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Review Article

The adaptor protein CARD9, from fungal immunity to tumorigenesis

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Abstract: The adaptor protein CARD9 is in charge of mediating signals from PRRs of myeloid cells to downstream transcription factor NF-κB. CARD9 plays an indispensable role in innate immunity. Both mice and humans with CARD9 deficiency show increased susceptibility to fungal and bacterial infections. CARD9 signaling not only activates but also shapes adaptive immune responses. The role of this molecule in tumor progression is increasingly being revealed. Our early study found that CARD9 is associated with the development of colon cancer and functions as a regulator of antitumor immunity. In this review, we focus on the upstream and downstream signaling pathways of CARD9, then we summarize the immunological recognition and responses induced by CARD9 signaling. Furthermore, we review the function of CARD9 in multiple aspects of host immunity, ranging from fungal immunity to tumorigenesis.

Keywords: CARD9, NF-κB, immunological recognition, CARD9-associated diseases, tumorigenesis

Introduction

Caspase recruitment domain-containing protein 9 (CARD9) is an intracellular adaptor protein from the CARD protein family and is identified because of its selective binding with the CARD domain of B cell leukemia-lymphoma 10 (BCL10) [1]. CARD9 is predominantly expressed by myeloid cells [1, 2]. In structure, it has an N-terminal CARD domain, and a coiled-coil region at the C terminus. The former mediates homophilic interactions among CARD-containing molecules, and the latter functions as an oligomerization domain [1, 3], which is also the action site of TRIM62-mediated ubiquitination [4] (**Figure 1**).

CARD9 functions as a scaffold protein that relays signals from Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) to mitogen-activated protein kinase (MAPK) and transcription factor nuclear factor-κB (NF-κB) [2, 5]. As a result, CARD9 is a link between innate and adaptive immunity, which has been well researched in microbial infections, especially invasive fungal infections [6]. CARD9-deficient (*Card9*$^{-/-}$) mice are more susceptible to microbial infections compared with control mice [2, 5-7]. Moreover, the cytokine production mediated by NF-κB is severely impaired in the absence of CARD9 [5]. As an inflammation-related molecule, CARD9 is implicated in several sterile inflammation diseases and current studies suggested that CARD9 signaling is capable of decreasing host susceptibility to colitis-associated colon cancer (CAC), which further revealed the role of CARD9 in oncogenesis [8, 9].

Box 1-The brief information of the CARD protein family

The CARD protein family is composed of a cluster of signaling proteins that contain the typical CARD domain. The CARD domain is characterized by no more than seven antiparallel α-helices. This domain is a homophilic interaction module that participates in activation or suppression of CARD containing members [1]. Thus, it plays a regulatory role in cell apoptosis, inflammation, and NF-κB signaling [10]. In addition to some caspase proteins, the family also includes the adaptor proteins CARD3 (also called RICK/RIP2/CARDIAK), CARD4 (also called NOD1), CARD5 (also called ASC), CARD8 (also

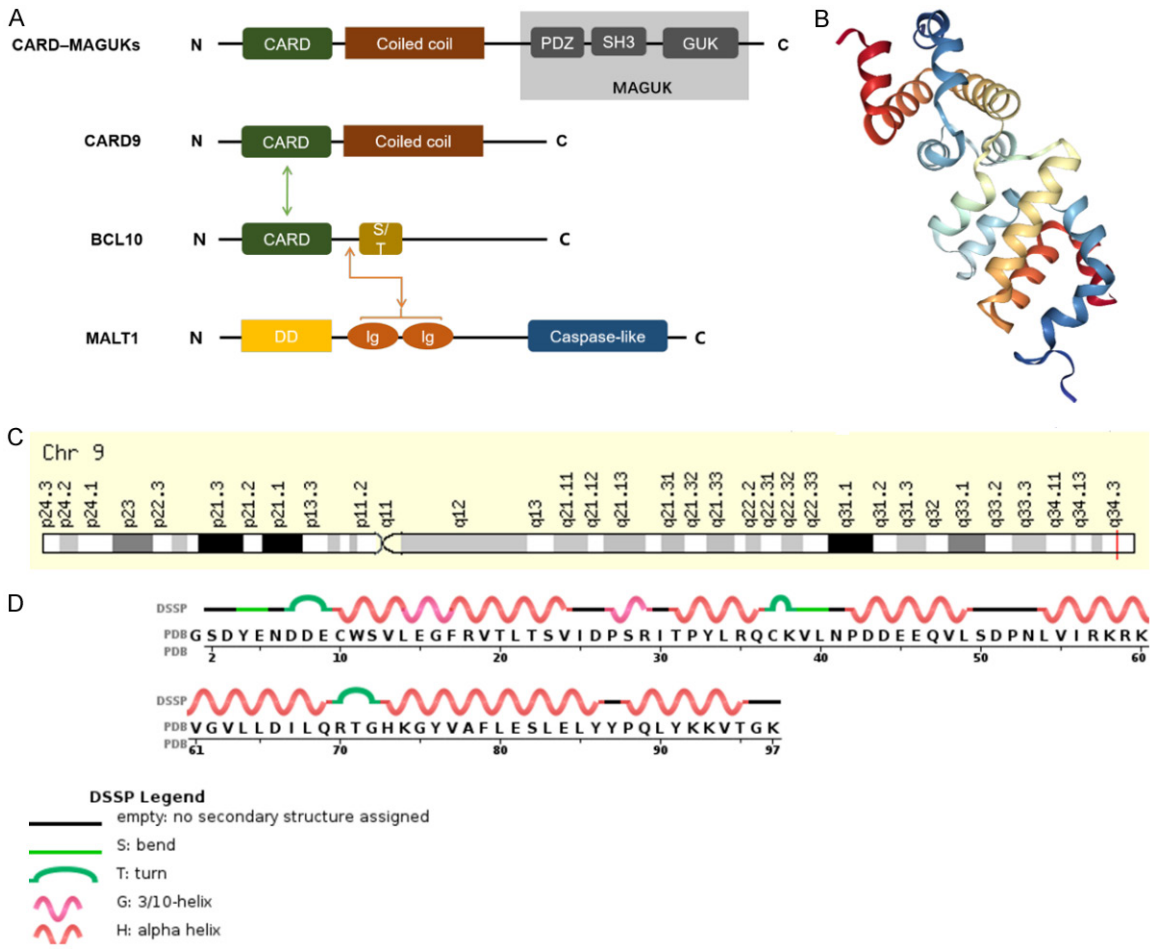

Figure 1. The fundamental characteristics of CARD9. In structure, CARD9 contains an N-terminal CARD domain and a C-terminal coiled-coil region; BCL10 is characterized by an N-terminal CARD domain and a C-terminal Ser/Thr-rich effector region; MALT1 consists of three Ig-like domains at the N terminus and a caspase-like domain at the C terminus. CARD9 interacts with BCL10 through their homologous CARD domain, while Ser/Thr-rich effector region of BCL10 binds to MALT1 via its Ig-like domains. The three molecules thereby forming a complex (A). The 3D view of CARD9 CARD domain-swapped dimer. Image from the RCSB PDB (rcsb.org) of PDB ID 6E28 (B). CARD9 Gene in genomic location: bands according to Ensembl, locations according to GeneLoc (C). The sequence chain view of CARD9. The showed Chain C includes one polymer and 97 residues. Image from the RCSB PDB (rcsb.org) of PDB ID 6E28 (D).

called TUCAN/CARDINAL), CARD9, CARD10 (also called CARMA3), CARD11 (also called CARMA1/BIMP3), CARD12 (also called IPAF/CLAN), CARD14 (also called CARMA2), and CARD15 (also called NOD2) [11-14].

Of the CARD protein family, CARD10, CARD11, and CARD14 belong to the membrane-associated guanylate kinase (MAGUK) family, and also have functional binding with downstream BCL10 [1, 11]. They share a PSD-95/Dlg/ZO-1 homologous (PDZ) domain, a Src-homology (SH3) domain and a guanylate kinase (GUK)-like domain in common, which are absent in CARD9 [11, 13] (**Figure 1**).

The intracellular-signaling networks of CARD9

CARD9 signals through a variety of innate pattern recognition receptors (PRRs) that mainly includes CLRs, TLRs, and intracellular nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) [7, 15]. Recognition of pathogenic microorganisms leads to recruitment of spleen tyrosine kinase (Syk) or receptor interacting protein 2 (RIP2 or CARD3) [16-18], further initiates the phosphorylation of CARD9. Activated CARD9 binds to BCL10 as well as to mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) to form a CARD9/BCL10/MALT1 (CBM) complex domain.

This allows the activation of NF-κB and MAPK, thus inducing the production of proinflammatory cytokines [1, 5].

