The Role of CARD9 in Metabolic Diseases*

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Summary: Caspase recruitment domain containing protein 9 (CARD9) is an adaptor protein that plays a critical role in pattern recognition receptors (PRRs)-mediated activation of NF- κ B and mitogen-activated protein kinase (MAPK). This elicits initiation of the pro-inflammatory cytokines and leads to inflammatory responses, which has been recognized as a critical contributor to chronic inflammation. Current researches demonstrate that CARD9 is strongly associated with metabolic diseases, such as obesity, insulin resistance, atherosclerosis and so on. In this review, we summarize CARD9 signaling pathway and the role of CARD9 in metabolic diseases.

Key words: caspase recruitment domain containing protein 9; pattern recognition receptors; metabolic diseases

Metabolic diseases are recognized as a group of diseases characterized by metabolic disorder, such as obesity, insulin resistance (IR), atherosclerosis and so on. In the modern era, metabolic diseases have become the leading risk factor affecting disability-adjusted life-years in many countries, with a skyrocketing incidence^[1]. Metabolic diseases have been validated to be strongly associated with inflammation^[2]. For example, elevated level of pro-inflammatory cytokines is detected in IR mice, and these cytokines drive macrophage polarization into M1 type. Besides, nuclear factor- κ B (NF- κ B) signaling pathway in macrophages is activated in mice with IR or atherosclerosis, which contributes to activation of macrophages and propagation of pro-inflammatory signals. Currently, extensive studies in terms of the potential involvement of caspase recruitment domain containing protein 9 (CARD9), an upstream molecule of NF- κ B, in inflammation have been conducted^[3,4]. As a result, CARD9 may be involved in metabolic diseases, which has been proved by some researches^[5,6].

CARD9, possessing a coiled-coil region in C terminus and a caspase recruitment domain (CARD) in N terminus, was originally identified via Millennium Pharmaceuticals proprietary database search for CARD-containing proteins^[7]. CARD9, a critical integrator of innate immunity, is highly expressed in myeloid cells, especially in macrophages and dendritic

cells (DCs)^[4]. In addition, CARD9 has been identified as a vital adaptor protein that integrates signals from pattern recognition receptors (PRRs) in myeloid cells^[8,9]. Following PRRs engagement, CARD9 can activate NF- κ B and mitogen-activated protein kinase (MAPK) signaling pathways by forming CARD9-B cell lymphoma 10 (BCL10)-mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) (CBM) complex, and then triggers an inflammatory immune response^[10,11].

Recently, accumulating evidence has proved that CARD9 plays an indispensable role in multiple metabolic diseases. In this review, we summarize CARD9 signaling pathway and the role of CARD9 in metabolic diseases, which is conducive to exploring the pathogenesis of metabolic diseases.

1 CARD9 SIGNALING PATHWAY

CARD9 is a critical adaptor protein that mainly functions in the innate immune system. Multiple signaling pathways mediated by PRRs, including transmembrane PRRs and cytoplasmic PRRs, converge on CARD9^[10,12-14].

1.1 CARD9 Integrates Signals from Transmembrane PRRs

Common transmembrane PRRs include C-type lectin receptors (CLRs) and Toll-like receptors (TLRs). Dectin-1, one of CLRs, initiates NF- κ B and MAPK signaling in CARD9-dependent manners^[15]. Ligation of Dectin-1 leads to the recruitment and activation of spleen tyrosine kinase (SYK), which subsequently phosphorylates protein kinase C δ (PKC δ) at position Tyr311. Activated PKC δ induces CARD9 phosphorylation at position Thr231 and the assembly of

