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Review

Caspase Recruitment Domain (CARD) as a Bi-functional Switch of Caspase Regulation and NF-κB Signals

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Over the last three years, increasing numbers of adaptor molecules that contain the caspase recruitment domain (CARD) have been identified. CARD was originally described as a protein-binding motif that interacts with caspase through a CARD-CARD interaction. However, CARD has now also been found in many adaptor proteins that do not interact with caspase, but mediate the assembly of CARD-containing proteins in apoptosis and NF-κB signaling. Apoptotic signaling is controlled by homo- and heterophilic interactions between CARD-containing molecules: Caspases exist as inactive zymogens, and are activated through interactions with adaptor molecules that contain CARD. CARD-containing molecules are also involved in the regulation of gene expression that is involved in cell survival and immune responses through NF-κB activation. Therefore, this report will describe the function and signaling cascade of recently identified CARD-containing proteins in apoptosis and NF-κB activation. CARD-containing molecules were divided into two major groups, based on their functional interactions with caspase and NF-κB signals (Fig. 1 and Table 1).

CARD-adaptor in NF-κB activation

Frequent mutation in tumors affecting NF-κB activation and apoptosis

Bcl10/CLAP/CIPER/mE10/CARMEN Bcl10 was recently cloned from the chromosome translocation t (1; 14) (p22; q32) in MALT B cell lymphoma. Inactivating mutations and the over-expression of Bcl10 were also frequently found in the lymphoid tumor of the B or T cell lineage, as well as cell lines that are derived from solid tumor types (Fakruddin *et al*., 1999; Lee *et al*., 1999; Willis *et al*., 1999; Zhang *et al*., 1999). This indicates that Bcl10 may be involved in the development of human malignancy. Bcl10 was also identified as CIPER by Koseki *et al*. (1999), mE10 by Yan *et al*. (1999), and CLAP by Srinivasa *et al*. (1999).

Bcl10 contains an N-terminal CARD and a C-terminal region that is rich in serine and threonine residues (STR) (Fig. 1). When overexpressed, Bcl10 activated NF-κB, which caused apoptosis of the transfected cells and suppressed transformation. However, the mutation of Bcl10 could not induce cell death, or activate NF-κB. This indicates that the loss of Bcl10 may confer a survival advantage to cells (Willis *et al*., 1999). Bcl10 forms homodimers, and interacts via its CARD with other CARD-containing molecules (including viral E10), but the binding of Bcl10 to caspase-9 needs to be further characterized (Koseki *et al*., 1999; Yan *et al*., 1999).

Recently, an analysis of $Bcl10^{-/-}$ mice demonstrated that Bcl10 was a positive regulator of antigen receptor-induced activation of NF-κB (Ruland *et al*., 2001). One-third of the $Bcl10^{-/-}$ embryos developed embryonic lethality, and the $Bcl10^{-/-}$ cells retained their susceptibility to various apoptotic stimuli, while an altered sensitivity to apoptosis was described in Bcl10 transgenic mice (Yoneda *et al*., 2000). However, severe immunodeficiency was observed in the surviving $Bcl10^{-/-}$ mice. Also, the antigen receptor or PMA/Ionomycin-induced NF-κB activation was defective in $Bcl10^{-/-}$ lymphocytes. Theses observations suggest that Bcl10 connects the antigen receptor signaling to NF-κB activation in B and T cells (Ruland *et al*., 2001).

Signaling net work mediated by CARD-CARD interactions

CARD9, CARD10/Carma3, CARD11/Carma1, CARD14/Carma2 While the upstream signaling pathway that links Bcl10 to extracellular receptors is not well-characterized, Bcl10 associates with many CARD-containing adaptor proteins, mainly through the CARD motif. Bcl10 binds CARD11 and CARD14, another CARD-containing membrane-associated guanylate kinase (MAGUK) family member (Bertin *et al*., 2001) (Table 1). CARD11 and CARD14 consist of an N-terminal CARD domain, a central coiled-coil domain, and a C-terminal tripartite domain that is

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Fig. 1. Schematic diagram showing respective domains of CARD-containing proteins.

comprised of a PDZ domain, a Src homology 3 domain (SH3), and a GUK domain with homology to the guanylate kinase (Bertin *et al.*, 2001) (Fig. 1). MAGUK family proteins are known to function as molecular scaffolds for the assembly of protein complexes. Interestingly, the expression of CARD11 and CARD14 induced the phosphorylation of Bcl10 and NF-κB activation. This suggests that CARD11 and CARD14 may function as upstream activators of Bcl10.

While CARD10 also belongs to the MAGUK family that interacts with Bcl10 and activates NF-κB, its ability to phosphorylate Bcl10 is still unknown (Wang *et al.*, 2001). In addition, CARD9, another CARD protein that interacts with Bcl10 and activates NF-κB, shows a similar structure with the MAGUK family, but lacks the tripartite structure at its C-terminal region (Bertin *et al.*, 2000) (Fig. 1). Though the functional significance of this modification is unclear, Yui *et al.* (2001) recently suggested that Bcl10 phosphorylation regulated its apoptotic activity. The phosphorylation of Bcl10 caused Bcl10 to bind cIAPs, and the Bcl10 mutant defective binding to cIAPs could not induce apoptosis (Yui *et al.*, 2001). Therefore, considering the observations that show the role of E10-mediated hyperphosphorylation of Bcl10 for NF-κB activation (Thome *et al.*, 2001), the modification of Bcl10 seems to be associated with the regulation of apoptosis and NF-κB activation.