CLR/CARD9 signaling in the immunological recognition of fungi

CARD9-related CLRs includes mainly Dectin-1, Dectin-2, Dectin-3 and Mincle. Upon recognition of carbohydrate agonists, CLRs recruits tyrosine kinases Syk under Src kinase-mediated tyrosine phosphorylation of the immunoreceptor tyrosine-based activation motif (ITAM)-like motif (hem-ITAM) or ITAM in their cytoplasmic tail [17, 19]. Syk is a pivotal mediator involved in coupling activated immunoreceptors in immune cells. Upon recruitment, Syk is phosphorylated and subsequently activates protein kinase Cδ (PKCδ), which leads to the recruitment and phosphorylation of CARD9 at Thr231 [20, 21]. Vav proteins are demonstrated to be key regulators that associate Syk and CARD9 similar to PKCδ [22]. In detail, Dectin-1 bears a hem-ITAM motif that only has a single Yxxl/L and transduces intracellular signaling in the form of homodimer directly via Syk [23], whereas Dectin-2, Dectin-3 and Mincle require additional association with FcRγ, which is an ITAM-containing adaptor molecule that possesses a tandem repeat of Yxxl/L, to indirectly signal through Syk [23, 24] (**Figure 2**).

CLRs play a major role in recognizing pathogenic microorganisms, especially fungi. The C-type lectin domain in the extracellular region of CLRs is a distinctive carbohydrate recognition domain [25] binding to carbohydrates found in fungi, including β-glucans and α-mannose [15, 19]. Dectin-1 participates in "phagocytic synapse" complexes [26] and specifically senses β-1,3-glucans in fungal cell walls [27], while Dectin-2 and Dectin-3 recognize α-mannose-type carbohydrates [24, 25, 28]. A recently noticed and less well-characterized receptor Mincle can bind to amphiphilic glycolipids from *Malassezia* [29].

Obviously, fungal cell walls are composed of more than one component; consequently, an array of PRRs are stimulated during fungal infections depending on the fungal species. Thus, the intact immune responses against fungal species require the simultaneous participation of several members of the CLRs [30]. Dectin-1 is the most representative Syk/CARD9-dependent pathway, which activates inflammasomes and production of proinflammatory cytokines and chemokines, including TNF-α, GM-CSF, CXCL2, IFN-γ, IL-1β, IL-2, IL-6, IL-10, and IL-23 [31] under the management of CARD9, to control the respiratory burst and suppress fungal pathogens [16]. Also, Dectin-1 signaling can polarize Th1 and Th17 type adaptive immunity against fungal infections [32]. CARD9 is indispensable for CLRs-induced antifungal immune responses, the implications of which are discussed later. In animal study, macrophages from Dectin-1-deficient mice failed to generate defensive inflammatory responses when stimulated with zymosan, a β-glucan-rich fungal component, and showed increased susceptibility to fungal infections with *C. albicans*, *Pneumocystis carinii*, and *A. fumigatus* [15, 33]. Consistent with this, human with Dectin-1 polymorphism were more susceptible to mucocutaneous infections caused by *C. albicans* due to defective cytokine release by monocytes and macrophages [34].

Dectin-1 can collaborate with TLR2 or TLR4 and result in increased CARD9-mediated NF-κB activation and cytokine production, such as TNF-α, IL-10 and IL-23, and decreased IL-12, especially when infected with *A. fumigatus*. Their interaction with each other initiates a much more powerful and comprehensive antifungal immunity that equips hosts with a stronger resistance to invasive pathogens [15, 35, 36]. In addition, Dectin-3, together with Dectin-2, composes heterodimers, which recognize α-mannans more effectively than pure homodimers during *C. albicans* hyphae infection [28].

TLR/CARD9 signaling in the immunological recognition of bacteria and other pathogens

Of all PRR families, TLRs are the best defined and similar to CLRs, play essential roles in both innate immunity and adaptive immunity. TLRs are expressed by macrophages and dendritic cells, as well as by specific B cells and T cells [37]. They can recognize a wide range of PAMPs, such as LPS, flagellin, and nucleic acids [37] (**Figure 2**). Myeloid differentiation primary response 88 (MyD88) is a critical intracellular signaling adaptor required for all TLRs except TLR3 [38]. Both *Card9*$^{-/-}$ and MyD88$^{-/-}$ bone marrow-derived DCs (BMDC) had impaired regulation of TLR-induced cytokine responses [2]. The TLR/MyD88 signaling pathway recruits

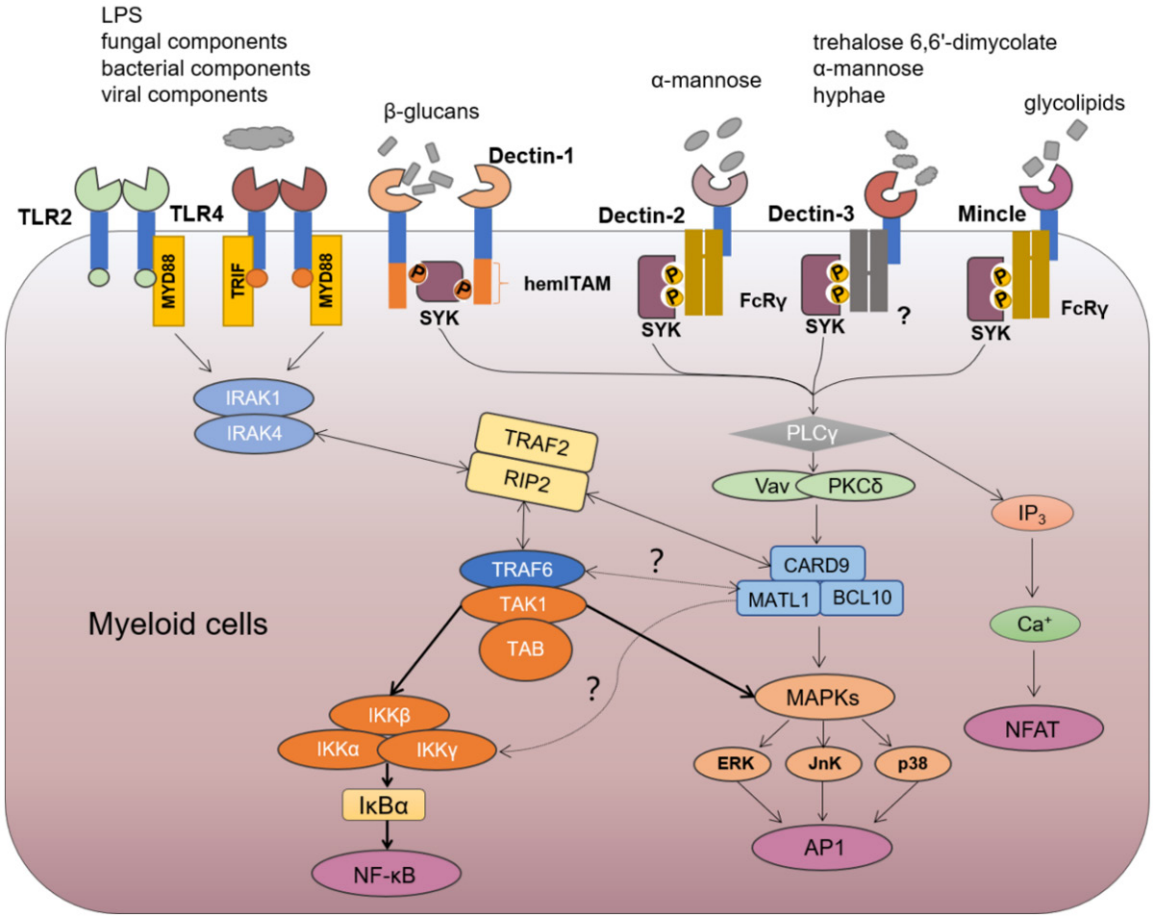

Figure 2. The upstream and downstream pathways of CARD9. Dectin-1 bears an hemITAM and mediates intracellular signaling directly via Syk, it mainly senses β-glucans in fungal cell walls; Dectin-2 and Mincle mediate intracellular signaling indirectly via association with an ITAM-containing adaptor molecule called FcRγ and Syk. And Dectin-2 mainly recognizes α-mannose, while Mincle majorly senses amphiphilic glycolipids. Dectin-3 couples with an unknown signaling adaptor to initiate Syk and can also recognize α-mannans. TLRs are also transmembrane receptors. They are capable of recognizing diverse PAMPs such as lipopolysaccharide (LPS), peptidoglycan and nucleic acids of fungal, bacterial and viral components. The signaling transmission mediated by TLRs demands specific intracellular signaling adaptors including MyD88. CARD9 transduces signals downstream of Syk to the canonical NF-κB pathway through cooperating with BCL10 and MALT1 for pro-inflammatory responses. Syk activates PKCδ and Vav proteins, which promote the activation of CARD9; RIP2 associates with CARD9, IRAK4, and TRAF6 to mediate activation of NF-κB and MAPKs. IKK complex consists of IKKα, IKKβ, and IKKγ, among which IKKγ is required for connecting with downstream molecules. MALT1 is responsible for relaying the signal from BCL10 to the IKK complex. Upon stimulation, IKKγ mediates ubiquitination and causes NF-κB to locate to the cell nucleus, thus inducing gene transcription of cytokine production.