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CBM complex^[15, 16]. Then, CBM complex triggers the production of pro-inflammatory cytokines, including IL-6, TNF- α and IL-1 β , via activating extracellular regulated protein kinases (ERK) and NF- κ B, the former of which happens through a RasGrf1–H-Ras pathway and the latter of which occurs via a classic NF- κ B signaling pathway^[17, 18]. There are numerous regulators of CARD9-dependent signaling pathways induced by CLRs, such as Vav proteins, tripartite motif-containing 62 (TRIM62), Rubicon and Casein kinase 2 (CK2)^[19–22]. Both Vav proteins and TRIM62 are indispensable to the activation of CARD9-mediated NF- κ B signaling pathway, while both Rubicon and CK2 are crucial in down-regulation of CARD9-mediated inflammatory responses. The phosphorylation of Vav proteins at position Tyr174 promotes the formation of CBM complex and subsequent NF- κ B activation. And TRIM62, facilitating K27-linked polyubiquitination of the CARD9 C-terminus, ubiquitinates CARD9 at position Lys125 and promotes CARD9-mediated NF- κ B activation, but is dispensable to the assembly of CBM complex. Rubicon, a critical autophagy regulator, competitively binds to CARD9 and leads to the disassembly of the CBM complex, thus turning off inflammatory signaling and preventing excessive inflammatory responses. In addition, CK2-mediated phosphorylation of CARD9 at Thr531 and Thr533 inhibits the formation of CBM complex, and the process is promoted by the VHL tumor suppressor protein (pVHL).

TLRs were observed to trigger CARD9-mediated MAPK activation^[23]. Hara *et al* reported that CARD9^{-/-} DCs could not be activated by Zymosan, the ligand of TLRs and Dectin-1. Mechanically, CARD9 was required for TLR-induced DCs activation through transducing MyD88-mediated signals and activating MAPK. Further researches revealed that CARD9 was required for innate immune responses to selective ligands^[13, 23]. For example, the generation of IL-6 and TNF- α in CARD9^{-/-} macrophage was induced by the ligands of TLR3 or TLR7, but not by the ligands of TLR2, TLR4, TLR5, and TLR9. In addition to inducing innate immune responses, CARD9 activates adaptive immunity to pathogens^[11]. For instance, Flagellin-specific T-cell responses were shown to require CARD9-expressing DCs.

1.2 CARD9 Integrates Signals from Cytoplasmic PRRs

Cytoplasmic PRRs include nucleotide-binding oligomerization domain (NOD) like receptors (NLRs) and retinoic-acid-inducible gene (RIG) like receptors (RLRs).

It has been validated that CARD9 is essential for NOD2-mediated activation of MAPK^[13]. Hsu *et al* reported that CARD9^{-/-} macrophages showed defective activation of p38 and c-Jun-NH2-terminal kinase (JNK)

following stimulation with muramyl-dipeptide (MDP), a ligand of intracellular NOD2 receptor. However, CARD9^{-/-} macrophages displayed normal NF- κ B activation after MDP treatment. Further evidence suggested that CARD9 could form trimolecular complex with NOD2 and receptor interacting protein 2 (RIP2), a CARD kinase, which further induced NOD2-mediated activation of p38 and JNK in innate immune responses.

RLRs, such as RIG-1, sense viral double-strand (ds) RNA in the cytoplasm and trigger the production of IL-1 β ^[24]. Loo *et al* showed that RLRs-mediated signals were relayed to interferon (IFN)-regulatory factor (IRF) and NF- κ B by interacting with the adaptor mitochondrial antiviral-signaling protein (MAVS). After selective RIG-I ligand dsRNA treatment, CARD9-BCL10 module was induced to activate NF- κ B to promote the synthesis of pro-IL-1 β , while RIG-I triggered caspase-1-dependent inflammasome activation by binding to apoptosis-associated speck-like protein containing CARD (ASC). Subsequently, caspase-1 processed pro-IL-1 β into mature and bioactive IL-1 β ^[14].

In addition to dsRNA, dsDNA in the cytoplasm also triggers IL-1 β production^[25, 26]. Following transfection with dsDNA, Rad50 sensed dsDNA and directly interacted with CARD9. And the formation of dsDNA-Rad50-CARD9 signaling complex was induced to recruit BCL10 for NF- κ B activation and subsequent pro-IL-1 β generation^[25]. Besides, absence in melanoma-2 (AIM2) was confirmed to bind dsDNA and promote ASC-mediated caspase-1-dependent inflammasome activation, which further cleaved pro-IL-1 β into mature IL-1 β ^[26].

