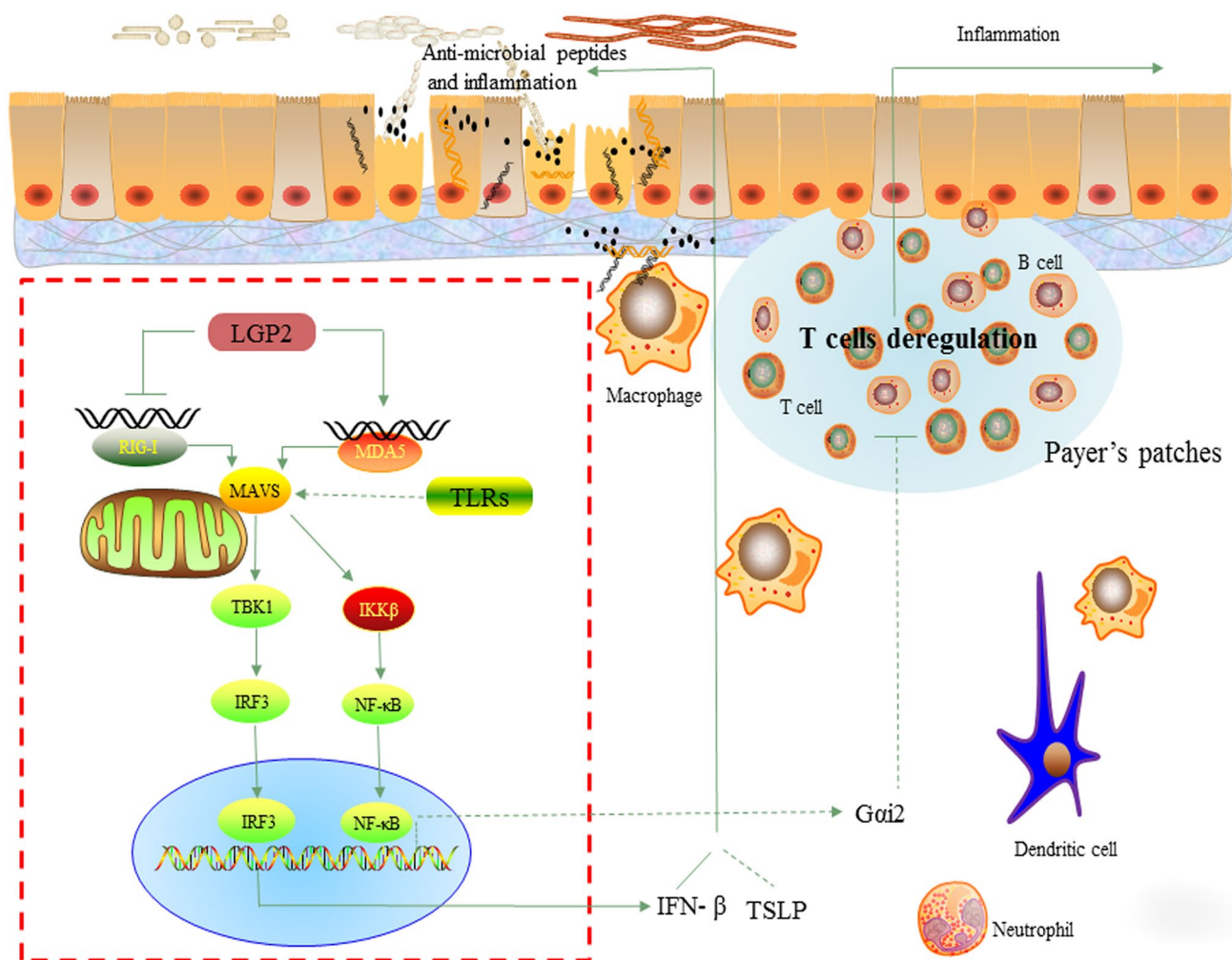
Fig. 3 The model of RLR signaling pathway in the development of colitis. RIG-I-like receptors (RLRs) are required for cytoplasmic RNA detection. Upon activation, both RIG-I and MDA5 recruit the adaptor-mitochondrial antiviral signaling protein (MAVS) via their CARD domains and form a platform to activate TRAF. This in turn leads to the phosphorylation of IRF3 and NF-κB signaling to promote the production of IFN-β and pro-inflammatory genes, respec-

tively. RIG-I enhances the promoter activity of Gαi2, partially through NF-κB, to reverses the deregulation of T cell subsets in Payer's patches—an important instigator for colitis development. On the other hand, MDA5 senses RNA from invasive gut microbiota to maintain intestinal homeostasis by inducing the expression of IFN-β and antimicrobial peptides. Moreover, IRF3 can also directly bind to TSLP to protect host from colitis