CARD-containing RIP2 to link with the interleukin-1 receptor-associated kinase 1 (IRAK1) and the ubiquitin ligase TNF receptor associated factor 6 (TRAF6). IRAK1 can recruit BCL10, which results in the activation of CARD9 and formation of the CBM complex [18, 39]. Interestingly, CARD9 is necessary for TLR-induced activation of MAPK but is dispensable for NF-κB activation [2].

TLRs play the dominant role in host defense against bacteria. MyD88-dependent TLR stimulation signals via a complex consisting of CARD9, RIP2 and IRAK1 [2]. TLR1, TLR2, TLR4, TLR5, and TLR6 are extracellular receptors that ensure the recognition of PAMPs derived from microbial membranes, such as lipids and lipoproteins, while TLR3, TLR7, TLR8, and TLR9 are intracellular receptors responsible for sensing nucleic acids coming from pathogens [40].

LPS, also known as endotoxin, is a component originating from the cell wall of gram-negative bacteria. TLR4 couples with MD-2 and forms an

LPS-binding complex on the cell surface. Upon bacterial invasion, LPS first interacts with LPS-binding protein (LBP) and then CD14, a glycosylphosphatidylinositol-linked and leucine-rich protein expressed by phagocytes. CD14 is in charge of delivering LPS/LBP to the TLR4/MD2 complex, while the activation of TLR4 commands signals to the CARD9 signalosome. In addition to LPS, TLR4 recognizes a very divergent collection of ligands, including respiratory syncytial virus fusion proteins, the plant-derived cytostatic drug paclitaxel, heat-shock proteins and so on [40, 41]. A reduced resistance to infection by *Salmonella typhimurium* or *Neisseria meningitis* has been identified in TLR4-mutated mice [37]. TLR2 normally forms heterodimers with TLR1 or TLR6. They recognize lipoteichoic acid, lipoproteins and peptidoglycan derived from gram-positive bacteria [37, 41]. Consistently, TLR2-deficient mice show an enhanced susceptibility to *Staphylococcus aureus* or *Streptococcus pneumoniae*, while humans with a polymorphism in the gene encoding TLR2 suffer from decreased defense to infection caused by gram-positive bacteria [37].

TLR2 and TLR4 are also implicated in the recognition of mycobacteria, one group of intracellular bacteria living within the macrophages. Of all cell wall components, lipomannan and lipoarabinomannan are relatively powerful stimulators that induce inflammatory cytokines in a TLR2/MyD88/CARD9-dependent manner. During the course of endolysosomal degradation, the DNA of mycobacteria can also be liberated and consequently activates TLR9, a bacterial genomic DNA receptor.

Different from those of fungi and bacteria, virus particles contain less kinds of components, which include genetic material and enzymes for synthesis or replication. In the detection of viral nucleotides, TLRs, especially TLR3, TLR7, TLR8, and TLR9, take a great deal of responsibility, while TLR2 and TLR4 are capable of recognizing viral glycoproteins. Components of parasitic worms can also be recognized by TLRs, including TLR2, TLR4, and TLR9. Following the activation of TLRs, the induction of the CARD9 signaling pathway results in Th1 responses, which can control the growth of parasites [37]. Similarly, CARD9 can mediate the recognition of bacteria, viruses and parasites through CLR-dependent pathways [42-47].

NLR/CARD9 signaling and inflammasome

The NLR family is located in the cytosol. They detect components from bacteria and injured or dying cells. NOD1 (or CARD4) and NOD2 (or CARD15) are the first two members discovered to have emerging roles as sensors of bacterial peptidoglycan. Upon stimulation by bacterial peptidoglycan, NOD1 and NOD2 form oligomeric proteins and recruit RIP2 via CARD-CARD interactions, which directly associates with CARD9 [48]. CARD9 is implicated in the selective regulation and control of NOD2-mediated activation of p38 and JNK signaling but not NF-κB [5].

The inflammasome is a multimeric signaling complex in the cytoplasm that mediates the proteolytic cleavage and maturation of IL-1β and IL-18 by controlling the activation of the inflammatory protease Caspase-1 and induces consequent cell death called pyroptosis [49]. Numerous inflammasomes have been described so far, among which the NLRP3 inflammasome is the best studied, which contains the NLR family, pyrin domain-containing 3 (NLRP3), the apoptosis-associated speck-like protein (ASC), and Caspase-1 [49]. During the inflammation formation, Syk is recruited and activated after stimulation of NLRP3 to trigger two downstream processes, CARD9-mediated NF-κB activation and ROS production. CARD9 signals result in pro-IL-1β and pro-IL-18 synthesis, while ROS production activates inflammasome that is required for proteolytic processing of pro-IL-1β to produce bioactive IL-1β [21, 50]. Although CARD9 regulates the pro-IL-1β synthesis, CARD9 is not necessary for inflammasome activation, due to the fact that pro-IL-1β synthesis and IL-1β production were blocked but NLRP3 activation was not affected in *Card9*$^{-/-}$ BMDCs in response to *C. albicans* [50]. The engagement of NLRP3 inflammasome is important in response to fungal infection. However, excessive activation of the NLRP3 inflammasome can lead to sterile inflammation and sometimes even promote the progress of autoimmune and inflammatory disease due to an excess of IL-1β and IL-18 [51] (**Figure 3A**).

The CARD9/BCL10/MALT1 complex is responsible for signaling relaying from PRRs to downstream signal components

CARD9 converges all signaling from various PRRs. The CBM complex is a cytosolic heterotri-

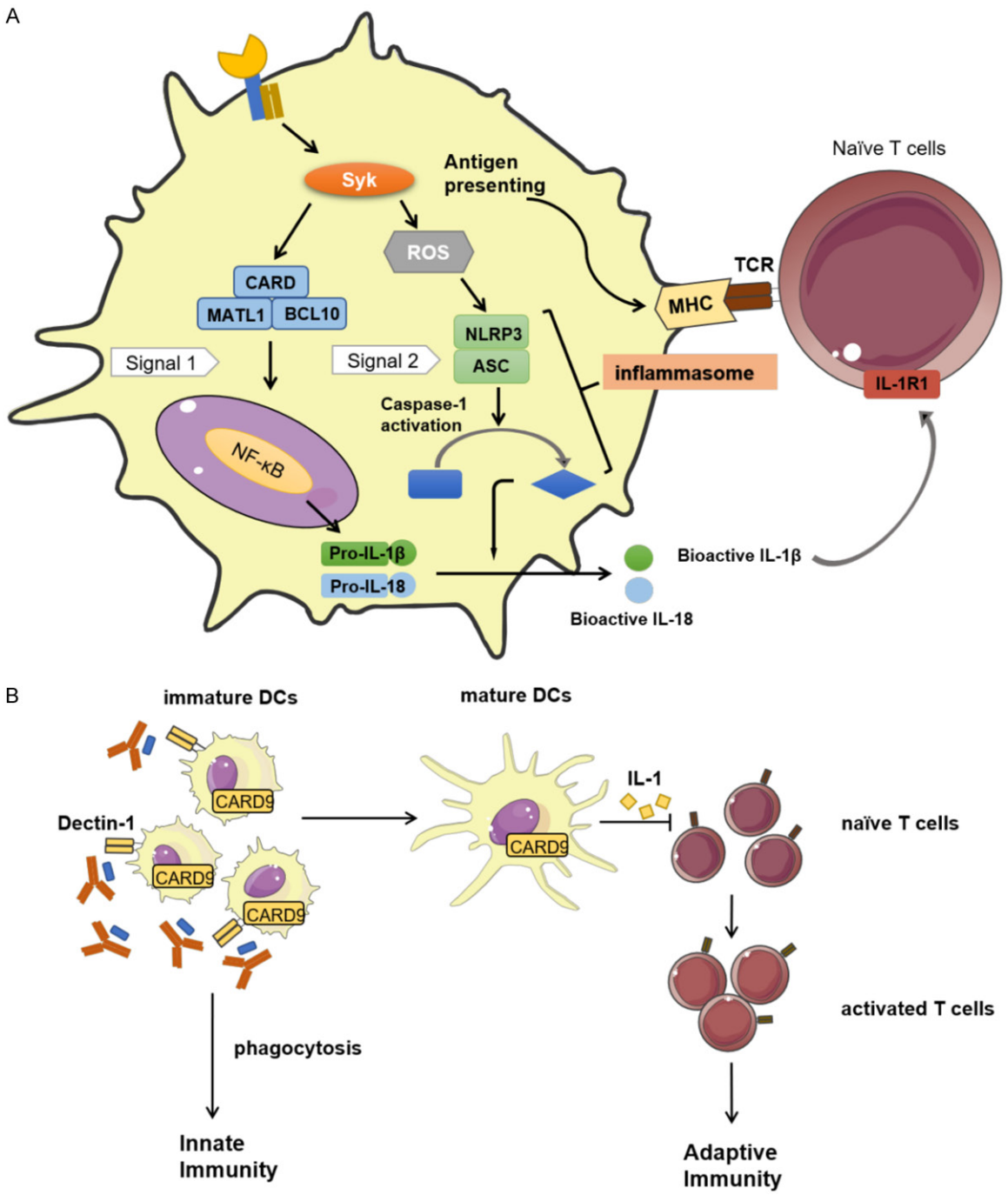

Figure 3. The maturation of DCs and IL-1α/β mediated by CARD9. The synthesis of IL-1α/β involves two signals, signal 1 comes from ITAM-Syk-mediated pro-IL-1α/β synthesis, and signal 2 comes from Syk-mediated NLRP3 inflammasome activation. Bioactive IL-1α/β can prime T cells via IL-1R1-MyD88 pathway (A). Immature DCs can recognize and take up pathogens, initiating the innate immunity. The maturation of DCs requires CARD9, and mature DCs could present antigens to naïve T cells and induce their differentiation, thus mediating the adaptive immunity (B).