2 THE ROLE OF CARD9 IN METABOLIC DISEASE

2.1 The Promotive Role of CARD9 in Obesity

Obesity is regarded as low grade chronic inflammation, with increased infiltration of immune cells into adipose tissue and heightened levels of circulating and localized pro-inflammatory cytokines^[27, 28]. Troglitazone (TGZ), a synthetic agonist of peroxisome proliferator-activated receptor-gamma (PPAR γ), improves insulin sensitivity in obese objects^[29]. Kock *et al* showed that TGZ inhibited Dectin-1-mediated activation of MAPK and NF- κ B signaling pathways in DCs through interfering with CARD9^[30], which suggests that TGZ may partially ameliorate obesity-related symptoms by suppressing CARD9-associated signaling pathways. And the activation of Dectin-1, inducing innate immune responses through activating CARD9-mediated NF- κ B signaling pathway^[17], aggravated adiposis and IR in MyD88^{-/-} mice^[31]. Furthermore, monocyte chemoattractant protein-1, the

serum level of which was closely correlated with SNPs in CARD9, was increased in obese human^[32]. These studies provide a possibility that CARD9 contributes to the development of obesity.

A recent study conducted by Yang *et al* indicated that CARD9 deficiency mitigated the high fat diet (HFD)-induced metabolic disorders and inflammation by suppressing p38 MAPK and NF- κ B signaling pathways^[5]. In HFD-treated mice, CARD9 deficiency ameliorated adiposis, diminished the excessive lipid accumulation, and alleviated adiposity-associated symptoms including glucose tolerance impairment, inflammation and hepatopathy. Compared to HFD-fed wild type (WT) mice, HFD-fed CARD9^{-/-} mice exhibited inhibited p38 and NF- κ B signaling pathways. In another study, CARD9^{-/-} mice preserved autophagy, suppressed p38 phosphorylation and reduced macrophages infiltration into heart, thus ameliorating myocardial dysfunction related to HFD-induced obesity^[6]. Similarly, zinc deficiency and HFD synergistically increased the activation of p38 signaling pathways through increasing the expression of BCL10 and CARD9, giving rise to exacerbating obesity-related cardiac hypertrophy^[33]. As a result, these data suggest that CARD9 is a potential target for attenuating obesity and obesity-associated symptoms.

2.2 The Promotive Role of CARD9 in Insulin Resistance

A large body of evidence suggests that high level of pro-inflammatory mediators in obese mice adversely affects insulin sensitivity and contributes to IR^[34, 35]. For example, TNF- α levels in bloodstream and peripheral tissues are increased in IR mice, and the neutralization of TNF- α prevents IR induced by HFD^[36]. CARD9 mediates the activation of NF- κ B signaling pathway, further promoting the production of pro-inflammatory cytokines^[37, 38]. In addition, increased infiltration of immune cells into adipose tissue is associated with IR^[28]. For instance, activation of DCs infiltrating into adipose tissue of obese mice, which is induced via SYK-CARD9 pathway, promotes inflammation and IR through inducing Th17 cell responses^[39, 40]. These results reveal the potential correlation between CARD9 and IR.

Yang *et al* revealed that CARD9 accelerated the development of IR and increased the activation of p38 and NF- κ B in HFD-fed mice^[5]. Besides, Cao *et al* found that CARD9 deficiency retarded the development of IR and decreased p38 activation^[6]. And TGZ, a drug for ameliorating IR, inhibited activation of DCs by suppressing CARD9-mediated activation of p38 and NF- κ B^[30]. Collectively, CARD9-mediated activation of p38 and NF- κ B plays a promotive role in IR.

