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TRANSLATIONAL PERSPECTIVE

CARD9-Mediated Signaling and Cardiovascular Diseases

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Inflammation and oxidative stress are critical to the pathogenesis of cardiovascular diseases (CVDs) including atherosclerosis and heart failure. Proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and interferon (IFN)-γ, play a critical role in the initiation and maintenance of inflammation. The adaptor protein caspase-recruitment domain 9 (CARD9) is one of the CARD-containing proteins and is expressed in many cells, especially in immunoreactive cells including macrophages, neutrophils, and dendritic cells, and is critically involved in inflammatory responses and oxidative stress through regulating the productions of a variety of proinflammatory cytokines and chemokines (Table 1).

CARD9 was first shown in 2000 to mediate the activation of nuclear factor κB (NF-κB) in 293T cells through binding to an upstream molecule B-cell lymphoma/leukemia 10 (BCL10), and was subsequently identified in 2006 as a key transducer of Dectin-1 (an important transmembrane pattern recognition receptors for extracellular signals). Accumulating data has now suggested that CARD9 plays a central role in regulating the productions of cytokines and chemokines including innate chemokines (CXCL1 and CXCL2), innate cytokines (TNF-α, IL-1β, and IL-6), and adaptive cytokines (IL-22, IL-17 and IFN-γ) via activation of NF-κB or mitogen-activated protein kinases (MAPKs)[1] in response to extracellular and intracellular proinflammatory signals through a complex and interactive mechanisms (Figure 1). CARD9 can interact with BCL10 and mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) to form interactive protein complexes in the cells, triggering the expressions of inflammatory mediators. There are extensive interactions among inflammatory cytokines, reactive oxygen species (ROS) production, and oxidative stress. Inflammatory cytokines trigger an excessive ROS formation, while ROS in turn regulates the expressions of proinflammatory cytokines. Rac1 is a small GTP-binding protein that functions as a molecular switch exchanging GDP for GTP and an important regulator for ROS formation through NADPH complex activation in macrophages. GDP-dissociation inhibitors (GDI) including LyGDI prevent Rac1 activation, while interactions of CARD9 with LYGDI activate Rac1 during bacterial and fungal infections, thus increasing NADPH oxidase-medicated ROS production (Figure 1).[2]

How are CARD9 and CARD9-mediated signaling contributing to the development and progression of CVDs? Animal studies have shown that global CARD9-deficient mice are resistant to developing viral myocarditis, cardiac hypertrophy, or vein graft failure.[3] CARD9 expression is significantly increased in viral myocarditis, and CARD9 deficiency prevents Coxsackie virus B3-induced excessive inflammation and production of transforming growth factor-β (TGF-β), IL-17A, and BCL-10 in mouse myocardium, with decreased serum levels of IL-6, IL-10, IFN-γ, TGF-β, and IL-17A. Angiotensin II and transverse aortic

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constriction markedly increase CARD9 expression in the mouse heart. CARD9 deficiency attenuates angiotensin II- or transverse aortic constriction-induced macrophage infiltration; activations of NF-κB, c-Jun N-terminal kinases, and p38 MAPKs; and expressions of IL-1β and TGF-β, preventing mouse cardiac fibrosis or hypertrophy. High-fat diet and zinc deficiency-induced oxidative stress activate CARD9/p38 MAPK signaling, an important mechanism for obesity-related cardiac hypertrophy.[3] Zinc supplement suppresses CARD9 expression and reduces oxidative stress in mice on a high-fat diet. CARD9 expression and production of proinflammatory cytokines are increased in vein grafts and associated with graft macrophage infiltration and neointima formation. CARD9 deficiency decreases macrophage population and prevents NF-κB activation and production of IL-1β, IL-6, and monocyte chemoattractant protein-1 (MCP-1), thus decreasing graft neointima formation.