mer assembled via a common CARD domain and they function as a central integrator to transmit signals from PRRs to downstream molecules. BCL10 is an adaptor protein that is required for CARD9-mediated NF-κB activation [52]. BCL10 consists of an N-terminal CARD and a C-terminal Ser/Thr-rich effector region, while MALT1 possesses two Ig-like domains and a death domain at the N terminus. Signal from BCL10 is relayed by MALT1 to the inhibitor

of NF-κB (IκB) kinase (IKK) complex through combination with the ubiquitin ligase TRAF6 and transforming growth factor β-activated kinase 1 (TAK1) [53-55]. The CARD9 C terminus is phosphorylated by PKCδ or casein kinase 2 (CK2) and integrate with BCL10 via CARD-CARD interaction [56], and BCL10 binds to the Ig-like domain of MALT1 via its C-terminal domain [57], leading to formation of the CBM signalosome (**Figure 2**).

NF-κB is the major transcription factor regulating the CARD9 signaling pathway

NF-κB is a pleiotropic transcription factor found in all cell types and is involved in the cellular responses to a vast array of stimuli. NF-κB plays a key role in regulating numerous proinflammation cytokines downstream to mediate immune responses. Due to its extensive participation in the immune system, including lymphoid organogenesis, hematopoiesis, inflammation and cancer biology, NF-κB activation is a tightly regulated process [58].

Previous studies have shown that the CBM complex mediates canonical NF-κB activation by controlling the activity of the IKK complex. IKK contains two kinases called IKKα and IKKβ and a regulatory subunit, IKKγ (also called NEMO). IKKγ is in charge of interacting with downstream regulatory molecules [57]. Generally, attachment with dephosphorylated IκB proteins inhibits the nuclear translocation and activation of NF-κB [59]. Antigen receptor triggering leads to a cascade of signals that activates CARD9, allowing for oligomerized form of BCL10 and MALT1 to activate IKK, which phosphorylates the IκB proteins. This results in degradation of the IκB proteasome and contribution to nuclear translocation of NF-κB [52]. The exact nature of functional interaction between BCL10 and MALT1 and how exactly does the CBM signalosome regulate NF-κB activation are not precisely understood. It is thought that BCL10 specifically enforces the oligomerization of the MALT1 caspase-like domain instead of directly interacting with IKKγ [57]. One hypothesis is that MALT1 works as a scaffolding component for the recruitment of TRAF2 and TRAF6 and induces their oligomerization, resulting in the ubiquitination of MALT1 and BCL10, which creates a docking site for TAK1 and TAB2. The IKK complex is recruited to the TAK1/TAB2 complex through ubiquitinated IKKγ by TRAF6, allowing TAK1 to phosphorylate IKKβ, ultimately resulting in activation of IKK [7, 52] (**Figure 2**).

Dectin-1/Syk signaling in macrophages and DCs can drive the activation of phospholipase Cγ (PLCγ), which further activates nuclear factor of activated T cells (NFAT), a family of calcineurin-dependent transcription factors that integrate with other transcriptional partners including activator protein 1 (AP1) and NF-κB signaling to promote gene transcription [60, 61]. This results in the production of COX2 and prostaglandin in macrophages, and cytokines IL-2, IL-10, and IL-23 in DCs [60].

MAPK signaling cascades of phosphorylation events

In general, MAPKs are involved in a wide range of cellular responses, including cell proliferation, differentiation, apoptosis, stress response, and cell death [62]. Each family of MAPKs consists of three conserved kinases that mediate the cascade of phosphorylation events. TAK1 has been identified as one of the best characterized MAPKKK. The formation of the CARD9/BCL10/TRAF6 complex provides a signal to stimulate TAK1 that sequentially induces the phosphorylation of MAPKs and activate a transcription factor, AP-1, which is an important target of MAPKs in the nucleus [63].

Previous studies have revealed that overexpression of CARD9 resulted in an increased activation of the p38 MAPK and JNK [64], while gene silencing of CARD9 significantly downregulated the expression of p38 MAPK [65]. Activation of the kinase p38 and JNK is apparently impaired in *Card9*$^{-/-}$ BMDC after zymosan stimulation. The downstream effectors that command CARD9-dependent MAPK activation are not well defined. After TLR stimulation, RIP2 and IRAK1 are recruited to associate with CARD9 and function together to activate MAPK [2]. Furthermore, Dectin-1 can initiate Ras-guanine-nucleotide-releasing factor 1 (Ras-GRF1) phosphorylation, thereby leading to the formation of a complex with CARD9, which further recruits H-Ras and results in activation of ERKs [66].

The negative regulation of the CARD9 signaling pathway

RUN domain Beclin-1-interacting cysteine-rich-containing (Rubicon) is a multidomain adaptor

protein downstream of PRRs, including TLR2, Dectin-1, and RIG-1 [67]. Rubicon engages in positive regulation of the NADPH oxidase complex and negative regulation of CARD9-mediated immune responses. By binding to $p22^{phox}$ of NADPH oxidase following TLR2 stimulation, Rubicon induces a burst of antimicrobial ROS and mediates the control of bacterial pathogens. In CARD9-dependent pathways, Rubicon can associate with CARD9, disassemble the CBM complex and competitively displace CARD9 depending on its serine phosphorylation status. Upon dephosphorylation, Rubicon is activated and able to prevent CARD9-mediated signal transduction and suppresses the inflammatory cytokine response [67, 68]. As is known, CARD9 deficiency is related to increased susceptibility to fungal diseases; however, aberrant activation of CARD9 may cause inflammatory diseases due to pathological immune cell activation. This inhibitory feedback mechanism is beneficial for balancing host immune responses to avoid deleterious effects caused by excessive inflammatory cytokines.

Innate immunity mediated by myeloid cells via CARD9

CARD9 recruitment to the phagosomes

Phagocytes, including mainly neutrophils, macrophages, monocytes and dendritic cells, are the main force in innate immunity that promote the clearance of fungi, and the immune response induced by phagocytes depend on the cell type involved. The identification of fungal components is mediated directly through multiple PRRs in the membrane of phagocytes as we discussed before or indirectly with the help of soluble PRRs, such as complement. Upon recognition, phagocytes initiate the internalization of fungi and recruit CARD9 to forming phagosomes, with which they mediate phagocytosis and direct fungi killing although CARD9 is redundant for phagocytosis [69, 70]. They kill internalized fungi through oxidative and nonoxidative mechanisms, such as the respiratory burst, and the reactive nitrogen intermediates [69]. However, aberrant CARD9 activation could induce pathologic activation of immune effector cells, resulting in inflammatory disorders or even cancers.

CARD9 drives neutrophil recruitment in inflammation and cancer

According to the ImmGen database, the expression of CARD9 in neutrophils is the highest among all immune cells [71]. Neutrophils are one of the first responders against invading pathogens, the effector functions of which includes phagocytosis, respiratory burst, degranulation, and release of neutrophil extracellular traps (NETs). During acute inflammation, they are also the first leukocytes to respond. Neutrophils are however as major players linking up inflammation with the pathogenesis of tumor progression and chronic syndromes [72]. The view of the role of neutrophils in the tumor microenvironment has switched from bystanders to contributors of the initiation and progression of cancer [72, 73]. Neutrophils recruitment mediated by CARD9 may lead to the generation of a permissive and proinflammatory premetastatic niches facilitating the survival and metastasis of cancer cells [72]. *Card9*$^{-/-}$ neutrophils demonstrated reduced accumulation at sites of inflammation *in vivo* due to defective generation of the inflammatory environment including remarkable decreased level of chemokines CXCL1, CCL3, CXCL2 and cytokine IL-1β. However, the intrinsic migratory capacity of *Card9*$^{-/-}$ neutrophils was not affected [71, 74]. Moreover, study of *in vitro* role of *Card9*$^{-/-}$ neutrophils through lineage-specific deletion studies indicated that the short-term effector functions of neutrophils were not influenced while a great reduction of gene expression was observed upon immune complex stimulation attributed to reduced phosphorylation and degradation of IκB and nuclear translocation of NF-κB [71]. Taken together, CARD9 is required for neutrophil-induced inflammation whereas several kinds of cancer have originated from infections that eventually progress to chronic inflammation, such as colitis, hepatitis and gastritis. Thus, neutrophil CARD9 could be also a potential target for antitumor therapy.

