World Journal of Biological Chemistry

Online Submissions: http://www.wjgnet.com/1949-8454office
wjbc@wjgnet.com
doi:10.4331/wjbc.v1.i12.353

World J Biol Chem 2010 December 26; 1(12): 353-361
ISSN 1949-8454 (online)

EDITORIAL

CARMA3: A novel scaffold protein in regulation of NF-κB activation and diseases

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Author contributions: Sun J solely contributed to this paper.
Supported by The National Institutes of Health through MD Anderson's Cancer Center Support Grant, No. CA016672
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Received: September 17, 2010 Revised: October 18, 2010
Accepted: October 25, 2010
Published online: December 26, 2010

Abstract

CARD recruited membrane associated protein 3 (CARMA3) is a novel scaffold protein. It belongs to the CARMA protein family, and is known to activate nuclear factor (NF)-κB. However, it is still unknown which receptor functions upstream of CARMA3 to trigger NF-κB activation. Recently, several studies have demonstrated that CARMA3 serves as an indispensable adaptor protein in NF-κB signaling under some G protein-coupled receptors (GPCRs), such as lysophosphatidic acid (LPA) receptor and angiotensin (Ang) Ⅱ receptor. Mechanistically, CARMA3 recruits its essential downstream molecules Bcl10 and MALT1 to form the CBM (CARMA3-Bcl10-MALT1) signalosome whereby it triggers NF-κB activation. GPCRs and NF-κB play pivotal roles in the regulation of various cellular functions, therefore, aberrant regulation of the GPCR/NF-κB signaling axis leads to the development of many types of diseases, such as cancer and atherogenesis. Recently, the GPCR/CARMA3/NF-κB signaling axis has been confirmed in these specific diseases and it plays crucial roles in the pathogenesis of disease progression. In ovarian cancer cell lines, knockdown of CARMA3 abolishes LPA receptor-induced NF-κB activation, and reduces LPA-induced ovarian cancer invasion. In vascular smooth cells, downregulation of CARMA3 substantially impairs Ang-Ⅱ-receptor-induced NF-κB activation, and *in vivo* studies have confirmed that Bcl10-deficient mice are protected from developing Ang-Ⅱ-receptor-induced atherosclerosis and aortic aneurysms. In this review, we summarize the biology of CARMA3, describe the role of the GPCR/CARMA3/NF-κB signaling axis in ovarian cancer and atherogenesis, and speculate about the potential roles of this signaling axis in other types of cancer and diseases. With a significant increase in the identification of LPA- and Ang-Ⅱ-like ligands, such as endothelin-1, which also activates NF-κB *via* CARMA3 and contributes to the development of many diseases, CARMA3 is emerging as a novel therapeutic target for various types of cancer and other diseases.

Key words: G protein-coupled receptor; β-arrestin; CARD recruited membrane associated protein 3; Nuclear factor-κB; Cancer; Atherogenesis

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Sun J. CARMA3: A novel scaffold protein in regulation of NF-κB activation and diseases. *World J Biol Chem* 2010; 1(12): 353-361 Available from: URL: http://www.wjgnet.com/1949-8454/full/v1/i12/353.htm DOI: http://dx.doi.org/10.4331/wjbc.v1.i12.353

INTRODUCTION

CARD recruited membrane associated protein 3 (CAR-

MA3) is a novel scaffold protein. CARMA3 belongs to the CARMA protein family, which includes CARMA1, CARMA2, and CARMA3[1,2]. CARMA protein is also referred to as Bimp (Bcl10-interacting membrane protein), because it was first identified as a protein that interacts with Bcl10, another CARD domain adaptor protein. Although the biology and function of the CARMA protein family has not been completely elucidated, it has been shown that all of them play important roles in nuclear factor (NF)-κB activation[1-3]. NF-κB was first identified as a transcription factor of immunoglobulin κ light chain in B cells and is characterized by its important roles in the immune system[4]. NF-κB is now known to be ubiquitously expressed in all cell types and has prominent roles in tumorigenesis and the development of neural, heart and immune diseases[4-8]. The NF-κB family has five members: p50, p52, RelA (p65), RelB, and c-Rel[4]. In resting cells, all five members form homodimers or heterodimers and are sequestered in the cytoplasm *via* coupling with the inhibitor of κB (IκB) proteins, such as IκBα[9]. IκB masks the nuclear localization signal of NF-κB and inhibits its function.