2.3 The Protective Role of CARD9 in Atherosclerosis

Inflammation, clearly implicated in CARD9, has been known as a crucial contributor to atherosclerosis^[41]. A recent research reported that

deletion of hematopoietic Mincle, a member of CLRs correlated with CARD9, reduced atherosclerotic lesion formation and lesion severity^[42]. Furthermore, atherosclerosis is associated with increased titers of oxidized low-density lipoprotein (LDL) immune complex^[43]. And oxidized LDL immune complex priming Nlrp3 inflammasome relies on CARD9 and involves TCR and Fc γ R synergy^[44]. And CARD9 has been recognized as a potential target in cardiovascular diseases^[45]. As a result, CARD9 may promote the development of atherosclerosis. However, a recent study conducted by Thiem *et al* indicated that hematopoietic CARD9 deletion increased atherosclerotic plaque formation in hyperlipidemic LDL receptor (LDLR)-knockout mice^[46]. In this study, deletion of hematopoietic CARD9 or Dectin-2 in LDLR^{-/-} mice did not affect plasma lipids, circulating immune cell composition and peripheral cytokines. But deletion of hematopoietic CARD9, not Dectin-2, promoted atherosclerotic plaque formation and increased severity. This research provides an intriguing possibility that CARD9 protects against atherosclerosis with an uncertain mechanism. But it is also surprising because hematopoietic deletion of several CLRs, such as Mincle and Dectin-2, has a protective or void effect on atherosclerosis in LDLR^{-/-} mice^[42, 46], and these CLRs could initiate signals by CARD9-dependent manners. We speculate that multiple signaling pathways are involved in pathogenesis of atherosclerosis, and deletion of hematopoietic CARD9 compensatorily activates or inhibits other signaling pathways, ultimately promoting atherosclerosis initiation. The accurate mechanism remains to be elucidated.

2.4 The Dual Role of CARD9 in Heart Diseases

Some studies demonstrate that heart diseases are associated with obesity and inflammation^[47-49]. For instance, mice fed on HFD were more likely to develop myocardial dysfunction than normal mice^[6]. And CARD9 deficiency alleviated obesity-induced myocardial dysfunction and inflammation through suppressing CARD9-dependent MAPK phosphorylation. Another research showed that CARD9-dependent MAPK signaling pathway was involved in the development of obesity-related cardiac hypertrophy (ORCH)^[33]. Zinc supplement alleviated ORCH, induced by HFD and zinc deficiency, via stimulating metallothionein to inhibit oxidative stress-activated CARD9-dependent MAPK signaling pathway. In addition, zinc modulates the assembly of CARD9-BCL10 complex by binding with CARD9^[50]. In summary, zinc supplement ameliorates CARD9-mediated inflammation by activating metallothionein to repress CARD9 activation and directly binding with CARD9, and further attenuates cardiac diseases. Furthermore, Ren *et al* demonstrated that CARD9 played a critical role in regulating angiotensin II

(Ang II)-induced cardiac remodeling^[51]. CARD9 deficiency alleviated Ang II-induced cardiac fibrosis and inflammation by inhibiting the activation of NF- κ B, JNK and p38 in macrophages.

In addition to mediating inflammation, CARD9 inhibits mitochondria-dependent apoptosis of cardiomyocytes under oxidative stress^[52]. During myocardial ischemia-reperfusion (I/R) injury, excessive ROS stimulates the release of cytochrome C from mitochondria into the cytoplasm. Then, cytochrome C, Apaf-1, and procaspase-9 assemble into an apoptosome which stimulates the activation of caspase-9. Activated caspase-9 subsequently activates caspase-3 inducing the apoptosis of cardiomyocytes. However, CARD9 competitively binds with Apaf-1 and disrupts apoptosomes formation through a CARD domain-dependent mechanism, which subsequently inhibits the activation of caspase-9 and caspase-3 and thus suppresses cardiomyocytes apoptosis induced by I/R injury.

These findings suggest that CARD9 has a dual role in heart diseases. On one hand, CARD9 deficiency in macrophages alleviates myocardial dysfunction, cardiac hypertrophy and cardiac remodeling via inhibiting the activation of CARD9-mediated inflammatory signals. On the other hand, CARD9 represses cardiomyocytes apoptosis induced by oxidative stress through interacting with Apaf-1.