CARD9 promotes macrophage polarization and tumor metastasis

CARD9 expression in macrophages is essential for immune responses against microbial insults. CARD9-deficient macrophages showed a total defect in killing *Listeria monocytogenes* and a partial defect in suppressing *C. albicans* because of impaired respiratory burst [75]. In addition, macrophages constitute a unique component of the complex tumor microenvironment and substantial clinical and experimental results suggest that macrophages promote the progression of invasion, metastasis and angiogenesis of tumor cells [76]. High expression of

CARD9 was found in increased infiltration of macrophages in the tumor microenvironment of human colon carcinoma. Further study into the molecular mechanism illustrated the tumor metastasis-promoting role of CARD9 in infiltrating macrophages and that CARD9 is responsible for macrophage polarization toward the metastasis-inducing phenotype by enhancing protumor cytokine levels through activation of NF-κB signaling pathway. On the other hand, tumor cells also have an effect on macrophages via the secretion of cytokine VEGF to drive Syk activity in macrophages, leading to formation of CBM complex. Additionally, CARD9 deficiency is proved to block the tumor metastasis mediated by macrophages, which provides a novel insight into the therapeutic target for cancer metastasis [77].

CARD9 is required for dendritic cells maturation

DCs, the best representatives of APCs, are capable of capturing and internalizing pathogens via CLRs or TLRs and inducing both innate and adaptive antimicrobial immunity [78]. CARD9 signaling is required for the DC maturation and induction of proinflammatory cytokines. Dectin-1 stimulation by microbial antigens leads immature DCs to undergo a maturation phase, which is induced by Dectin-1/Syk/CARD9 signaling and independent of TLR signaling [32], triggering secretion of different immune effectors, including TNF-α, IL-1β, IL-2, IL-6, IL-10, IL-12, IL-23 and various chemokines that recruit various immune effector cells, such as neutrophils [32, 79]. As demonstrated in a study, CARD9-deficient DCs failed to activate NF-κB, and the transcriptional induction of TNF-α was remarkably constrained to zymosan stimulation [5, 36]. Signals through TLRs also facilitate DC activation and maturation possibly due to the shared CARD9 signaling. CARD9 deletion in DCs has been reported to reverse the development of experimental colitis and autoimmunity in Lyn-deficient mice by weakening TLR-induced cytokine production and DC-driven inflammation [80]. Moreover, upregulation of the costimulatory molecules after DC maturation promotes their capacities to present antigens to naïve T lymphocytes. DCs play an important role in shaping adaptive T cell responses by triggering the differentiation of $CD4^+$ lymphocytes into Th1/Th17 effector cells through specific cytokine secretion [79] (**Figure 3B**). The impact of Th17 responses on humans will be discussed in the following sections.

CARD9 and myeloid-derived suppressor cells (MDSCs)

MDSCs are immature cells of myeloid origin characterized by their immune suppressive activity. Actually, MDSCs are a heterogeneous population of immune cells which consist of a monocytic and a neutrophilic subset. A systematic expansion of MDSCs was detected in patients with microbial infections, inflammation and different types of cancer. Their contribution to immune suppression and tumorigenesis is well-established, basically through the increased production of arginase 1, ROS and nitrogen species to suppress T cell-based responses [81, 82]. MDSC expansion is governed by two groups of signals, one is induced by microbial products or other danger signals that provide for the pathologic activation of MDSCs, such as IFN-γ, TGFβ and IL-1β; the another one is produced by tumor cells, including COX2, GM-CSF and VEGF, which promote the accumulation of immature myeloid cells [81]. Accumulating evidences suggest that CARD9 signaling is involved in MDSC differentiation. Our early study had observed that CARD9 deficiency subjected mice to increased tumor burden in the model of AOM-DSS-induced CAC. CARD9 deficiency led to modified intestinal fungal composition with a remarkable growth of *C. tropicalis*. $CARD9^{-/-}$ macrophages failed to clear *C. tropicalis*, resulting in enhanced intestinal fungal burden and tumor progression, while *C. tropicalis* was responsible for inducing the differentiation and accumulation of MDSCs, which suppressed effector T cell responses and facilitated the progress of colon cancer [8] (**Figure 6B**). In lung cancer research, $CARD9^{-/-}$ mice suffered from heavier tumor burden with an increased number of MDSCs in tumor tissues. CARD9 was found to suppress the expansion of MDSCs and indoleamine 2,3-dioxygenase (IDO) production, an immune suppressive factor of MDSCs [83]. Particularly, neutrophilic MDSCs has also been reported to play a beneficial role in NK and Th17-induced hyperinflammatory responses during systemic *C. albicans* infection through Dectin-1/CARD9 signaling-mediated production of ROS and IL-1β [84].

CARD9 and Innate lymphoid cells (ILCs)

CARD9 is essential for the activation and recruitment of IL-22-producing ILCs in intestinal homeostasis after epithelial injury [85]. ILCs are major innate source of IL-22 (see below) that plays a role in maintaining mucosal homeostasis of both mice and human beings [86]. Mice with CARD9 deficiency showed attenuated production of chemokine CCL20 and cytokine IL-1β by myeloid cells, resulting in impaired recruitment of ILC3s and IL-22 expression, promoting inflammation-driven tumor growth [85, 87] (**Figure 4A**). CARD9 signaling has been demonstrated to specifically regulate the generation of IL-22 by ILC3s through the production of myeloid-derived IL-1β during colonic inflammation [87]. ILC3s express IL-1 receptor IL-1R1 and it is proved that IL-1β can stabilize IL-22 secretion by ILC3s [88].

Box 2--Innate lymphoid cells

Innate lymphoid cells are defined as a group of tissue-resident lymphocytes that share a great similarity with T cells, particularly in the secretion of certain cytokines and dependence on some transcription factors, while the diversification compared with T cells lies in the lack of cell-surface molecules. At the early stage, ILCs were separated into three dissimilar subsets. Group 1 ILCs are composed of ILC1s and NK cells, which are characterized by the production of IFN-γ and the dependence on the transcription factor T-bet; group 2 ILCs are indicated as ILC2s, which are reliant on GATA3 and RORα and generate cytokines such as IL-5 and IL-13; and group 3 ILCs are dependent on RORγt, secrete IL-17 and/or IL-22, similar to Th17 cells in the gut (**Figure 4B**) [89]. The most recent classification divides ILCs into five subcategories, including ILC1s, ILC2s, ILC3s, NK cells, and LTi cells [90].

ILC2s and ILC3s, both of which are capable of expressing MHCII molecules, have been found to present antigens from the commensal microbiota to antigen-specific T cells [91]. Interestingly, in the development of Crohn's disease, patients were discovered to have a decreased number of MHCII-expressing ILC3s, which might account for the dysregulation of T cell responses and result in intestinal inflammation [92]. ILCs play both favorable and unfavorable roles such as conducting the immune response against incursive microbes, limiting infection and reinforcing host defense to tissue development and repair, whereas under certain conditions, they can also facilitate the pathogenesis and inflammation of microbiota-induced disorders. For instance, they can promote the development of inflammatory bowel diseases (IBD), obesity, other metabolic disorders, and autoimmune diseases, as well as tumors [90, 93, 94].

IL-22 expressed by ILC3s has an important impact on maintaining tissue homeostasis, especially in the gut. In addition to managing epithelial cells to produce antibacterial peptides and antiviral proteins, IL-22 can also complement epithelial cells from intestinal stem cells and shield the intestinal barrier from a series of injuries, such as acute inflammation and irradiation. In colorectal cancer, presumably, the defect in IL-22 is relatively responsible for the severe inflammation, given that IL-22 is required for the excitation and proliferation of epithelial cells and that it is also involved in promoting the progression of colon cancer [90, 95].