Cytokines and chemokines are important for atherosclerosis development and progression. Thus, it was hypothesized that inhibiting CARD9 expression could reduce atherosclerosis. However, studies show that hematopoietic CARD9 deletion increases aortic atherosclerosis independent of plasma lipid levels in hyperlipidemic low-density lipoprotein receptor deficient mice, while reducing macrophage population and collagen content in atherosclerotic lesions without affecting lesion size in hyperglycemic mice.[3] Hematopoietic CARD9 deletion does not have a significant impact on the productions of IL-6 and TNF-α or gene expressions of TNF-α, MCP-1, IL-1β, and IL-10. This is not surprising because the CIRT (Cardiovascular Inflammation Reduction Trial) has shown that there are no cardiovascular benefits in patients with myocardial infarction or type 2 diabetes or metabolic syndrome when there are no reductions in CRP, IL-1β, or IL-6 levels.[4] In consistent with these observations, the data from CANTOS study shows that the patients with the greatest reductions in IL-6 and CRP levels benefited the most from canakinumab treatment with reduced adverse cardiovascular events.[4] Apparently, hematopoietic CARD9 deletion alone is not enough to suppress cytokine productions, and is naturally unable to decrease atherosclerosis. Local CARD9 expression in other cells and organ systems like endothelial cells and spleen may be increased as a compensation, leading to increased vascular inflammation and atherosclerosis.

Cardiac ischemia/reperfusion (I/R) injury significantly contributes to sudden cardiac death and ischemic cardiomyopathy. Inflammatory responses are activated immediately when cardiac ischemic injury occurs and persist for the first week, then resolve in about 2 weeks following cardiac damage. A mouse model with 45 minutes of ischemia followed by 24 hours of reperfusion demonstrates that CARD9-deficient mice have a smaller infarct size than wild-type control, with decreases in neutrophil infiltration, phosphorylated p38 MAPK, and levels of TNF-α, IL-6, CXCL1, and MCP-1 in the heart and serum. Activation of autophagy or inhibition of

ABBREVIATIONS AND ACRONYMS

BCL10 = B-cell lymphoma/leukemia 10

CARD9 = caspase-recruitment domain 9 protein

CVDs = cardiovascular diseases

CXCL = CXC-chemokine ligand

GDI = GDP-dissociation inhibitors

IFN = interferon

I/R = ischemia/reperfusion

MAPK = mitogen-activated protein kinase

MCP = monocyte chemoattractant protein

NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells

ROS = reactive oxygen species

TGF = transforming growth factor

TNF = tumor necrosis factor

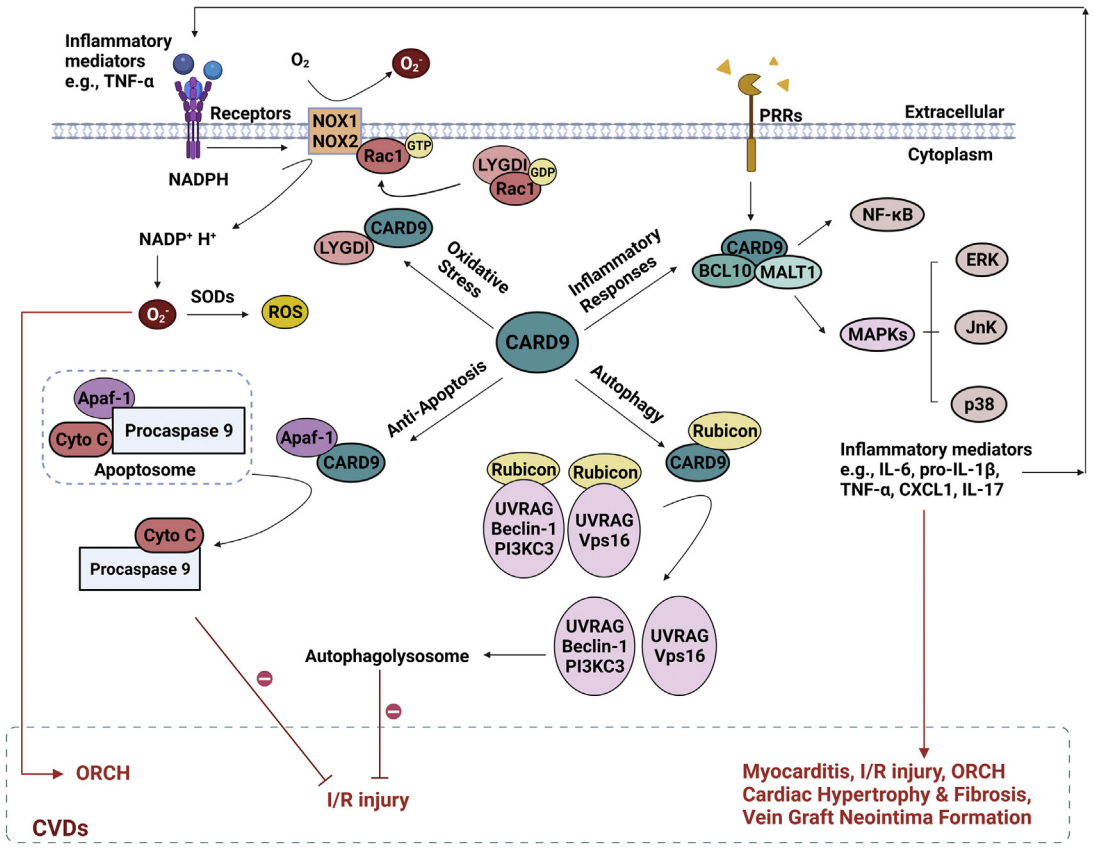
TABLE 1 Role of CARD9-Mediated Signaling in Cardiovascular Diseases and Mechanisms