2.5 The Promotive Role of CARD9 in Neointima Formation of Vein Grafts

CARD9 deficiency not only alleviates the obesity-associated heart diseases through suppressing CARD9-dependent MAPK phosphorylation, but ameliorates the intimal hyperplasia of vein grafts by inactivating CARD9-dependent NF- κ B signaling pathway^[6, 33, 53]. Vein grafts are generally used for vascular reconstruction. However, the graft patency is limited by neointima formation^[54]. Inflammation plays a crucial role in intimal hyperplasia of vein grafts. Increased production of pro-inflammatory cytokines and infiltration of inflammatory immunocytes in grafted vein precede neointima formation and are throughout intimal hyperplasia of vein grafts^[53, 55, 56]. Liu *et al* found that CARD9-dependent NF- κ B activation in macrophages contributed to necrotic smooth muscle cells (SMCs)-induced inflammation and facilitated intimal hyperplasia of vein grafts^[53]. Necrosis of SMCs was induced in early vein grafts and induced production of IL-1 β , IL-6 and MCP-1 in macrophages. These pro-inflammatory cytokines ulteriorly recruited macrophages and exacerbated inflammation. Further study found that CARD9 was highly expressed in infiltrated macrophages of grafted veins. Furthermore, depletion of CARD9 alleviated SMC-induced inflammation by inactivating NF- κ B and finally ameliorated neointima formation of vein

grafts. Therefore, CARD9 contributes to neointima formation of vein grafts.

3 CONCLUSIONS AND PERSPECTIVES

A surge of interest in the role of innate immune in metabolic regulation has revealed the close relation between innate immune and metabolic disease. As a key adaptor transducing PRRs-mediated signals in innate immune, CARD9 is involved in the pathogenesis of multiple metabolic diseases via activating p38 and NF- κ B signal pathways and inducing cascaded inflammatory reaction. TGZ was reported to interfere with CARD9 and inhibited Dectin-1-mediated secretion of cytokines and chemokines via p38 and NF- κ B pathway in DCs^[30]. Pharmacological inhibition of SYK in inflammatory macrophage, which may indirectly inhibit CARD9, ameliorated fibrosis, inflammation and steatosis in mice with non-alcoholic steatohepatitis^[57]. Also, CARD9 participates in the regulation of cell apoptosis. Li *et al* found that CARD9 protects against myocardial I/R injury by inhibiting cardiomyocytes apoptosis^[52]. Interestingly, the researchers found that CARD9 induced cell apoptosis in human leukemic cells^[58, 59], such as NB4 and HL60 cells, but inhibited that in oral squamous cell carcinoma (OSCC) and cardiomyocytes. Collectively, CARD9 is not only a critical adaptor of innate immunity, but an apoptosis-regulating protein (fig. 1).

In conclusion, this review summarizes the role of CARD9 in immunity-based metabolic diseases. It

Fig. 1 CARD9 involved in multiple metabolic diseases

As an adaptor of innate immunity, CARD9 accelerates inflammation by activating MAPK and NF- κ B signaling pathway in innate immune cells, which promotes multiple metabolic diseases, including obesity, IR, cardiac fibrosis, neointima formation of vein grafts, and inhibits atherosclerosis via the unknown mechanism. As an apoptosis-regulating protein, CARD9 suppresses caspase-9 activation by combining with Apaf-1, which inhibits apoptosis of cardiomyocytes and further relieves I/R injury.

not only facilitates the further study of pathogenesis of metabolic diseases, but provides a theoretical foundation for the study of drugs preventing or treating metabolic diseases. As a signal transducer in innate immune, CARD9 regulates immune-based metabolic diseases through delivering inflammatory signals to p38 and NF- κ B pathways, but some blind spots still exist. Upon the role in metabolic disease, it is unclear whether CARD9 directly regulates metabolic pathways such as mTOR and AMPK pathways. As an apoptosis-regulating protein, how CARD9 affects the apoptosis of immune cells and whether it affects the crosstalk between cascaded inflammatory reaction and the programmed cell apoptosis remain unclear.

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Conflict of Interest Statement

The authors declare that there is no conflict of interest.