NF-κB is activated *via* the classical or the alternative pathway[4]. Most receptors, such as the T-cell receptor (TCR) and B-cell receptor (BCR)[4], activate NF-κB through the classical pathway. Only a small number of receptors activate NF-κB through the alternative pathway[4,10]. Upon receptor activation, downstream adapters bind to these receptors and recruit kinases to activate the IκB kinase (IKK) complex[11,12]. IKK comprises IKKα, IKKβ, and IKKγ [NF-κB essential modulator (NEMO)] in the classical pathway and an IKKα dimer in the alternative pathway. The IKK complex directly phosphorylates IκBα at serines 32 and 36, which leads to IκBα polyubiquitination by the E3 ubiquitin ligase[4]. IκBα is then degraded by the 26S proteasome, and the NF-κB dimer is released from the cytoplasm and translocated to the nucleus, where it transactivates its target genes[13].

Although the CARMA protein family has been shown to activate NF-κB, it is still unknown which receptors function upstream of CAMRA3 to trigger NF-κB activation. Recently, it has been shown that some G protein-coupled receptors (GPCRs), like lysophosphatidic acid (LPA) and angiotensin (Ang) Ⅱ receptors trigger NF-κB activation *via* CARMA3. GPCRs comprise a large protein family of transmembrane receptors that sense molecules outside the cell and activate inside signal transduction pathways, and ultimately, cellular responses. GPCRs are the largest cell surface receptors. Up to 2% of the human genome encodes GPCRs[14]. GPCRs are expressed throughout the body, including the central nervous system, cardiovascular system, gastrointestinal tract, musculoskeletal system, genitourinary system, reproductive system, and almost all organs controlled by the autonomic nervous system[15]. GPCRs are activated by a diverse array of ligands and play crucial roles in physiology. Furthermore, they are involved in almost all types of stimulus-response pathways and are important targets of 40%-50% of modern drugs[16].

GPCRs signal *via* heterotrimeric G proteins (Gα, Gβ, and Gγ) or β-arrestins[17,18]. G proteins are heterotrimeric and include eighteen α subunits that are classified into four groups (Gs, Gi, Gq, and G12/13), twelve β subunits, and five γ subunits[19]. These G proteins independently or cooperatively activate their downstream signaling cascades[19]. β-arrestins also function to relay signals rather than simply desensitize GPCR-induced signals[20]. Upon activation, GPCRs activate numerous downstream effectors. One important target is NF-κB. Constitutive activation of NF-κB contributes to various diseases, including cancer and atherogenesis[5,21]. In this review, we summarize the biology of CARMA3 and the CARMA protein family, discuss the role of the GPCR/CARMA3/NF-κB signaling axis in ovarian cancer and atherosclerosis, and speculate about the potential roles of this signaling axis in other types of cancer and diseases.

CARMA PROTEIN FAMILY

Structure

CARMA proteins are caspase recruitment domain (CARD)-containing members of the membrane-associated guanylate kinase (GUK) family. The CARMA protein family has three members: CARMA1, CARMA2, and CARMA3. The three members share similar structures (Figure 1A): an N-terminal CARD followed by a coiled-coil domain; a linker region; a PDZ domain; an Src homology 3 (SH3) domain; and a GUK-like domain[1,3]. The CARD domain is found in a variety of proteins, especially those involved in apoptosis and inflammation. This domain consists of six or seven antiparallel α helices with a hydrophobic core and outer surface. It mediates the interaction of larger protein complexes by association with different individual CARD domains[22-24]. The coiled-coil domain mediates dimerization[25]. The linker region contains crucial phosphorylation sites[26]. Upon phosphorylation of the linker region, CARMA protein is activated, unfolds, and recruits downstream molecules (Figure 1B). The PDZ, SH3 and GUK domains are membrane-associated domains that act in membrane localization. Therefore, they are also known as membrane-associated GUK domains[27]. Although the structure of the GUK domain is similar to that of GUK itself, it does not have any kinase activity.

