CARD10 Is a Novel Caspase Recruitment Domain/Membrane-associated Guanylate Kinase Family Member That Interacts with BCL10 and Activates NF-κB

Lin Wang‡, Yin Guo§, Wann-Jeng Huang‡, Xiaoling Ke‡, Jean-Luc Poyet§, Gulam A. Manji‡, Sarah Merriam¶, M. Alexandra Glucksmann‡, Peter S. DiStefano‡, Emad S. Alnemri§, and John Bertin‡

From ‡Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts 02139 and §The Center for Apoptosis Research and the Department of Microbiology and Immunology, Kimmel Cancer Institute, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

© 2001 by The American Society for Biochemistry and Molecular Biology, Inc.

BCL10 belongs to the caspase recruitment domain (CARD) family of proteins that regulate apoptosis and NF-κB signaling pathways. Analysis of BCL10-deficient mice has revealed that BCL10 mediates NF-κB activation by antigen receptors in B and T cells. We recently identified a subclass of CARD proteins (CARD8, CARD11, and CARD14) that may function to connect BCL10 to multiple upstream signaling pathways. We report here that CARD10 is a novel BCL10 interactor that belongs to the membrane-associated guanylate kinase family, a class of proteins that function to organize signaling complexes at plasma membranes. When expressed in cells, CARD10 binds to BCL10 and signals the activation of NF-κB through its N-terminal effector CARD domain. We propose that CARD10 functions as a molecular scaffold for the assembly of a BCL10 signaling complex that activates NF-κB.

Protein modules play an important role in the assembly of signaling proteins into specific signal transduction pathways (1). The death domain, death effector domain, caspase recruitment domain (CARD), and the recently identified PYRIN (DAPIN/PAAD) domain are protein modules found in many proteins that regulate apoptosis (2–5). The CARD domain consists of six or seven antiparallel α-helices and forms highly specific homophilic interactions between signaling partners. Thus far, 21 human CARD-containing proteins have been identified. Members of this family include caspases 1, 2, 4, 5, 9, and 13, Apaf-1, CARD4 (Nod1), Nod2, CARD7 (DEFCAP/NAC), c-IAP-1, c-IAP-2, RICK (RIP2/CARDIAK), ARC, BCL10, RAIDD, ASC, Iceberg, CARD9, CARD11, and CARD14 (6–13). CARD domains mediate the assembly of family members into discrete signaling complexes. For example, Apaf-1 and caspase-9 specifically associate with each other through their CARD domains in the presence of cytochrome c and dATP resulting in caspase oligomerization and activation (14).

Besides regulating apoptosis, numerous CARD proteins have been found to play an important role in signaling pathways that lead to the activation of NF-κB. The CARD protein RICK assembles into signaling complexes with its upstream activators CARD4 and Nod2 and activates the IKK complex through IKKγ (13, 15–17). The CARD protein BCL10 (also known as CLAP/CIPER/eE10/CARMEN) signals the activation of NF-κB and has been implicated in B cell lymphomas of mucosa-associated lymphoid tissue (18–23). Targeted disruption of its gene in mice has implicated a role for BCL10 in NF-κB activation by antigen receptors in B and T cells (24). We recently identified several CARD proteins (CARD9, CARD11, and CARD14) that function to transduce upstream signals to the activation of BCL10 (10, 11). Interestingly, CARD11 and CARD14 are members of the membrane-associated guanylate kinase (MAGUK) family that function to assemble signaling complexes at the plasma membrane. We report here that CARD10 is a novel CARD/MAGUK family member that signals the activation of NF-κB through BCL10. CARD10 and other members of the CARD/MAGUK family likely play an important role in receptor-mediated activation of BCL10 and NF-κB.

EXPERIMENTAL PROCEDURES

Expression Plasmids and Antibodies—Plasmids expressing CARD10 with C-terminal FLAG epitope were constructed using pCMV-Tag 4A (Stratagene). Constructs encoding epitope-tagged BCL10 were described previously (19). For mammalian two-hybrid assays, the pCMV-CARD10-CARD/BD plasmids were constructed by inserting the CARD domain of CARD10 (residues 1–138) into pCMV-BD (Stratagene). The panel of CARD domains used for the mammalian two-hybrid screen was described previously (10, 11). CARD7-CARD/AD contains the CARD domain (residues 1335–1429) of CARD7(3), CARD8-CARD/AD contains the CARD domain (residues 318–431) of CARD82 (accession number CA85421 (to E. S. A.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s)AY028896.‡

†To whom correspondence should be addressed: Millennium Pharmaceuticals, Inc., 640 Memorial Dr., Cambridge, MA 02139. Tel.: 617-679-7215; Fax: 617-679-7071; E-mail: bertin@mpi.com.