CARD11, 14 → Bcl10-(p) → apoptosis & NF-κB
CARD9, CARD10 ?↗

Nod1/CARD4 and Nod2/CARD15 Recent studies identified new members of the Apaf-1 family, which is a mammalian counterpart of *C. elegance* CED4, an important apoptosis regulator. Nod1 and Nod2 are Apaf-1-like molecules that are composed of CARD, nucleotide-binding domain (NBD), and leucine-rich repeats (LRR) (Inhara *et al.*, 1999; Liu *et al.*,

Table 1. CARD-containing proteins.

Protein	Function	Binding partner
Caspase Regulation		
Pseudo-ICE/ COP/ CARD16	Inhibitor of caspase-1 activation	caspase-1, RIP2
ICEBERG	Inhibitor of caspase-1 activation	caspase-1
CARD12/ CLAN/ Ipaf-1	Regulator of caspase-1 activation	ASC, caspase-1, Nod2, CARD7, Bcl10
DEFCAP/ CARD7/ NAC	Regulator of caspase-2 activation	caspase-2, -9
Apaf-1	Regulator of caspase-9 activation	caspase-9
CARD8/ TUCAN/ CARDINAL	Negative regulator of caspase-9 and/or NF-κB activation	caspase-9
ARC	Inhibitor of caspase-8 activation	caspase-2, -8
ASC (?)	Pro-apoptotic	
CARD-adaptor family in NF-κB activation		
RIP2/ RICK/ CADIAK	NF-κB activation	cIAP-1, TRAF-1, -5, -6, Nod1, CARD8, CARD16, p75
Bcl10/ CLAP/ CIPER/ mE10/ CARMEN	NF-κB activation	CARD9, 10, 11, 12, 14
Nod1/ CARD4	NF-κB activation, caspase-9 activation	caspase-9 and RIP2
Nod2/CARD15	NF-κB activation	RIP2
CARD9	NF-κB activation	Bcl10
CARD10/ Carma3	NF-κB activation	Bcl10
CARD11/ Carma1	NF-κB activation	Bcl10
CARD14/ Carma2	NF-κB activation	Bcl10

1999; Ogura *et al.*, 2001) (Fig. 1). Nod1 and Nod2 may regulate both apoptosis and NF-κB activation (Inhara *et al.*, 1999). Nod1 bound to the prodomain of caspases and promoted caspase-9-induced apoptosis (Inhara *et al.*, 1999; Liu *et al.*, 1999). Unlike Apaf-1, however, Nod1 and Nod2 induced the activation of NF-κB through its LRR motif that interacts with the CARD-containing kinase RIP2 (Table 1). Self-association of Nod1 induced the interaction of the associated RIP2/RICK with IKK-γ, which activated NF-κB (Liu *et al.*, 1999; Ogura *et al.*, 2001).

In contrast to Nod1, the expression of Nod2 is highly restricted to monocytes. Frameshift and missense mutations of Nod2 were reported in Crohn's disease, a chronic inflammatory disorder of the gastrointestinal tract (Ogura *et al.*, 2001) and in the Blau syndrome, a second granulomatous disorder (Miceli-Richard *et al.*, 2001), respectively. Loss of the function mutation of the Nod2 gene that is found in Crohn's disease produces a truncated protein that is defective in NF-κB activation (Ogura *et al.*, 2001), indicating the important role of Nod2 in pathogenesis.

NF-κB ← Nod1, Nod2 → caspase-9

RIP2/RICK/CARDIAK RIP2 is homologous to RIP that is a component of both the TNFR-1 and CD40 signaling. RIP2 contains an N-terminal domain that is homologous to Ser/Thr kinases and a C-terminal CARD (McCarthy *et al.*, 1998) (Fig. 1). RIP2 was implicated in the activation of NF-κB and cell death. The pro-apoptotic function of RIP2 was restricted to its C-terminal CARD, whereas the intact molecule was necessary for NF-κB activation (McCrathy *et al.*, 1998). RIP2 interacts with cIAP1 and TRAFs in TNF-R1 signaling (McCarthy *et al.*, 1998) and with CLARP, a caspase-like molecule that is known to bind to FADD and caspase-8 in CD95-mediated apoptosis (Inohara *et al.*, 1998) (Table 1). RIP2 also binds to the death domain of the p75 nerve growth factor-receptor via its CARD domain in order to induce death in a subset of cell types (Khursigara *et al.*, 2001). These observations implicate that RIP2 is recruited to the receptor-signaling complexes in order to regulate the signals that lead to NF-κB activation and apoptosis (Inohara *et al.*, 1998; McCarthy *et al.*, 1998; Navas *et al.*, 1999).