Distribution

Although the three CARMA proteins have similar structures, they are transcribed by different genes and expressed in different tissues[1,3,28]. Specifically, CARMA1 is predominantly expressed in the spleen, thymus, and peripheral blood leukocytes[3]; CARMA2 is expressed only in the placenta[1]; and CARMA3 is expressed in a broad range of tissues, especially at high levels in the liver, kidney, heart, and brain, but is not expressed in the spleen, thymus, or peripheral blood lymphocytes[28].

Function

Overexpression of CARMA proteins induces robust NF-

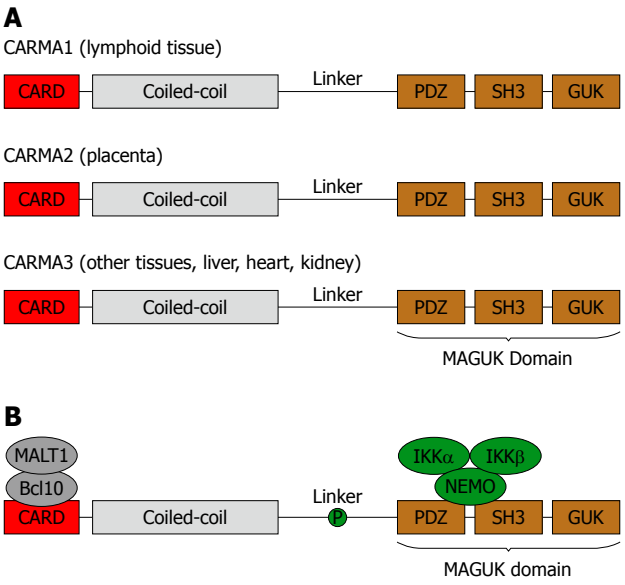

Figure 1 CARD recruited membrane associated protein family members. A: The CARD recruited membrane associated (CARMA) protein family has three members: CARMA1, CARMA2 and CARMA3. Each member shares similar structures: an N-terminal caspase-recruitment domain (CARD), followed by a coiled-coil domain (CC), a linker region, a PDZ domain, an SH3 domain, and a GUK-like domain. Although all CARMA protein members share similar structures, they are transcribed by distinct genes, and expressed in different tissues. Specifically, CARMA1 is predominantly expressed in spleen, thymus, and peripheral blood leukocytes; CARMA2 is expressed only in placenta; and CARMA3 is expressed in a broad range of tissues, especially highly in liver, kidney, heart, and brain, but not in spleen, thymus, or peripheral blood lymphocytes; B: Upon activation, the linker region is phosphorylated. The CARD domain of CARMA protein interacts with Bcl10, which further binds MALT1, while PDZ and SH3 domains associate with the IκB kinase complex *via* NF-κB essential modulator (NEMO). Additionally, different CARMA proteins also interact with other unique signaling molecules. For example, CARMA3 interacts with β-arrestin 2, whereas CARMA1 associates with ADAP.

κB activation[1,2]. However, the receptors that employ CARMA proteins to activate NF-κB remain unknown. Recently, studies have demonstrated that CARMA1 is required for TCR- and BCR-induced NF-κB activation[29-31]. Also, we and other groups have shown that CARMA3 is indispensable for induction of NF-κB activation by some GPCR ligands (LPA, endothelin-1, and Ang Ⅱ)[32,33]. CARMA1 and CARMA3 activate NF-κB by recruiting the same downstream molecules: Bcl10 (B-cell chronic lymphocytic leukemia-lymphoma 10), MALT1 (mucosa-associated lymphoid tissue lymphoma translocation gene 1), and TRAF6 (tumor necrosis factor receptor-associated factor 6)[34-36]. Bcl10 and MALT1 are two indispensable proteins thought to synergize in NF-κB activation. TRAF6 is an E3 ubiquitin ligase that catalyzes the formation of polyubiquitin chains on IKK and facilitates NF-κB activation[37,38]. In addition, overexpression of CARMA2 activates NF-κB. However, because CARMA2 is only expressed in placental tissues, its function remains undetermined.