‡The abbreviations used are: CARD, caspase recruitment domain; MAGUK, membrane-associated guanylate kinase; CMV, cytomegalovirus; wt, wild-type; GST, glutathione S-transferase.

§This work was supported by National Institutes of Health Grant CA5421 to E. S. A.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AY028896. §

†To whom correspondence should be addressed: Millennium Pharmaceuticals, Inc., 640 Memorial Dr., Cambridge, MA 02139. Tel.: 617-679-7215; Fax: 617-679-7071; E-mail: bertin@mpi.com.

ßThe abbreviations used are: CARD, caspase recruitment domain; MAGUK, membrane-associated guanylate kinase; CMV, cytomegalovirus; wt, wild-type; GST, glutathione S-transferase.

*J. Bertin, unpublished information.
FIG. 1. The CARD domain of CARD10 interacts selectively with the CARD of BCL10 by mammalian two-hybrid analysis. A. 293T cells were transfected with the mammalian two-hybrid reporter construct pFR-Luc (Stratagene), and the CARD of CARD10 fused to the DNA-binding domain of GAL4 was screened against a panel of individual CARDs fused to the activation domain of murine NF-κB. After 24 h, cells were collected and assayed for relative luciferase activity as a measure of protein-protein interaction. B. Amino acid sequence of CARD10 (1032 residues). CARD, coiled-coil, PDZ, SH3, and GUK domains are highlighted.

FIG. 2. Domain structure and tissue distribution of human CARD10. A. Domain structure of CARD10 in comparison to CARD11, CARD14, and CARD9 (10, 11) showing CARD, coiled-coil, PDZ, SH3, and GUK domains. B. Alignment of the CARD of CARD10 (residues 31–117) with CARDs found in CARD11 (residues 19–105), CARD14 (residues 23–109), CARD9 (residues 14–100), BCL10 (residues 21–103), RAIDD (residues 9–95), and caspase-9 (residues 9–93). C. Alignment of the PDZ domain of CARD10 (residues 607–680) with PDZ domains found in CARD11 (residues 660–742), CARD14 (residues 570–653), PSD-95 (residues 355–430), ZO-1 (repeat 3; residues 411–485), and ZO-2 (repeat 3; residues 511–587). D. Alignment of the SH3 domain of CARD10 (residues 704–772) with SH3 domains found in CARD11 (residues 766–834), CARD14 (residues 676–744), PSD-95 (residues 471–540), ZO-1 (residues 504–571), and ZO-2 (residues 604–668). E. Alignment of the GUK domain of CARD10 (residues 833–1018) with GUK domains found in CARD11 (residues 954–1142), CARD14 (residues 814–999), P55 (residues 269–460), and PSD-95 (residues 564–761). F. Tissue distribution of human CARD10 transcripts. Expression was determined by Northern blot analysis using CLONTECH human multiple tissue Northern blots. PBL, peripheral blood leukocytes.
to normalize transfection efficiencies.

Co-immunoprecipitation Assays—293T cells transfected with plasmids were lysed in 50 mM Tris, pH 8.0, 120 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40 buffer and incubated with indicated antibodies. The immune complexes were precipitated with protein G-Sepharose (Amersham Pharmacia Biotech), washed extensively, and then subjected to SDS polyacrylamide gel electrophoresis and immunoblotted with polyclonal anti-FLAG (Santa Cruz Biotechnology, Inc.).

In vitro Binding Assays—In vitro binding assays between BCL10 and CARD10 proteins were performed as described previously (26). In brief, BCL10 wt and the L41R mutant were expressed in DH5-α bacteria as GST fusion proteins, and equal amounts of protein were immobilized on glutathione-Sepharose (Amersham Pharmacia Biotech). An equal amount of CARD10 protein, labeled with [35S]methionine, was incubated with the protein-bound Sepharose beads bound to an equal amount of GST (lane 2), GST-BCL10 (lane 3), and GST-BCL10-L41R (lane 4) and then analyzed by SDS polyacrylamide gel electrophoresis and autoradiography. The point mutation L41R within the CARD domain abrogates CARD-CARD interactions.