REFERENCES

- Gakidou E, Afshin A, Abajobir AA, *et al.* Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*, 2017,390(10100):1345-1422
- Baker RG, Hayden MS, Ghosh S. NF-kappaB, inflammation, and metabolic disease. *Cell Metab*, 2011,13(1):11-22
- Luo P, Yang Z, Chen B, *et al.* The multifaceted role of CARD9 in inflammatory bowel disease. *J Cell Mol Med*, 2020,24(1):34-39
- Roth S, Ruland J. Caspase recruitment domain-containing protein 9 signaling in innate immunity and inflammation. *Trends Immunol*, 2013,34(6):243-250
- Zeng X, Du X, Zhang J, *et al.* The essential function of CARD9 in diet-induced inflammation and metabolic disorders in mice. *J Cell Mol Med*, 2018,22(6):2993-3004
- Cao L, Qin X, Peterson MR *et al.* CARD9 knockout ameliorates myocardial dysfunction associated with high fat diet-induced obesity. *J Mol Cell Cardiol*, 2016,92:185-195
- Bertin J, Guo Y, Wang L *et al.* CARD9 is a novel caspase recruitment domain-containing protein that interacts with BCL10/CLAP and activates NF-kappa B. *J Biol Chem*, 2000,275(52):41 082-41 086
- Zhong X, Chen B, Yang L *et al.* Molecular and physiological roles of the adaptor protein CARD9 in immunity. *Cell Death Dis*, 2018,9(2):52
- Vaezi A, Fakhim H, Abtahian Z *et al.* Frequency and Geographic Distribution of *CARD9* Mutations in Patients With Severe Fungal Infections. *Front Microbiol*, 2018,9:2434-2440
- Gross O, Gewies A, Finger K *et al.* Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. *Nature*, 2006,442(7103):651-656
- Atif SM, Lee SJ, Li LX *et al.* Rapid CD4+ T-cell responses to bacterial flagellin require dendritic cell expression of Syk and CARD9. *Eur J Immunol*, 2015,45(2):513-524
- Ma J, Abram CL. CARD9 mediates dendritic cell-induced development of Lyn deficiency-associated autoimmune and inflammatory diseases. *Front Microbiol*, 2019,12(602):eaa03829
- Hsu YM, Zhang Y, You Y *et al.* The adaptor protein CARD9 is required for innate immune responses to intracellular pathogens. *Nat Immunol*, 2007,8(2):198-205
- Poeck H, Bscheider M, Gross O *et al.* Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. *Nat Immunol*, 2010,11(1):63-69
- Drummond RA, Saijo S, Iwakura Y *et al.* The role of Syk/CARD9 coupled C-type lectins in antifungal immunity. *Eur J Immunol*, 2011,41(2):276-281
- Strasser D, Neumann K, Bergmann H, *et al.* Syk kinase-coupled C-type lectin receptors engage protein kinase C-sigma to elicit Card9 adaptor-mediated innate immunity. *Immunity*, 2012,36(1):32-42
- Jia XM, Tang B, Zhu LL, *et al.* CARD9 mediates Dectin-1-induced ERK activation by linking Ras-GRF1 to H-Ras for antifungal immunity. *J Exp Med*, 2014,211(11):2307-2321
- Wagener M, Hoving JC, Ndlovu H, *et al.* Dectin-1-Syk-CARD9 Signaling Pathway in TB Immunity. *Front Immunol*, 2018,9:225-231
- Roth S, Bergmann H, Jaeger M, *et al.* Vav Proteins Are Key Regulators of Card9 Signaling for Innate Antifungal Immunity. *Cell Rep*, 2016,17(10):2572-2583
- Cao Z, Conway KL, Heath RJ, *et al.* The Ubiquitin Ligase TRIM62 Regulates CARD9-Mediated Antifungal Immunity and Intestinal Inflammation. *Immunity*, 2015,43(4):715-726
- Yang CS, Rodgers M, Min CK, *et al.* The autophagy regulator Rubicon is a feedback inhibitor of CARD9-mediated host innate immunity. *Cell Host Microbe*, 2012,11(3):277-289
- Yang H, Minamishima YA, Yan Q, *et al.* pVHL acts as an adaptor to promote the inhibitory phosphorylation of the NF-kappaB agonist Card9 by CK2. *Mol Cell*, 2007,28(1):15-27
- Hara H, Ishihara C, Takeuchi A, *et al.* The adaptor protein CARD9 is essential for the activation of myeloid cells through ITAM-associated and Toll-like receptors.