Mechanism of activation

Upon receptor activation, CARMA proteins are recruited to the membrane proximal regions of receptors by adaptor proteins, where they can be further phosphorylated by specific protein kinase C (PKC) isoforms, which results in activation and recruitment of downstream effectors. In T cells, adhesion- and degranulation-promoting adaptor protein (ADAP) links CARMA1 with the membrane proximal region of TCR and facilitates its phosphorylation and activation by PKC[39]. In ADAP-deficient T cells, TCR-stimulated assembly of the CARMA1/Bcl10/MALT1 complex and activation of NF-κB are substantially impaired[39]. Upon GPCR activation, CARMA3 is linked with the GPCR *via* β-arrestin 2. In β-arrestin-2-deficient mouse embryonic fibroblasts, GPCR (LPA)-induced NF-κB activation is completely abolished[40]. Although it has been reported that β-arrestins inhibit GPCR-induced NF-κB activation[41,42], it is proposed that the phosphorylation status of β-arrestin 2 might critically regulate and determine its function in NF-κB activation.

After CARMA proteins are linked to the receptor proximal region, PKC is engaged in phosphorylation of CARMA proteins. In T and B cells, PKCθ and PKCβ, respectively, phosphorylate CARMA1 and play indispensable roles in TCR- and BCR-induced NF-κB activation. In both pathways, PKCθ and PKCβ phosphorylate similar residues on the link region of CARMA1 and contribute to NF-κB activation[43,44]. PKC also functions in GPCR-induced NF-κB activation. Several groups have revealed that PKCα or PKCδ might be required for GPCR-induced NF-κB activation[33,45]. Also, PKCδ deficiency reportedly impairs LPA-induced NF-κB-dependent interleukin (IL)-8 secretion[46], and dominant-negative PKCα substantially attenuates LPA-induced NF-κB activation[47], which indicates that PKCδ or PKCα is the key PKC isoform in phosphorylation and activation of CARMA3.

In particular, in response to TCR and BCR activation, PKCθ and PKCβ are activated and phosphorylate S552 and S564/S649/S657, respectively, on the CARMA1 linker region. Mutations on these residues abolish TCR- and BCR-induced NF-κB activation[48,49]. Similar to CARMA1, it has been proposed that some PKC isoforms activate NF-κB through CARMA3 in the presence of GPCR[32,33,47]. In determining which residue of CARMA3 is phosphorylated, we have demonstrated that CARMA3 mutant S520A, an analog of CARMA1 S552A, does not rescue TCR-induced NF-κB activation[49]; however, wild-type CARMA3 does rescue TCR-induced NF-κB activation in CARMA1-deficient Jurkat T cells[49]. Therefore, CARMA3 S520 might be the crucial site for CARMA3 phosphorylation and activation.

Upon phosphorylation, CARMA1 and CARMA3 contribute to NF-κB activation by regulating the activity of the IKK complex through IKK NEMO polyubiquitination[33,38]. Although reports have suggested that phosphorylation of IKK indicates its activation, we have shown that phosphorylation of IKK is not sufficient to induce its kinase activity[33]. Only after both IKKα and IKKβ are phosphorylated and IKK NEMO is ubiquitinated, is IKK activated and able to phosphorylate downstream IκBα[33,38]. In GPCR-induced NF-κB signaling, IKKα/β phosphorylation is controlled by a PKC-dependent but