CARD9 signaling shapes adaptive Th1/Th17 immunity

The inflammatory mediators released from CARD9-induced DCs greatly influence the polarization of Th cell responses. CARD9 deficiency has been shown to result in altered Th1 and Th17 responses [32] and the majority of CARD9 mutations lead to decreased circulating Th17 cells [96]. Th17 cells are characterized by the secretion of IL-17 and are considered to have central roles in host adaptive defense against fungi [97]. IL-1β together with IL-6 and IL-23 driven by CARD9 signaling has a strong preference for Th17 responses. Dectin-1/Syk/CARD9 pathway can induce greater Th17 immunity than TLRs signaling in response to *C. albicans* probably because DCs stimulated by Dectin-1 agonists are inclined to produce more IL-23 rather than IL-12, which drives Th1 immunity [32]. IL-17 and IL-22 generated by Th17 cells are critically required for induction of inflammatory responses through mobilizing neutrophils and mediating epithelial cells to produce antimicrobial peptides, respectively, as well as maintaining antifungal defense [98] (**Figure 5A**). In humans, the deficiency of Th17 immunity, including Dectin-1/Syk/CARD9 signaling

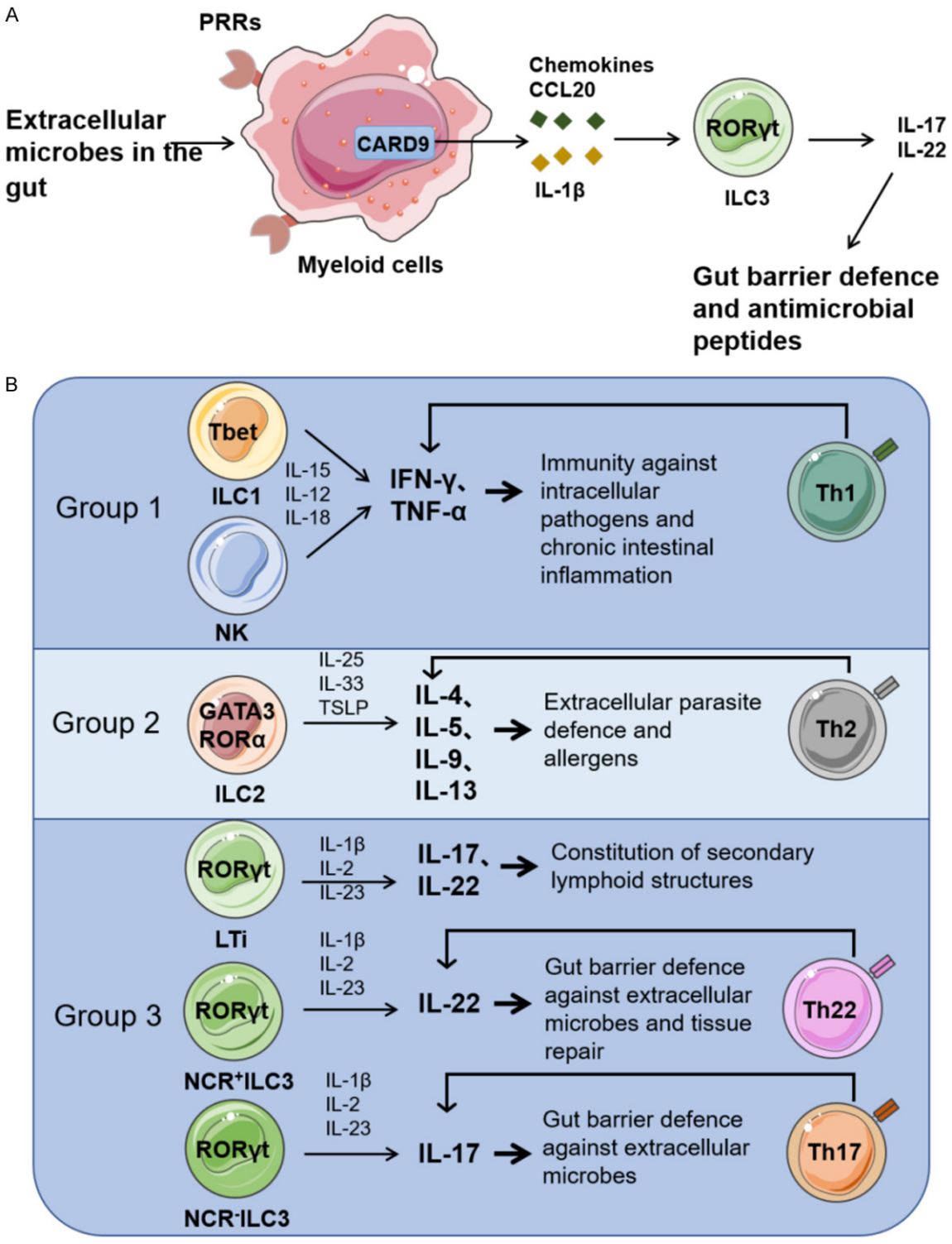

Figure 4. CARD9 and ILCs. CARD9 in myeloid cells is required for providing chemokines CCL20 and cytokines IL-1β, which are needed for the recruitment of ILC3s. The constant exposure to IL-1β maintains the expression of IL-22 in ILC3s (A). Typical ILCs are separated into three subsets mainly. ILCs bear a striking resemblance to T cells in secretin cytokines and dependence of transcription factors. ILC1s and NK cells are similar to Th1 cells, ILC2s resemble Th2 cells, while ILC3s appear to like Th17 and Th22 cells. NK cells and ILC1s are responsive to IL-15, IL-12, and IL-18, and depend on the transcription factor T-bet. When activated, they secrete IFN-γ and TNF-α to command defensive immunity against intracellular pathogens. ILC2s rely on GATA3 for their differentiation. They are activated in response to IL-33, IL-25 or TSLP, then express IL-4, IL-5, IL-9, and IL-13 to defend against extracellular parasites and allergens. ILC3s consist of LTi cells, NCR negative and positive ILC3s, all of them express RORγt. With the stimulation of microbes, as well as the existence of IL-1β, IL-2, and IL-23, ILC3s are activated and produce IL-17 and IL-22 (B).

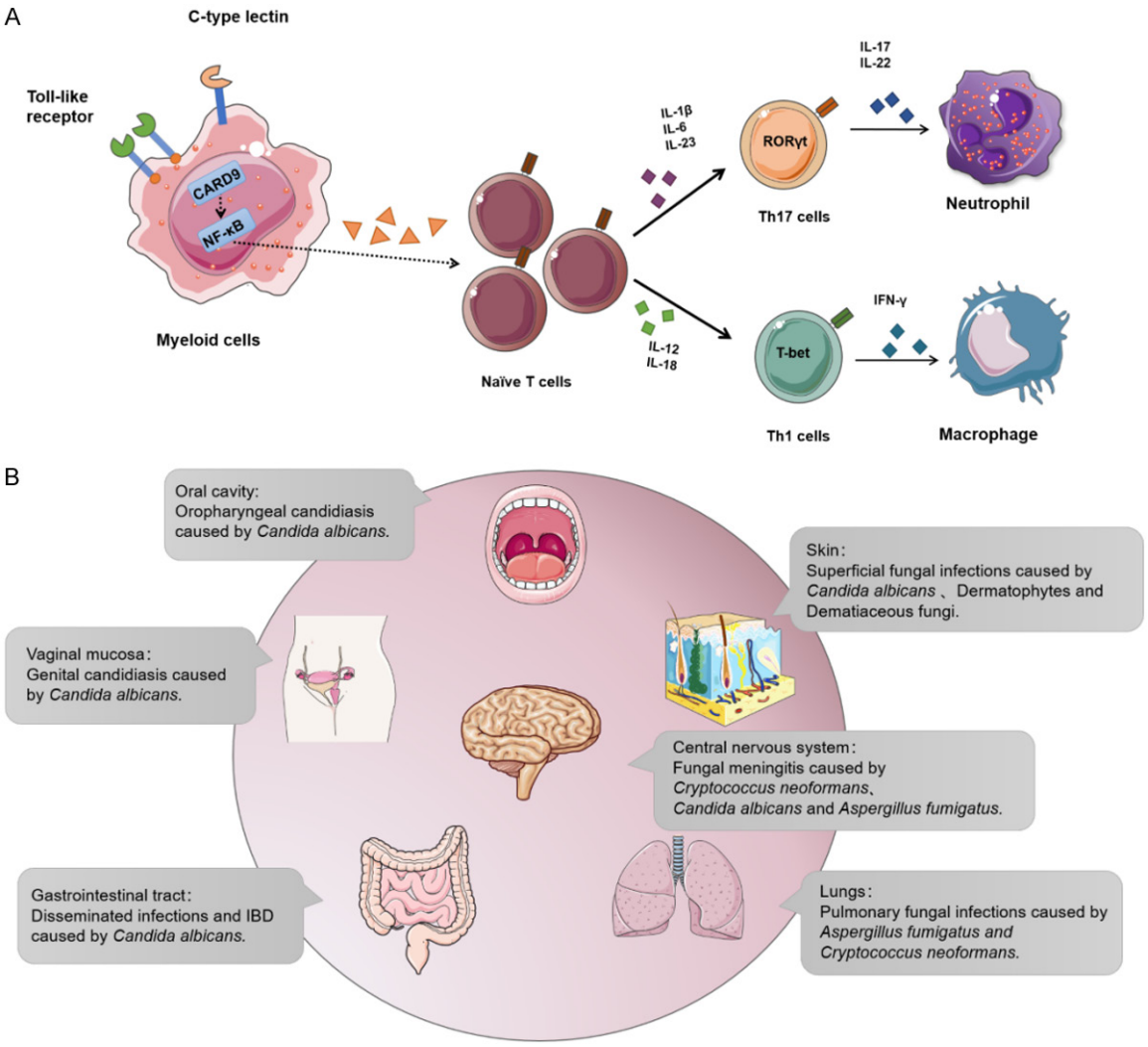

Figure 5. CARD9 regulates the differentiation of Th cells and summary of fungal diseases caused by CARD9 deficiency. IL-1β, IL-6, and IL-23 are required for the differentiation of Th17 cells, which can produce IL-17 and IL-22. IL-17 and IL-22 are critically important for mobilizing neutrophils. IL-12 and IL-18 play an essential role in the differentiation of Th1 cells. The IFN-γ produced by Th1 cells is demanded for phagocytes activation in response to fungal infection (A). Brief summary of organ-specific fungal species and relevant diseases in humans with CARD9 deficiency (B).