pathways, including Wnt/β-catenin signaling pathway, epithelial-mesenchymal transition (EMT) signaling pathways, and IFN-β signaling pathway. The Wnt/β-catenin/T-cell factor (TCF) target genes, such as Snail1, matrix metalloproteinase 7 (MMP7), plasminogen activator inhibitor 1 (PAI1), and RAS, are often associated with tumor invasion [106]. DDX3 can directly interact with CK1ε to facilitate β-catenin trafficking to nuclear and trigger Wnt/β-catenin/TCF signaling [107]. EMT is a hallmark event that contributes to tumor invasion and is characterized by an increase in mesenchymal markers and a decrease in epithelial markers (such as E-cadherin). EMT may be induced by Wnt/β-catenin, notch, and some other signaling pathways [108]. DDX3 can promote EMT program by suppressing the

expression of E-cadherin directly [109]. In addition, DDX3 is required for replication of RNA virus and is involved in inducing the production of IFN-β by interacting directly with the IFN-β promoter or interacting with IKKε, TBK1, or MAVS after binding to RNA virus [95].

The role of DDX3 in CRC is still controversial. One recent study indicated that the low level of DDX3 on CRC tissues is closely associated with a poorer prognosis and more severe metastasis [110]. *In vivo* and *in vitro* experiments confirmed that DDX3 silencing promotes the invasion of CRC that may be partially attributed to an increase in *Snail* expression and a decrease in *E-cadherin* expression [110]. These indicated that DDX3 might act as a CRC suppressor. However, another study demonstrated that

the positive expression of DDX3 in CRC tissues is associated with increased level of β-catenin in nuclear [111]. In addition, DDX3 silencing in CRC cell lines resulted in decreased proliferation rates, cell cycle arrest, and reduced production of target genes of Wnt/β-catenin signaling [111]. These indicated that DDX3 serves as an oncogene for CRC that may be partially attributed to the regulation of Wnt/β-catenin signaling. Consistently, subsequent studies demonstrated that DDX3 could promote the CRC invasion by increasing activity of CK1ε/Dvl2/β-catenin/TCF signaling or by increasing activity of KRAS/ ERK/PTEN/AKT/β-catenin signaling [112, 113]. Another recent study showed that DDX3 inhibition is safe and effective for restriction of a range of virus infection, which provides potentials for targeting DDX3 in CRC [114].

DHX9

DHX9 can unwind both RNA and DNA and regulate the gene expression by interacting with transcription factors such as NF-κB, p53, and c-Myc [115]. In addition, DHX9 plays an important role in maintaining gene stability [116]. Recent study suggested that DHX9 can respond to both CpG-B DNA and dsRNA via MAVS and MyD88, respectively [95]. Since DHX9 is implicated in a range of cellular processes, deregulation of DHX9 may also have great effects on cell growth or viability, and even leads to various diseases. One previous study demonstrated that *DHX9* level significantly increased in lung cancer tissues, and in comparison with non-small lung cancer, it was higher in small cell lung cancer, which was associated with a poorer prognosis [117, 118]. Subsequent studies showed that DHX9 is crucial for tumor survival, and DHX9 silencing restricts the tumor growth by regulating the transcript activities induced by p53 and c-Myc [119–121]. At the same time, DHX9 silencing in mice did not affect the vitality of normal cells, and *DHX9* expression was upregulated in human CRC tissues and cells [98, 122, 123], indicating that DHX9 promotes the development and progression of CRC, and may serve as a safe and effective molecular target for CRC therapy. Better understanding on the expression pattern and function of DHX9 in human CRC and its association with the tumor growth, metastasis, and prognosis is urged.

DDX5/6/17 and DHX36

Compared to DDX3 and DHX9, the signaling pathways of other CRC-associated helicases are less studied. The role of DDX5/6/17 in tumors was discussed previously [124]. DDX5 (p68), a founding member of the DExD/H box family, forms complex with DDX17 and is involved in multiple cellular processes [125]. Studies showed that both the expression of DDX5 and DDX17 increased in human CRC

cell lines and tissues, which were associated with more cellular differentiation [126, 127]. The detail mechanisms of increased DDX5 in tumor setting are still largely unknown, although there is a possible link with poly-ubiquitylation [127]. In addition, it was reported that DDX5 and DDX17 can promote the proliferation of CRC cells via regulating oncogenes, such as c-Myc [126]. DDX6 (p54) level is also increased in CRC tissues and cells [128]. Silencing of DDX6 may inhibit the proliferation of CRC cell lines, which is attributed to the downregulation of Wnt/β-catenin signaling pathway [129]. DHX36 was identified as a member of DExD/H box family in 2002. Later studies indicated that it forms complex with DDX1 and DDX21 to recognize dsRNA, and signals via TIR domain-containing adaptor-inducing IFNβ (TRIF), which eventually induces the production of type I IFN [130, 131]. However, the role of DHX36 in CRC is less studied, although one study reported that the overexpression of DHX36 can reduce the migration of CRC cells [132].