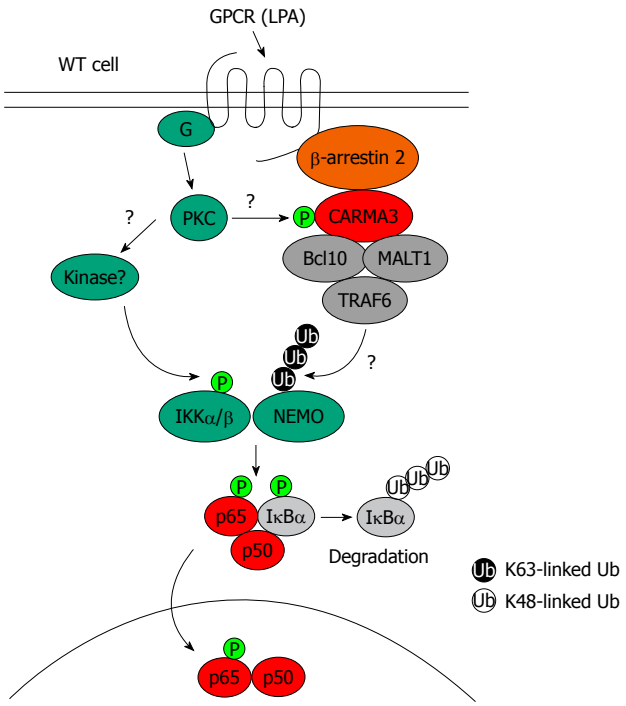

Figure 2 Working model of CARD recruited membrane associated protein 3-dependent nuclear factor-κB activation in the G protein-coupled receptor (lysophosphatidic acid) signaling pathways. G protein-coupled receptor (GPCR) [lysophosphatidic acid (LPA)]-induced nuclear factor (NF)-κB activation involves the recruitment of CARD recruited membrane associated protein 3 (CARMA3) to the receptor by β-arrestin 2, which leads to formation of the CARMA3/Bcl10/MALT1/TRAF6 complex, which results in polyubiquitination of the IκB kinase (IKK) complex. A CARMA3-independent, PKC-dependent signal induces phosphorylation of the IKK complex by an unknown kinase in the presence of GPCR. After IKK is both polyubiquitinated [NF-κB essential modulator (NEMO)] and phosphorylated (IKK), it is activated, which leads to NF-κB activation. In the absence of CARMA3, GPCR (LPA)-induced polyubiquitination of the IKK complex is defective, which results in defects in IKK and NF-κB activation. WT: Wild-type; Ub: Ubiquitin.

CARMA3-independent pathway, and IKK NEMO polyubiquitination is controlled by a CARMA3-dependent pathway (Figure 2). Therefore, in CARMA3-deficient murine embryonic fibroblasts, IKKα/β phosphorylation remains intact, but IKK NEMO polyubiquitination is impaired. Consequently, IKK is not activated and is unable to phosphorylate IκBα, thus, LPA-induced NF-κB activation is completely abolished[33,38].

THE GPCR/CARMA3/NF-κB SIGNALING AXIS

At the molecular level, GPCR activates CARMA3, which in turn further activates NF-κB *via* multiple pathways[19]. However, which pathway is important in relaying signals to NF-κB *via* CAMRA3 remains to be determined. Upon ligand binding to receptors, G proteins such as Gαq, Gαi, and Gα12/13 are activated[50]. Gαq then activates phospholipase Cβ, which hydrolyzes phosphatidylinositol 4 5-bisphosphate. With consequent production of diacylglycerol and release of calcium from endoplasm, PKC is activated[51], thereby leading to NF-κB activation. This pathway promotes cell survival. In addition, Gαi activates the phosphatidylinositol 3-kinase (PI3K)/AKT and SOS/RAS/ERK pathways[52,53], which activate NF-κB and promote cell spread, migration, invasion, and DNA synthesis. Furthermore, Gα12/13 activates NF-κB *via* the G12/13/RHO/GEF/RHOA pathway and contributes to contraction and cell rounding[54].

CARMA3 is an indispensable signaling component in GPCR-induced NF-κB activation, therefore, it plays a crucial role in the development of diseases that result from the aberrant regulation of GPCR/NF-κB signaling, such as tumor progression and atherogenesis. GPCR activates NF-κB *via* CARMA3, and NF-κB in turn upregulates the expression of numerous genes that are involved in cell proliferation, anti-apoptosis, angiogenesis, migration, invasion, metastasis and inflammation, such as cyclin D1[55], bcl-2[56], vascular endothelial growth factor (VEGF)[57,58], cyclooxygenase-2[59], matrix metalloproteinase (MMP)-2[58], MMP-9[58], urokinase plasminogen activator (uPA)[57,58,60], growth-regulated oncogene α[61-63], IL-6[64], and IL-8[58,65]. Thus, sustained NF-κB activity has emerged as a hallmark of many diseases[5,21].