and impaired Th17 signaling pathway (mutations in STAT1 and STAT3), has been found to promote predisposition to mucocutaneous fungal infections [15, 99]. In spite of protective functions, the immune effectors initiated by Th17 cells depending on CARD9 also contribute to some autoimmune and allergic disorders [71, 100, 101]. Furthermore, Dectin-1 also synergizes with TLR2 to induce high production of IL-12 and favors Th1 responses [17] (**Figure 5A**). Th1 responses play a key role in the control of systemic fungal infections [15, 99], which are characterized by the production of IFN-γ required for the optimal activation of neutrophils and macrophages in CARD9-dependent pathways [102].

The essential role of the adaptor protein CARD9 in diseases

CARD9 and infectious diseases

Genetic polymorphisms and deletion in the gene encoding CARD9 is reported to be linked with a wide range of infectious diseases, which might be attributed to decreased production of inflammatory cytokines and chemokines, leading to impaired regulation of pathogen infec-

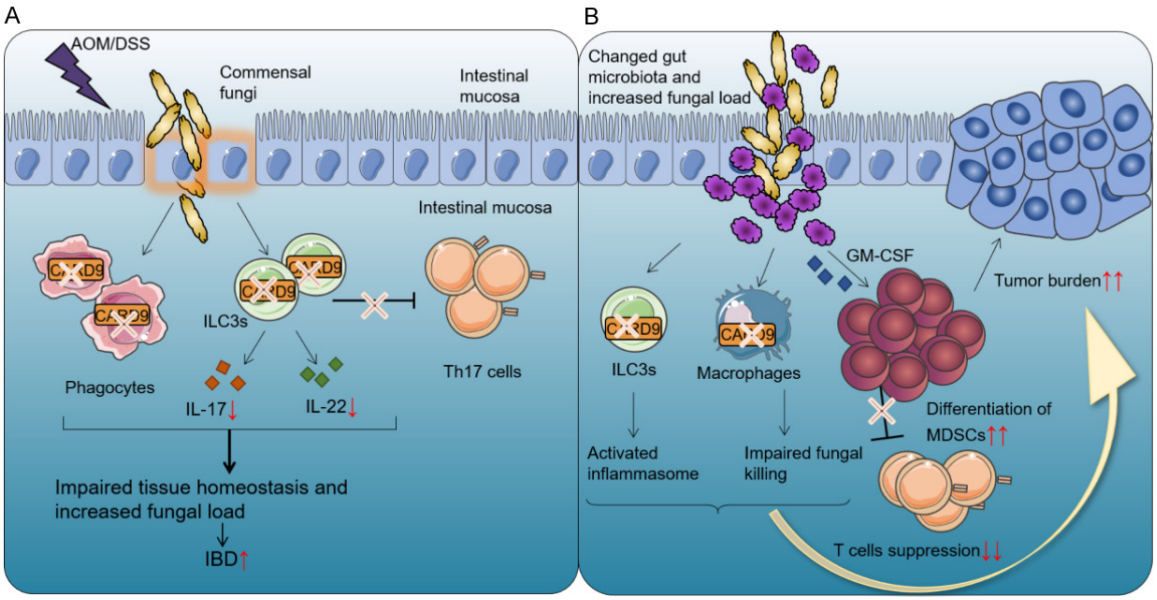

Figure 6. CARD9 in IBD and CAC. In the model of DSS induced colitis, when faced with invasion of fungi, *Card9*$^{-/-}$ phagocytes lose the ability to devour pathogens, and ILCs deficient of Card9 produce impaired IL-17 and IL-22 and may also show impaired function to present antigens to T cells. The lack of IL-17 is responsible for defective recruitment of neutrophils, while the reduction of IL-22 is related to the decrease of tissue homeostasis, both of which can also lead to impaired production of the antimicrobial peptide by epithelial cells. As a result, the increased fungal load was found in the gut of *Card9*$^{-/-}$ mice, and promoting the susceptibility to IBD (A). Changed gut microbiota and increased fungal load can activate inflammasome through Syk/CARD9 axis to provide defense against colitis, which would also conversely promote the growth of the intestinal tumor. *Card9*$^{-/-}$ macrophages showed impaired fungal killing. In the development of colon cancer, increased fungal load cause overproduction of GM-CSF, thus promoting the differentiation and activation of MDSCs, which accelerates the development of CAC (B).

tion. Human with CARD9 mutation has only been found to be susceptible to fungal disease while CARD9-deficient mice show susceptibility to both fugal and intracellular bacterial infections. In addition, mutation of PRRs upstream of CARD9 can also result in susceptibility to fungal or bacterial challenge, which are listed in the table below (**Table 1**).

The CARD9 pathway is the first line in host resistance to fungal infection in innate immunity, including *C. albicans*, *Dermatophytes*, *C. neoformans*, *A. fumigatus* and so on [74, 103-106]. Autosomal recessive (AR) CARD9 deficiency affects human organs ranging from the central nervous system to the gastrointestinal tract [7, 107] (**Figure 5B**). Four cases of patients with inherited CARD9 deficiency were diagnosed with subcutaneous phaeohyphomycotic caused by *Phialophora verrucose*, CARD9-deficient cells from those patients showed selectively impaired NF-κB activation, production of proinflammatory cytokine and chemokine, and Th17 immunity [108]. Other case of CARD9-mutated patients who suffered from cutaneous infection and deep dermatophytosis as a result of *Corynespora cassiicola* and *Dermatophytes* respectively were also reported [105, 109]. In addition, human AR CARD9 deficiency was also identified to be associated with extrapulmonary aspergillosis due to defective neutrophil accumulation at sites of infection [106]. Consistent with the results found in human, the importance of CARD9 in antifungal responses is emphasized by animal model studies. In response to *C. albicans* infections in central nervous system, CARD9 is required for CXCR2^{+} neutrophils accumulation through CXC chemokines production by glial cells in mice [74]. CARD9 deficiency leads to a selective impairment in neutrophils killing toward *C. albicans* and defective generation of Th17 responses for lack of cytokines, especially IL-1β, resulting in uncontrolled fungal growth during *C. albicans* brain infections [110]. In the case of *A. fumigatus* infection, the total clearance of *A. fumigatus* requires the activation of Th1 and Th17 cells dependent on CARD9 signaling. Resident alveolar macrophages in the lung phagocytose inhaled *A. fumigatus* conidia through Dectin-1,

Table 1. Mutation of the CARD9 signaling pathway and fungal or bacterial diseases

PRRs	PAMP Ligands	Correlated Pathogens	Diseases and Characteristics Associated with the Immunodeficiency or Mutation	References
Dectin-1	β-glucan	*C. albicans*	Candidiasis, colitis; impaired recruitment of myeloid cells	[33]
		S. cerevisiae	IBD	[123]
		C. posadasii	Coccidioidomycosis	[124]
		A. fumigatus	Invasive pulmonary aspergillosis (IPA), allergic bronchopulmonary aspergillosis	[125, 126]
		P. carinii	Pneumonia, bacterial pneumonia in HIV patients; impaired functions of alveolar macrophages	[127]
Dectin-2	α-mannose	*C. albicans*	Candidiasis, colitis; impaired IL-1β and IL-23 secretion and Th17 cell differentiation	[128]
	α-mannose	*A. fumigatus*	Invasive pulmonary aspergillosis (IPA)	[126]
	O-linked mannobiose-rich glycoprotein	*Malassezia*	Skin diseases	[29]
	Msg/gpA and β-glucans	*P. carinii*	*Pneumocystis* pneumonia (PCP); decreased production of the proinflammatory cytokines IL-6 and TNF-α	[129]
Dectin-3	α-mannose	*C. albicans*	Candidiasis, colitis	[28]
	TDM	*M. tuberculosis*	Tuberculosis	[130]
		C. tropicalis	Colitis	[131]
	Glucuronoxylomannan (GXM)	*C. neoformans* and *C. gattii*	Meningoencephalitis and pneumonia; abolished proinflammatory cytokine production	[104]
Mincle	α-mannose	*C. albicans*	Candidiasis, colitis	[132]
	TDM	*M. tuberculosis*	Tuberculosis	[45]
	Glyceroglycolipid and unique mannosyl fatty acids	*Malassezia*	Skin diseases	[133, 134]
TLR2	Bacterial peptidoglycan	Bacteria	Reduced TNF-α produced by $TLR2^{-/-}$ macrophages	[135]
	Bacterial lipoprotein	Gram-negative bacteria		[135]
	Zymosan	*A. fumigatus*	Impaired production of TNF-α, IL-12, and MIP-2-α	[135-137]
TLR4	LPS	Gram-negative pathogens		[136]
	Cryptococcal polysaccharide	*C. neoformans*		[136]
	Aspergillus hyphae	*A. fumigatus*	Invasive aspergillosis; chronic pulmonary aspergillosis; impaired production of TNF and IL-1 from macrophages	[137, 138]
TLR9	Fungal DNA	*C. albicans, A. fumigatus, C. neoformans*		[69, 139-141]