Disease	Animal Model (All With Mouse)	Role of CARD9	Mechanism	First Author[a]
Cardiac hypertrophy and fibrosis	Angiotensin II treatment transverse aortic constriction surgery	↑ Cardiac remodeling and dysfunction	↑ Mac-2, α-smooth muscle actin, NF-κB/p65, p38, and c-Jun N-terminal kinases 1/2	Peterson et al,[S1] Ren et al[S2]
CVB3-induced myocarditis	CVB3 infection	↑ Acute viral myocarditis	↑ IL-6, IL-10, IFN-γ, TGF-β, and IL-17A	Sun et al[S3]
Neointima formation of grafted veins	Inferior venae cavae grafted into carotid artery	↑ Neointima formation in the vein grafts	↑ Proinflammatory cytokine secretion and NF-kB activation	Liu et al[S4]
Obesity-related heart hypertrophy	High-fat diet, zinc supplement	Zinc supplement prevented high-fat diet–induced expression of CARD9 in heart	↑ Macrophage infiltration, p38 MAPK phosphorylation, IL-6, IL-1β, and TNF-α production	Wang et al[S5]
Atherosclerosis	Western diet	Hematopoietic CARD9 deletion ↑ Atherosclerotic lesion	↑ Lesion macrophage, MCP-1	Thiem et al[S6]
	Streptozotocin injections	Hematopoietic CARD9 deletion had no effect on lesion size	No changes in serum glucose, TNF-α, MCP-1, IL-1β, IL-10	Thiem et al[S7]
Cardiac ischemia/reperfusion (I/R) injury	45 min of ischemia/24 h reperfusion	↑ Cardiac I/R injury	↑ Neutrophil infiltration, p38 MAPK, TNF-α, IL-6, CXCL1, and MCP-1	Qin et al[S8]
	30 min of ischemia/12 h of reperfusion	↑ Animal survival ↓ Myocardial I/R injury	↑ Autophagy, ↓ Apoptosis	Li et al[S9,S10]

↑ indicates promote or increase; ↓ indicates inhibition or decrease. [a]References in **Table 1** are listed in the Supplemental Appendix.

CARD9 = caspase-recruitment domain 9 protein; CXCL = CXC-chemokine ligand; IFNγ = interferon-γ; I/R = ischemia/reperfusion; MAPK = mitogen-activated protein kinase; MCP-1 = monocyte chemoattractant protein-1; NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells; TGF = transforming growth factor; TNF = tumor necrosis factor.