Recently, we have discovered that CARMA3 is indispensable for GPCR-induced NF-κB activation in murine embryonic fibroblasts[33]. However, whether the GPCR/CARMA3/NF-κB signaling axis is found in some specific diseases has yet to be completely elucidated. Below, we briefly describe the role of the GPCR/CARMA3/NF-κB signaling axis in ovarian cancer and atherogenesis, and speculate about its potential roles in other types of cancer and diseases (Figure 3).

GPCR/CARMA3/NF-κB signaling axis in ovarian cancer

Ovarian cancer is among the four most common cancers worldwide. In 90% of patients, LPA expression level is significantly elevated[66]. LPA is a typical GPCR ligand that activates NF-κB and leads to tumor progression. LPA receptors LPA1, LPA2, and LPA3 are aberrantly expressed in ovarian cancer cells[50]. Consequently, LPA serves as a diagnostic marker for ovarian cancer[67].

As a major active constituent of serum, LPA is a water-soluble phospholipid derivative of an intermediate in intracellular metabolism[50] or it is produced extracellularly from lysophosphatidylcholine by phospholipase A1/A2 or autotaxin (lysophospholipase D)[50,68,69]. Autotaxin is a widely expressed extracellular exophosphodiesterase that contributes to synthesis of LPA and promotes tumor invasion and metastasis[70]. LPA activates NF-κB and exerts striking wide hormone- and growth-factor-like effects, such as proliferation, apoptosis, differentiation, and chemotaxis[50]. Mechanistically, LPA activates NF-κB and transactivates numerous NF-κB target genes, such as cyclin D1, VEGF, uPA, IL-6, and IL-8. All of these genes play crucial roles in tumor progression.

Hu *et al*[55] have demonstrated that, at concentrations found in ascitic fluid, LPA can directly promote ovarian tumor growth by increasing the expression of cyclin D1, a key G1-phase checkpoint regulator, which results