Taken together, the mechanisms of DExD/H box helicases on CRC are quite complicated. The DDX3 mainly interacts with Wnt-β-catenin signaling pathway and Snail/EMT signaling pathway to direct the development of CRC. DHX9 serves as an important role in tumor cell survival by regulating the transcript activities of oncogenes or tumor suppressor gene, such as p53 and c-Myc (Fig. 4; Table 1). Although studies indicated that the expression of DHX9 is increased in CRC tissues, additional studies are needed to characterize the underlying mechanisms. As for the DDX5/6/17 and DHX36, the mechanisms of their cancer promoting effects on CRC are more unshaped. Overall, some DExD/H box helicases exhibit a pro-tumor effect.

Conclusions and future prospects

The immune cells and some other cells are armed with a range of nucleic acid sensing PRRs. Activation of these receptors by diverse nucleic acids of microbes leads to inflammatory responses and the activation of innate as well as adaptive immune cells, which plays critical roles in host defense against intracellular bacteria and virus infection. Aberrant activation of nucleic acid sensing PRRs signaling pathway by self-nucleic acids also contributes to inflammatory diseases. Additionally, recent accumulating studies from others and ours broaden the previous understanding on the function of nucleic acid sensing PRRs in tumors.

The nucleic acid sensing PRRs in IECs can be activated by invasive gut microbiota or the accumulated/damaged DNA in cytoplasmic or nucleus, and further influence the development of colitis and CRC by regulating the following aspects: inflammasome, gut microbiota, AKT, type I IFNs, Wnt-βcatenin, Snail/EMT, and oncogenes or tumor
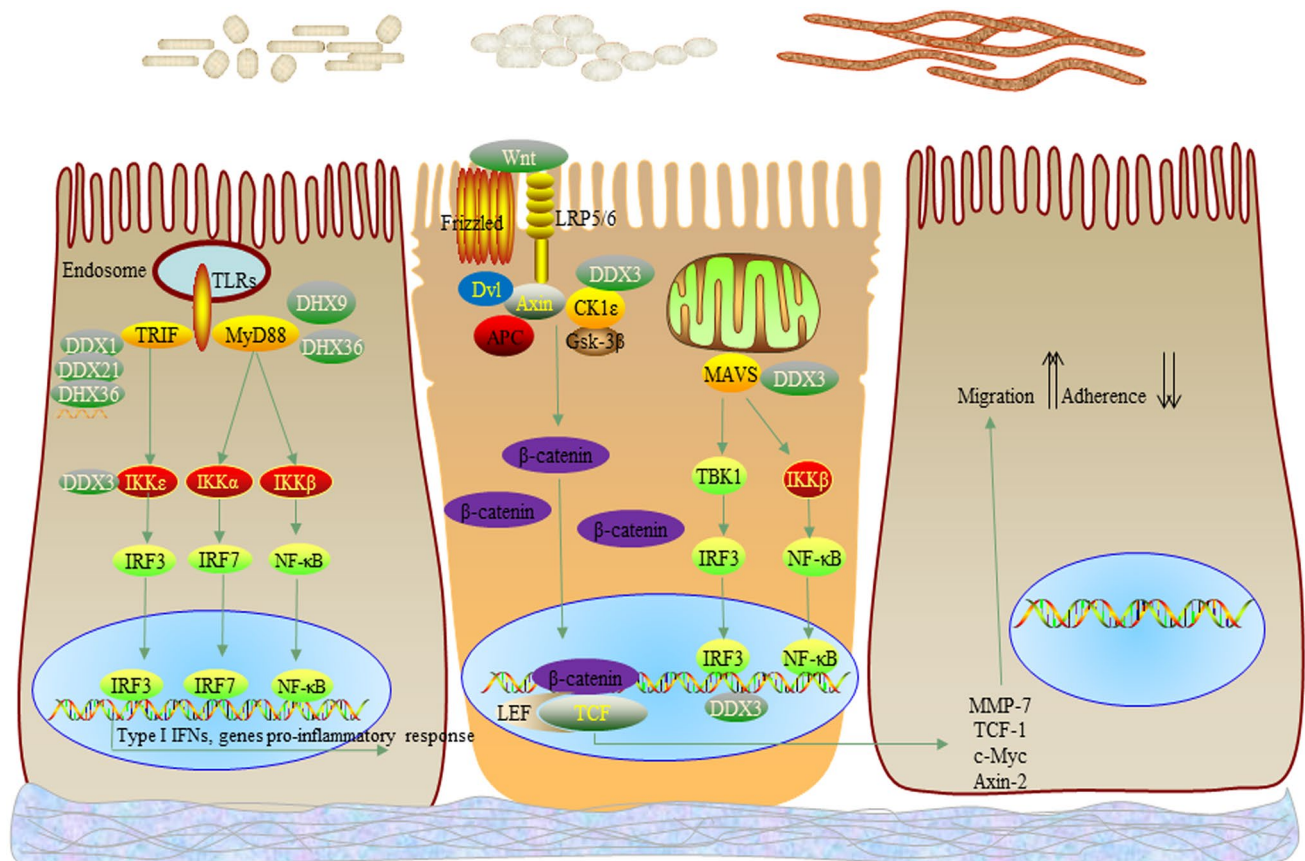
Fig. 4 DExD/H box helicases signaling pathway in the development of colorectal cancer. DExD/H box helicases, including DDX1, DDX3, DDX5, DDX6, DDX17, DDX21, DDX41, DHX9, DHX36, DDX41, and DDX60, are involved in pathogen recognition, immune responses, and cancers (including CRC) progression, in addition to their functions in the regulation of nucleic acid metabolism. DHX36 responds to dsRNA and forms a complex with DDX1 and DDX21. The complex can signal via TRIF, or detect CpG-A DNA and trigger immune response via MyD88. DHX9 can respond to both CpG-B DNA and dsRNA, and signal via MAVS and MyD88, respectively. DDX60 interacts with RIG-I and sensitizes the binding of RIG-I and dsRNA. DDX3 can interact with dsRNA and signal via MAVS, in addition to acting as a mediator of downstream of TBK, IKKε and a transcriptional regulator of the IFN-β promoter. Furthermore, DDX3 can promote the CRC invasion by increasing activity of CK1ε/Dvl2/β-catenin/TCF signaling pathway. Except DDX3, the signaling pathways of DExD/H box helicases in CRC are largely unknown