NF-κB ← RIP2/RICK/CARDIAK → CLARP → caspase-8

CARD-adaptor in caspase regulation

Pseudo-ICE/COP/CARD16 and ICEBERG Pseudo-ICE and ICEBERG show a 92% and 53% sequence identity, respectively, to the prodomain of caspase-1 (Druilhe *et al.*, 2001; Lee *et al.*, 2001). Pseudo-ICE binds to both RIP2 and the prodomain of caspase-1, and inhibits RIP2-induced caspase-1 oligomerization and processing for its activation (Lee *et al.*, 2001; Druilhe *et al.*, 2001). ICEBERG is similar to Pseudo-ICE, and it prevents the association of caspase-1 with RIP2, thereby its activation (Humke *et al.*, 2000; Druilhe *et al.*, 2001). These observations indicate that Pseudo-ICE and ICEBERG are intracellular regulators that play a role in down-regulating inflammatory responses through the negative feedback loop. Furthermore, like caspase-1, Pseudo-ICE, but

not ICEBERG, interacts with the CARD-containing kinase RIP2 and activates NF-κB (Table 1). This suggests that Pseudo-ICE and ICEBERG are intracellular regulators of NF-κB activation during the inflammatory response.

NF-κB ← Pseudo-ICE/COP/CARD16, ICEBERG-1 caspase-1

CARD12/Ipaf-1/CLAN CARD12 is composed of an N-terminal CARD, NBS, and C-terminal LRR (Fig. 1). The CARD domain of CARD12 interacts selectively with the CARD domain of ASC and caspase-1. As suggested, it may also be involved in the activation of caspase-1 in response to inflammatory and apoptotic stimuli (Poyet *et al.*, 2001; Geddes *et al.*, 2001; Damiano *et al.*, 2001). This indicates that CARD12 contributes to a number of intracellular processes, including apoptosis and the inflammatory response, including cytokine processing.

CARD12/Ipaf-1/CLAN → ASC, caspase-1

DEFCAP/NAC/CARD7 Similar to Apaf-1 and Nod1, DEFCAP contains CARD and NBD, but unlike Apaf-1 and Nod1, it contains pyrim-like motif (PLM) and a proline-rich sequence (PR) at its N-terminus (Hlaing *et al.*, 2001) (Fig. 1). The PLM of DEFCAP is conserved with other apoptotic relating molecules, such as ASC. CARD of DEFCAP also interacts with caspase-2 and caspase-9. Alternative splicing of DEFCAP generates two splice-variants-long (L) and short (S) forms. Overexpression of DEFCAP-L, but not of DEFCAP-S, induced significant levels of apoptosis, whereas only the CARD or NBD of NAC suppressed Apaf-1-dependent apoptosis (Chu *et al.*, 2001).

CARD7/DEFCAP/NAC → caspase-2

CARD8/TUCAN/CARDINAL CARD8 contains a CARD motif to the C-terminus like DEFCAP and RIP2 (Bouchier-Hayes *et al.*, 2001; Pathan *et al.*, 2001) (Fig. 1). Interestingly, CARD8 failed to promote apoptosis or NF-κB activation, but rather inhibited Apaf-1/caspase-9-mediated apoptosis and NF-κB activation that is triggered by the forced-expression of TRAIL-R, RIP, Bcl10, IL-1R, or TNF-R via an interaction with IKK-γ (Bouchier-Hayes *et al.*, 2001; Pathan *et al.*, 2001). Together with the up-regulation of CARD8 in some types of cancer, CARD8 as a negative regulator of apoptosis and NF-κB activation may contribute to tumorigenesis.

NF-κB |— CARD8/TUCAN/CARDINAL —| Apaf-1/caspase-9

ARC ARC contains a N-terminal CARD that is fused to a C-terminal region that is rich in proline/glutamic acid residues (Koseki *et al.*, 1998) (Fig. 1). The expression of ARC was detected in the heart and muscle, and likely regulates cell death that is induced by a variety of death signals. ARC inhibited cell death that is induced by death receptor signaling (probably via an interaction with caspase-8) and suppressed caspase-independent events (including oxidant stress) by preserving the mitochondrial function (Neuss *et al.*, 2001).

ASC ASC is a CARD-containing proapoptotic molecule that forms insoluble aggregates during apoptosis. ASC contains a CARD motif in its C-terminal half and PLM in the N-terminus (Masumoto *et al.*, 1999; Richards *et al.*, 2001) (Fig. 1). ASC interacts with pyrin, the product of the MEFV locus of familial Mediterranean fever, whose patients suffer sporadic inflammatory attacks (Richards *et al.*, 2001). Thus, through linking pyrin to ASC-mediated apoptosis, the modulation of cell survival via ASC may be a component of familial Mediterranean fever (Richards *et al.*, 2001).

Many CARD-containing proteins have been identified from the mutation screening studies. However, the CARD-CARD binding assays and their roles in the signaling still need to be solved. Analyzing their activities in apoptosis network and NF-κB activation will be crucial to understanding the fine-tuning mechanism that regulates cell death and survival.

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