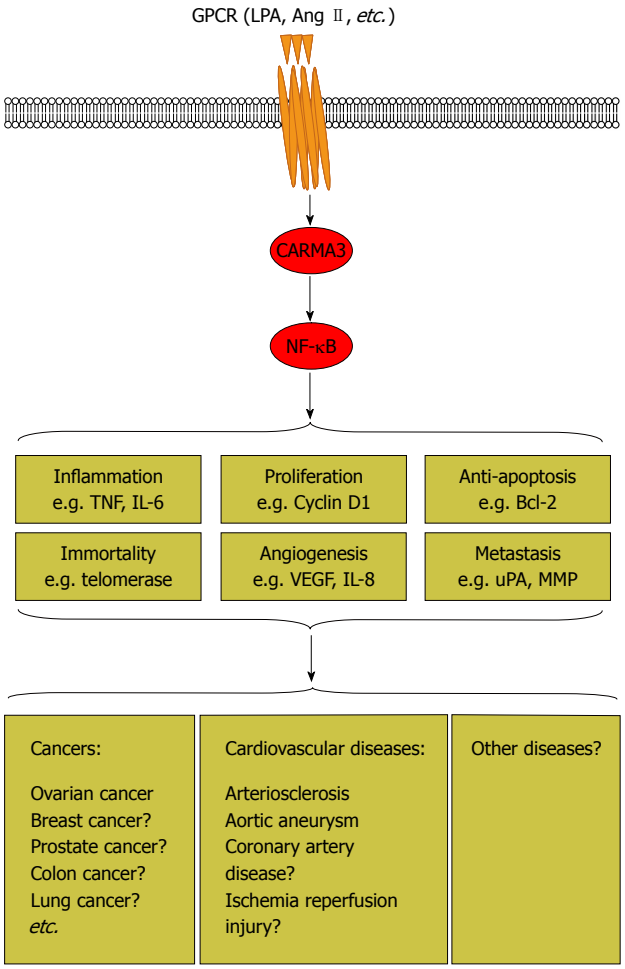

Figure 3 Proposed working model of G protein-coupled receptor/CARD recruited membrane associated protein 3/nuclear factor-κB signaling pathways in cancer, cardiovascular diseases, and other diseases. G protein-coupled receptor (GPCR) [lysophosphatidic acid (LPA), angiotensin (Ang) Ⅱ] activates CARD recruited membrane associated protein 3 (CARMA3), which in turn triggers nuclear factor (NF)-κB activation. NF-κB plays an important role in regulation of many physiological and pathological processes. Aberrant regulation of the GPCR/CARMA3/NF-κB signaling axis results in cancer, cardiovascular diseases, and probably other diseases. Mechanistically, it promotes cell proliferation, angiogenesis and metastasis, and inhibits apoptosis. In addition, it also induces inflammation. CARMA3 is indispensable for GPCR (LPA, Ang Ⅱ)-induced NF-κB activation. Consequently, it plays pivotal roles in GPCR-induced tumor progression and cardiovascular diseases. Full definition of GPCR/CARMA3/NF-κB signaling events could aid the discovery of new drug targets and production of profoundly significant clinical therapies for cancer, cardiovascular diseases, and many other diseases. TNF: Tumor necrosis factor; IL: Interleukin; VEGF: Vascular endothelial growth factor; uPA: Urokinase plasminogen activator; MMPs: Matrix metalloproteinases.

in cell proliferation. In addition, LPA stimulates secretion of VEGF[71] and promotes ovarian cancer angiogenesis, migration, and invasion[72]. Furthermore, LPA enhances secretion of IL-6, a pleiotropic cytokine that is involved in ovarian carcinogenesis *via* the Gi/PI3K/AKT/NF-κB pathway[64].

Recently, Li *et al*[60] have shown that LPA-induced, NF-κB-mediated ovarian cancer migration and invasion is partially dependent on expression of the NF-κB target gene uPA. Mutation of an NF-κB binding site in the uPA promoter region results in reduction of LPA-induced activation of the uPA promoter by > 80%. Li *et al*[60] have concluded that the Gi/Ras/Raf/NF-κB/uPA signaling cascade is responsible for LPA-induced ovarian cancer cell migration and invasion.

More recently, Mahanivong *et al*[47] have demonstrated that the LPA/CARMA3/NF-κB signaling axis is found in ovarian cancer cells. In this study, they observed that CARMA3 nucleated the LPA/NF-κB signaling pathway. LPA-induced NF-κB activation and ovarian cancer cell migration and invasion are substantially attenuated upon silencing CARMA3, Bcl10, and MALT1 with specific siRNAs. Mechanistically, Mahanivong *et al*[47] have found that the Ras/PKCα signaling cascade is involved and PKC might phosphorylate CARMA3. Thus, they delineated the entire GPCR/CARMA3/NF-κB signaling pathway in ovarian cancer cells.

Before the discovery of CARMA3, accumulating evidence suggested that the LPA/NF-κB signaling axis contributes to ovarian cancer tumorigenesis and progression, whereas the precise signaling components and mechanisms are not well defined. The study by Mahanivong *et al*[47] has provided the first evidence that the LPA/CARMA3/NF-κB signaling axis exists in ovarian cancer, plays important roles in ovarian cancer cell progression, and is a novel therapeutic target for ovarian cancer. In addition, it offers insight into other types of cancer. For example, aberrant regulation of LPA/NF-κB/IL-6/8 signaling pathways has been confirmed in breast cancer[57,61,73-82], colon cancer[83-88], prostate cancer[89-94], and lung cancer[95,96]. Inhibition of LPA activation or NF-κB signaling has been shown to prevent tumor progression and enhance sensitivity of chemotherapy[82]. Although the roles of CARMA3 in tumor progression have yet to be confirmed in these cancer types, the LPA/CARMA3/NF-κB signaling axis might also be found in these cancer types due to the high conservation of signaling pathways in most cell types. Future research will focus on the role of CARMA3 in these cancer types.