suppressor genes. Activation of AIM2 in IECs acids to maintain gut microbiota homeostasis in an inflammasome/IL-1β/IL-18/antimicrobial peptide-dependent manner to reduce pathogenic microflora such as *Escherichia coli* and thus protect against colitis. AIM2 binds to DNA-PK and inhibits the activation of AKT signaling pathway that is crucial for IECs proliferation. The STING signaling pathway mainly prevents the CRC development in a type I IFNs-CD8⁺T cell-dependent manner. Notably, the situation of DExD/H box helicases in CRC is much more complicated. Apart from RIG-I and MDA5 signaling pathway, the other members mentioned above exhibit an oncogenic activity though the underlying mechanisms are still largely unknown.

Despite accumulating evidences indicating that nucleic acid sensing PRRs are involved the development of CRC, more animal and clinical experiments are needed to

illuminate their specific mechanisms in CRC and make their clinic application to be possible. Firstly, apart from AIM2 and DDX3, studies on the correction between the expression profile of the other nucleic acid sensing PRRs in CRC and tumor biological behaviors as well as prognosis are still lacking. In addition, it is of interest to known whether it could be more precise to predict the CRC prognosis when combined nucleic acid sensing PRRs with other clinical features such as tumor stages. Secondly, the signaling pathway of DExD/H box helicases is poorly defined thought they tend to interact with a range of transcription factors and modulate RNA metabolism throughout the whole life activities. Thirdly, most of the nucleic acid sensing PRRs shuttle between cytoplasm and nucleus, and the experiments are required to understand their function in different subcellular localization in the development of diseases. Fourthly, accumulated evidences showed that AIM2,

STING, DHX9, LGP2, and DDX3 respond to chemoradiotherapy DNA damage and mediate different immunity responses. For instance, AIM2 responds to ionizing radiation-induced break DNA in nucleus and drives the pyroptosis of IECs, while STING senses dying tumor DNA after radiation potent leveraging radiotherapeutic management. These raise the questions that whether most of the nucleic acid sensing PRRs are involved in radiation-induced DNA responses. It is necessary to determine toxicity and synergy between chemoradiotherapy and nucleic acid sensing PRRs and, thus, optimize the treatment plans. Fifthly, in light of that most of the nucleic acid sensing PRRs play an important role in guarding pathogens invasion, whether most of them aid to maintain gut microbiota homeostasis like AIM2 and RLRs should be conceived. Lastly, it seems that some of the sensors such as AIM2 and STING can regulate each other and the cross-talk among these nucleic acid sensing PRRs is of interest. Overall, although some achievements have made in understanding the complicated role of nucleic acid sensing PRRs in colitis as well as CRC, there is still a long way to translate these discoveries into practice.

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