- Nat Immunol, 2007,8(6):619-629
- 24 Loo YM, Gale M, Jr. Immune signaling by RIG-I-like receptors. *Immunity*, 2011,34(5):680-692
 - 25 Roth S, Rottach A, Lotz-Havla AS, *et al.* Rad50-CARD9 interactions link cytosolic DNA sensing to IL-1 β production. *Nat Immunol*, 2014,15(6):538-545
 - 26 Rathinam VA, Jiang Z, Waggoner SN, *et al.* The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nat Immunol*, 2010,11(5):395-402
 - 27 Sell H, Habich C, Eckel J. Adaptive immunity in obesity and insulin resistance. *Nat Rev Endocrinol*, 2012,8(12):709-716
 - 28 McLaughlin T, Ackerman SE, Shen L, *et al.* Role of innate and adaptive immunity in obesity-associated metabolic disease. *J Clin Invest*, 2017,127(1):5-13
 - 29 Frias JP, Yu JG, Kruszynska YT, *et al.* Metabolic effects of troglitazone therapy in type 2 diabetic, obese, and lean normal subjects. *Diabetes Care*, 2000,23(1):64-69
 - 30 Kock G, Bringmann A, Held SA, *et al.* Regulation of dectin-1-mediated dendritic cell activation by peroxisome proliferator-activated receptor- γ ligand troglitazone. *Blood*, 2011,117(13):3569-3574
 - 31 Castoldi A, Andrade-Oliveira V, Aguiar CF, *et al.* Dectin-1 Activation Exacerbates Obesity and Insulin Resistance in the Absence of MyD88. *Cell Rep*, 2017, 19(11):2272-2288
 - 32 Voruganti VS, Laston S, Haack K, *et al.* Genome-wide association replicates the association of Duffy antigen receptor for chemokines (DARC) polymorphisms with serum monocyte chemoattractant protein-1 (MCP-1) levels in Hispanic children. *Cytokine*, 2012,60(3):634-638
 - 33 Wang S, Gu J, Xu Z, *et al.* Zinc rescues obesity-induced cardiac hypertrophy via stimulating metallothionein to suppress oxidative stress-activated BCL10/CARD9/p38 MAPK pathway. *J Cell Mol Med*, 2017,21(6):1182-1192
 - 34 Biobaku F, Ghanim H, Batra M *et al.* Macronutrient-Mediated Inflammation and Oxidative Stress: Relevance to Insulin Resistance, Obesity, and Atherogenesis. *J Clin Endocrinol Metab*, 2019,104(12):6118-6128
 - 35 Lackey DE, Olefsky JM: Regulation of metabolism by the innate immune system. *Nat Rev Endocrinol*, 2016,12(1):15-28
 - 36 Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- α : direct role in obesity-linked insulin resistance. *Science*, 1993,259(5091):87-91
 - 37 Pandori WJ, Lima TS. Toxoplasma gondii activates a Syk-CARD9-NF- κ B signaling axis and gasdermin D-independent release of IL-1 β during infection of primary human monocytes. *PLoS Pathog*, 2019,15(8):e1007923
 - 38 Yang ZW, Meng XX, Zhang C *et al.* CARD9 gene silencing with siRNA protects rats against severe acute pancreatitis: CARD9-dependent NF- κ B and P38MAPKs pathway. *J Cell Mol Med*, 2017,21(6):1085-1093
 - 39 Bertola A, Ciucci T, Rousseau D, *et al.* Identification of adipose tissue dendritic cells correlated with obesity-associated insulin-resistance and inducing Th17 responses in mice and patients. *Diabetes*, 2012,61(9):2238-2247
 - 40 LeibundGut-Landmann S, Gross O, Robinson MJ, *et al.* Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat Immunol*, 2007,8(6):630-638
 - 41 Libby P. Inflammation in atherosclerosis. *Nature*, 2002,420(6917):868-874
 - 42 Clement M, Basatemur G, Masters L. *et al.* Necrotic Cell Sensor Clec4e Promotes a Proatherogenic Macrophage Phenotype Through Activation of the Unfolded Protein Response. *Circulation*, 2016,134(14):1039-1051