FIGURE 1 Schematic Illustration of CARD9-Mediated Signaling Pathways and Relations With Cardiovascular Diseases



CARD9 mediates inflammatory responses through extensive interactions with a complex network of extracellular and intracellular molecules, and associates with cardiac hypertrophy/fibrosis, I/R injury, ORCH, myocarditis, and vein graft neointima formation. Apaf-1 = apoptotic protease activating factor 1; BCL10 = B-cell lymphoma/leukemia 10; CARD9 = caspase-recruitment domain 9 protein; CVD = cardiovascular disease; CXCL = CXC-chemokine ligand; GDI = GDP-dissociation inhibitors; I/R = ischemia/reperfusion; MAPK = mitogen-activated protein kinase; NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells; ORCH = obesity-related cardiac hypertrophy; PRRs = pattern recognition receptors; ROS = reactive oxygen species; Rubicon = RUN domain Beclin-1-interacting cysteine-rich-containing; TNF = tumor necrosis factor.

apoptosis is an important protective mechanism against myocardial I/R injury and cardiac remodeling. A recent study, using a mouse model with 30 minutes of ischemia and 12 hours of reperfusion, shows that CARD9 deficiency significantly decreases mouse survival and aggravates cardiac dysfunction with impaired autophagy and increased apoptosis, whereas CARD9 overexpression increases autophagic flux and decreases apoptosis in cardiomyocytes.[5] Mechanistically, CARD9 binds with apoptotic protease activating factor 1 and disassociates cytochrome C-apoptotic protease activating factor 1-procaspase-9 apoptosome complex, thus attenuating apoptosis (Figure 1). CARD9 also interacts directly with RUN domain Beclin-1-interacting cysteine-rich-containing (Rubicon) protein and promotes the formations of UV-irradiation-resistance-associated gene (UVRAG)-PI3KC3 complex and UVRAG-Vps16 complex, leading to autophagosome formation, maturation, and endocytosis, thereby alleviating I/R injury (Figure 1).[5] The apparently inconsistent role of CARD9 in cardiac I/R injury is possibly because of different I/R time and resultant cardiac inflammatory/redox status. Excessive inflammation is detrimental to tissue recovery, whereas a balanced inflammation is necessary for anti-inflammatory response and healing. The cytokines released from inflammatory cells promote M2 macrophages (anti-inflammatory) polarization. CARD9 is important for macrophage differentiation; thus, CARD9-defective macrophages exhibit a significant decrease in the transcriptional expressions of M2 macrophage marker (Arginase 1). CARD9 may

function differently in different disease states or pathological conditions. Further studies are needed to define the mechanisms for the different roles of CARD9 in different pathological conditions including cardiac I/R injury.

Can CARD9 and CARD9-mediated signaling emerge as a new target for prevention and/or treatment of CVDs? Antioxidant therapies with vitamin E and C or β-carotene failed to achieve clinical benefits in patients with CVDs. New therapies including IL-1β antibody canakinumab and anti-inflammatory drug colchicine significantly reduce major adverse cardiovascular events, including nonfatal myocardial infarction, nonfatal stroke, and cardiovascular death. However, canakinumab therapy does not decrease all-cause mortality, and is associated with a significant increase in fatal infection (including sepsis).[4] Although it decreases the risk of major adverse cardiovascular events, colchicine therapy is associated with increased noncardiovascular mortality. Anti-inflammatory therapy by targeting CARD and CARD9-mediated signaling with optimal modification of production of inflammatory mediators could be a promising option. Indeed, a small-molecule BRD5529 has been recently developed to inhibit CARD9 function. Another key challenge of anti-inflammatory therapy is to achieve a delicate balance between inhibiting pathological inflammation and compromising immune response with increased risk for serious infections, because global CARD9 ablation indeed increases infection risk. Clearly, it is necessary to define the roles and mechanisms of CARD9 in specific cells and organ systems to formulate the optimal strategy for CVDs.

In conclusion, CARD9 is critically involved in the production of pro-inflammatory cytokines through extensive interactions with a complex network of extracellular and intracellular signaling molecules. There are important and complex relationships between CARD9-mediated signaling and CVDs. CARD9 could be a potential novel target for prevention and/or treatment of CVDs with delicate modification of inflammatory responses and oxidative stress. However, further studies are needed to define the complex, and yet critical, roles and mechanisms of CARD9-mediated signaling in the pathophysiology of CVDs including atherosclerosis and I/R injury.

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APPENDIX For supplemental references, please see the online version of this article.