this finally leads to the activation of epithelial cells, which recruit neutrophils for fungal killing by generating CXCL1. Mice with CARD9^{S12N} are prone to have a small number of Th1 and Th17 cells when challenged with *Aspergillus* spores, as well as a greater number of Th2 cells than wild-type (WT) mice by facilitating IL-5 secretion from aveolar macrophages, which tends to result in allergic responses in the lung [111, 112].

CARD9 has been identified to suppress certain kinds of bacterial infections, including *Mycobacterium*, *Salmonella*, and *L. monocytogenes* [45, 75, 113, 114]. CARD9-deficient mice showed heavy bacterial burdens and severe systemic inflammatory response in the lung after *Mycobacterium tuberculosis* infection because of the failure in establishing an anti-inflammatory feedback path [113]. GWAS analysis confirmed that leprosy caused by *Mycobacterium leprae* has a strong relationship with risk SNPs in CARD9 gene variants [115]. During *L. monocytogenes* infection, another intracellular bacterium, CARD9 promotes ROS production in macrophages by interacting with Rac GTPases-related inhibitory protein LyGDI in the phagosome and thus regulating Rac1 activation [75]. In contrast to the proinflammatory role in fungal infection, CARD9 has been identified as a negative regulator of the IL-1β production when infected with *Salmonella enterica* through downregulating pro-IL-1β production and suppress NLRP3 activation [114]. In addition to common fungal and bacterial infections, CARD9 also plays an important role in viral and parasitic infection [42-44], which will not be discussed further here.

CARD9 and inflammatory diseases

CARD9 is one of the major players in intestinal homeostasis and emerging evidence has demonstrated that the genetic diversity of CARD9 is closely related to the risk of IBD, which includes Crohn's disease and ulcerative colitis [116]. *Card9*$^{-/-}$ mice with DSS-induced colitis were associated with defective intestinal epithelial restoration, accompanied by defective IL-6, IL-17A, IL-22 expression and antimicrobial peptides secretion as a result of decreased Th17 cells and IL-22-producing ILCs, those changes made them more susceptible to intestinal inflammation [85]. Alterations in the intestinal microbiota have been confirmed as a motivator in the pathogenesis of IBD. In addition to tissue homeostasis, CARD9 is necessary for maintaining a balanced gut microbiota and suppressing the fungal dysbiosis [8], which is achieved through harmonizing immune responses activated by ILC3s and Th17 cells [85]. Moreover, the altered gut microbiota in *Card9*$^{-/-}$ mice fails to turn tryptophan into aryl hydrocarbon receptor ligands, consequently downregulating IL-22 production and promoting susceptibility to colitis [117] (**Figure 6A**). In addition to IBD, some other inflammation-related diseases, such as severe acute pancreatitis [65], cardiovascular disease [118] and obesity [119], have been reported to be closely related to the expression of CARD9.

CARD9 and cancer

Previous studies have primarily focused on the function of CARD9 in inflammation-related diseases while recent years have witnessed the role of CARD9 in the pathogenesis of tumors. Our early study identified CARD9 as an antitumor molecular in the development of CAC by shaping the intestinal microbiota ecosystem and restricting MDSCs expansion [8] (**Figure 6B**), meanwhile, *Malik et al.* also reported that Syk/CARD9 controlled IL-18 maturation and inflammasome activation to protect against colitis and colon cancer [9]. In contrast, under inappropriate activation, the expression of CARD9 is increased in tumor-infiltrating macrophages and this promotes the growth and liver metastasis of colon carcinoma cells [77]. Additionally, *Bergmann et al.* observed that the existence of CARD9 promoted CAC progression by inducing IL-1β-mediated IL-22 production from ILC3s and STAT3 activation, a transcription factor for tumor survival and metastasis [87]. CAC poses a threat to numerous individuals, especially those with IBD, who have an increased risk. Herein lies an assumption that the gut inflammasome activated by commensal fungi initially provides defense against colitis and CAC, whereas overactive inflammasome would conversely promote the growth of intestinal tumors, the specific balance between the two status is worth exploring. Therefore, either CARD9 deficiency or aberrant expression could influence the tumorigenesis through modifying the composition of gut microbiota [8, 9, 87].

CARD9 pathway has also been involved in renal cell carcinoma (RCC), mostly caused by mutations of von Hippel-Lindau tumor suppressor protein (pVHL), which can promote the inhibito-

ry phosphorylation of CARD9 by CK2. Therefore, pVHL deficiency can lead to increased JNK and NF-κB activity due to overactive CARD9, bringing about proapoptotic cytokine resistance and uncontrolled tumor growth [56, 120]. Consistently, the downregulation of CARD9 in pVHL-deficient cancer cells can partly restore the sensitivity to cytokines and restrain tumorigenesis [56].

In addition, abnormal CARD9 upregulation might contribute to the development of gastric mucosa-associated lymphoid tissue lymphomas [121] and other digestive system neoplasms, including primary gastric lymphoma and hepatocellular carcinoma, especially HCV-associated neoplasms, implying the specific role of CARD9 in the contribution to oncogenesis and revealing a novel point that is worth exploring in the future to develop possible therapies [122].

Discussion and conclusion

Recently, insight into CARD9 has not only been limited to infective and inflammatory diseases but has also been associated with a great deal of cancers. However, even though the mechanism and function of CARD9 have been well explored, the specific but common cellular and molecular mechanisms underlying the development of certain diseases, such as malignant tumors, remain to be thoroughly resolved.

In view of the central function of the CARD9 signaling pathway in innate recognition and immunity, regulation and intervention aimed at this signaling pathway could shed significant light on the conceivable therapy of certain diseases. For example, activators and agonists of the CARD9 pathway could be applied in the development of immune preparation, such as vaccines against some harmful bacteria and viruses. On the other hand, antagonists and inhibitors could be introduced into the treatment of immunopathology and neoplastic diseases.

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Disclosure of conflict of interest

None.

Abbreviations

CARD9, caspase recruitment, domain-containing protein 9; BCL10, B cell leukemia-lymphoma 10; NF-κB, nuclear factor-κB; Card9-/-, CARD9-deficient; CAC, colitis-associated colon cancer; DCs, dendritic cells; MAGUK, membrane-associated guanylate kinase; PRRs, pattern recognition receptors; CLRs, C-type lectin receptors; TLRs, Toll-like receptors; NOD, nucleotide-binding oligomerization domain; NLRs, NOD-like receptors; RLHs, RIG-I-like helicases; Syk, spleen tyrosine kinase; RIP2, receptor interacting protein 2; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; CBM, CARD9/BCL10/MALT1; MAPK, mitogen-activated protein kinase; ITAM, immunoreceptor tyrosine-based activation motif; PKCδ, protein kinase Cδ; MyD88, Myeloid differentiation primary response 88; BMDC, bone marrow-derived DCs; RIP2, receptor interacting protein 2; IRAK1, interleukin-1 receptor-associated kinase; TRAF6, TNF receptor associated factor 6; TAK1, transforming growth factor β-activated kinase 1; IκB, inhibitor of NF-kB; IKK, IκB kinase; CK2, casein kinase 2; AP-1, activator protein 1; Ras-GRF1, Ras-guanine-nucleotide-releasing factor 1; PLCγ, phospholipase Cγ; Rubicon, RUN domain Beclin-1-interacting cysteine-rich-containing; ASC, apoptosis-associated speck-like protein; LBP, LPS-binding protein; MDSCs, myeloid-derived suppressor cells; IDO, indoleamine 2,3-dioxygenase; ILC, innate lymphoid cells; IBD, inflammatory bowel diseases; AR, autosomal recessive; WT, wild-type; EAU, experimental autoimmune uveitis; RCC, renal cell carcinoma; pVHL, von Hippel-Lindau tumor suppressor protein.

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