 - 43 Salonen JT, Yla-Herttuala S, Yamamoto R, *et al.* Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *Lancet*, 1992,339(8798):883-887
 - 44 Rhoads JP, Lukens JR, Wilhelm AJ, *et al.* Oxidized Low-Density Lipoprotein Immune Complex Priming of the Nlrp3 Inflammasome Involves TLR and Fc γ R Cooperation and Is Dependent on CARD9. *J Immunol*, 2017,198(5):2105-2114
 - 45 Peterson MR, Haller SE, Ren J, *et al.* CARD9 as a potential target in cardiovascular disease. *Drug Des Devel Ther*, 2016,10:3799-3804
 - 46 Thiem K, Hoeke G, van den Berg S, *et al.* Deletion of hematopoietic Dectin-2 or CARD9 does not protect against atherosclerotic plaque formation in hyperlipidemic mice. *Sci Rep*, 2019,9(1):4337-4347
 - 47 Lassale C, Tzoulaki I, Moons KGM, *et al.* Separate and combined associations of obesity and metabolic health with coronary heart disease: a pan-European case-cohort analysis. *Eur Heart J*, 2018,39(5):397-406
 - 48 Choi S, Kim K, Kim SM, *et al.* Association of Obesity or Weight Change With Coronary Heart Disease Among Young Adults in South Korea. *JAMA Intern Med*, 2018,178(8):1060-1068
 - 49 Wannamethee SG, Shaper AG, Rumley A, *et al.* Lung function and risk of type 2 diabetes and fatal and nonfatal major coronary heart disease events: possible associations with inflammation. *Diabetes Care*, 2010,33(9):1990-1996
 - 50 Holliday MJ, Ferrao R, de Leon Boenig G *et al.* Picomolar zinc binding modulates formation of Bcl10-nucleating assemblies of the caspase recruitment domain (CARD) of CARD9. *J Biol Chem*, 2018,293(43): 16 803-16 817
 - 51 Ren J, Yang M, Qi G, *et al.* Proinflammatory protein CARD9 is essential for infiltration of monocytic fibroblast precursors and cardiac fibrosis caused by Angiotensin II infusion. *Am J Hypertens*, 2011,24(6): 701-707
 - 52 Li Y, Liang P, Jiang B, *et al.* CARD9 inhibits mitochondria-dependent apoptosis of cardiomyocytes under oxidative stress via interacting with Apaf-1. *Free Radic Biol Med*, 2019,141:172-181
 - 53 Liu Y, Wang Y, Shi H, *et al.* CARD9 mediates necrotic smooth muscle cell-induced inflammation in macrophages contributing to neointima formation of vein grafts. *Cardiovasc Res*, 2015,108(1):148-158
 - 54 Bourassa MG. Fate of venous grafts: the past, the present and the future. *Journal of the J Am Coll Cardiol*, 1991,17(5):1081-1083

- 55 Shi HT, Wang Y, Jia LX, *et al.* Cathepsin S contributes to macrophage migration via degradation of elastic fibre integrity to facilitate vein graft neointimal hyperplasia. *Cardiovasc Res*, 2014,101(3):454-463
- 56 Sterpetti AV, Cucina A, Lepidi S, *et al.* Formation of myointimal hyperplasia and cytokine production in experimental vein grafts. *Surgery*, 1998,123(4):461-469
- 57 Sterpetti AV, Cucina A, Lepidi S, *et al.* Formation of myointimal hyperplasia and cytokine production in experimental vein grafts. *Surgery*, 1998,123(4):461-469
- 58 Yang J, Chai L, Gao C, *et al.* SALL4 is a key regulator of survival and apoptosis in human leukemic cells. *Blood*, 2008,112(3):805-813
- 59 Canestraro M, Galimberti S, Savli H, *et al.* Synergistic antiproliferative effect of arsenic trioxide combined with bortezomib in HL60 cell line and primary blasts from patients affected by myeloproliferative disorders. *Cancer Genet Cytogenet*, 2010,199(2):110-120
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