GPCR/CARMA3/NF-κB signaling axis in atherogenesis

Ang Ⅱ is another type of GPCR ligand. It is a seven-amino-acid oligopeptide that is derived from Ang I and angiotensinogen. Ang Ⅱ is a powerful hormone in the blood and regulates blood pressure[97]. In addition, it plays a crucial role in atherogenesis[98]. Atherogenesis results from vascular inflammation[99-101]. Epidemiologically, the hallmark of vascular inflammation is the elevation of IL-6[21]. IL-6 leads to the recruitment of circulating leukocytes and macrophages into the vascular wall, thereby leading to oxidation of lipoprotein, and atherogenesis[21]. It has been shown that Ang Ⅱ infusion induces IL-6 production, which results in atherogenesis and vascular diseases[102]. IL-6 is an NF-κB targeted gene. Accumulating evidence has also illuminated the central role of NF-κB as a signal regulator that controls the process of vascular inflammation[21]. Therefore, the NF-κB/IL-6 signaling pathway plays a crucial role in atherogenesis and vascular inflammation.

Although Ang Ⅱ is known to activate NF-κB and

IL-6, the detailed molecular mechanism has remained elusive. Recently, McAllister-Lucas *et al*[32] have revealed that CARMA3 is an essential mediator of Ang-Ⅱ-dependent NF-κB signaling. They have shown that all components of the CARMA3/Bcl10/MALT1 signaling pathway are present within the liver in the Ang-Ⅱ-responsive HepG2 hepatocyte cell model. Ang-Ⅱ-induced NF-κB activation is significantly abolished, upon knocking down of CARMA3, Bcl10 or MALT1. This study first provided the evidence that the CARMA3/Bcl10/Malt1 signalosome does exist and is indispensable for NF-κB activation upon Ang Ⅱ receptor activation in hepatic cells.

To explore further the physiological and pathological function of CARMA3 signaling in the cardiovascular system, McAllister-Lucas *et al*[98] recently have revealed that CARMA3, Bcl10 and MALT1 are also expressed in vascular tissues. Consistent with previous results, Ang-Ⅱ-induced NF-κB activation was significantly impaired upon knocking down of CARMA3, Bcl10, and MALT1 in vascular smooth muscle cells. Most importantly, when they crossed the $Bcl10^{-/-}$ and $ApoE^{-/-}$ mice, they found that Bcl10 deficiency protected $ApoE^{-/-}$ mice from Ang-Ⅱ-dependent atherosclerosis and aortic aneurysms. Mechanistically, they revealed that serum levels of several pro-inflammatory mediators, which have all been implicated in the pathogenesis of atherogenesis, were also lower in $ApoE^{-/-}$ $Bcl10^{-/-}$ mice than in $ApoE^{-/-}$ mice ($P < 0.01$)[98]. Together, these results first demonstrated that Ang Ⅱ/CARMA3/NF-κB signaling also exists and plays an important role in atherogenesis in addition to cancer. Further research will focus on its function and therapeutic application in atherogenesis and other vascular diseases, such as LPA- and NF-κB-induced ischemia-reperfusion injury[103,104] and coronary artery disease[104,105].

OUTLOOK

The GPCR/CARMA3/NF-κB signaling axis is a novel signaling pathway. GPCRs belong to a large family, which comprises more than 1000 receptors. Characterization of the role of CARMA3 in the GPCR-induced NF-κB activation signaling pathway will help create a holistic view of GPCR-induced NF-κB activation in the progression of various types of cancer (ovarian, colon, prostate, breast, and head and neck)[50,84], atherogenesis, as well as other diseases, such as LPA- and NF-κB-induced ischemia-reperfusion injury[103,104] and coronary artery disease[104,105]. With a strongly increasing tendency to identify more LPA-like ligands, such as the recently identified Ang Ⅱ and endothelin-1, which also activate NF-κB *via* CARMA3 and contribute to many diseases, CARMA3 is expected to play crucial roles in a broad range of physiological and pathological conditions.

Future research will define the molecular mechanisms underlying induction of NF-κB activation by GPCR, β-arrestin 2, CARMA3, PKC, IKK, and whether CARMA3 signaling induces NF-κB activation under non-GPCR receptors. Also, we will determine what other novel signaling pathways CARMA3 mediates, and investigate the aberrant regulation of signaling cascades in diseases. Characterization of the roles and mechanisms of CARMA3 signaling will aid the discovery of new drug targets and be of major significance for many diseases and therapies.

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S- Editor Cheng JX **L- Editor** Kerr C **E- Editor** Zheng XM