RESULTS AND DISCUSSION

We performed a mammalian two-hybrid analysis and screened Millennium Pharmaceuticals’ collection of CARD domains for a selective interaction with the CARD domain of BCL10. The CARD domain of CARD10 interacted with the CARD of BCL10 resulting in a 340-fold increase in relative luciferase activity (Fig. 1A). Co-expression of CARD10-CARD with 22 other CARD domains failed to activate luciferase expression indicating that the CARD of CARD10 interacts selectively with the CARD of BCL10. Human CARD10 is a novel CARD family member of 1032 amino acids with a predicted molecular mass of 116 kDa (Fig. 1B). Amino acid sequence analysis revealed that CARD10 has a domain structure that is
Our finding that the CARD domain of CARD10 interacts selectively with the CARD domain of BCL10 by mammalian two-hybrid analysis suggests that CARD10 is a specific signaling partner of BCL10. We therefore examined the interactions between these two proteins when overexpressed in cells. Immunoprecipitation of T7-tagged BCL10 quantitatively co-precipitated FLAG-tagged CARD10 (Fig. 3A). This interaction was dependent on the CARD domains of both proteins, because CARD10 failed to associate with a variant BCL10 with a point mutation (L41R) that disrupts CARD-mediated homodimerization (19). In addition, a CARD10 truncation mutant lacking its CARD domain failed to co-precipitate with BCL10. We next tested whether CARD10 interacts with endogenous BCL10 when overexpressed in cells (Fig. 3B). Immunoprecipitation of endogenous BCL10 co-precipitated FLAG-tagged CARD10 but not a FLAG-tagged CARD10 truncation mutant lacking its CARD domain. We also examined the interaction of radiolabeled CARD10 with GST-BCL10 in vitro and found that CARD10 associates directly with BCL10 through a CARD-CARD interaction (Fig. 3C).

Our finding that CARD10 specifically associates with BCL10 prompted us to examine whether CARD10 induces NF-κB activity, using a luciferase reporter gene system. When CARD10 was expressed in 293T cells, NF-κB activity was induced 90-fold compared with empty vector (Fig. 4A). NF-κB activity was dependent on the IKK complex, because dominant-negative versions of IKKγ and IKKβ blocked the ability of CARD10 to induce the activation of NF-κB (data not shown). To determine the role of individual domains in NF-κB signaling, we constructed a series of N- and C-terminal truncation mutants of CARD10 (Fig. 4B). The N-terminal CARD domain of CARD10 was essential for NF-κB signaling, because deletion of this domain eliminated the induction of NF-κB activity (Fig. 4C). Immunoblot analysis revealed that the truncation mutant was expressed at a level comparable with wt protein that induces NF-κB activity 30- to 40-fold (compare Fig. 4, A and C). Deletion of the C-terminal GUK domain did not interfere with the ability of CARD10 to induce NF-κB activity. However, further deletion of the SH3 and PDZ domains reduced the levels of NF-κB activity to levels 2- to 3-fold less than that obtained with wt protein. Immunoblot analysis revealed that the C-terminal truncated proteins were expressed at levels similar to each other and wt protein indicating that reductions in activity were not because of reduced levels of expression (Fig. 4C). Thus, the PDZ and SH3 domains are required for maximal activation of NF-κB activity by CARD10.

We have identified CARD10 as a specific regulator of BCL10. Our finding that CARD10 both binds to BCL10 and signals NF-κB activation through its N-terminal CARD domain suggests that this molecule functions as an upstream activator of BCL10. CARD10 is one of four CARD proteins identified thus far that assemble with BCL10 and signal the activation of NF-κB (10, 11). These molecules (CARD10, CARD9, CARD11, and CARD14) likely function to transduce distinct upstream stimuli to the activation of BCL10 and NF-κB. Interestingly, this subclass of CARD proteins is related in both sequence and structure (Fig. 2). Besides containing closely related N-terminal CARD domains that interact specifically with BCL10, each molecule contains coiled-coiled domains that could mediate self-association resulting in aggregation and activation of BCL10 in response to upstream signals. BCL10 might then engage and oligomerize IKKγ resulting in the activation of the IKK complex and NF-κB (17, 30; see Fig. 5). Thus, CARD10 and the other BCL10 activators (CARD9, CARD11, and CARD14) likely function in a manner analogous to Apaf-1 and CARD4 that induce oligomerization and activation of their respective signaling partners.

Fig. 5. Model for CARD10 activation of NF-κB. In response to upstream signals CARD9, 10, 11, and 14 self-assemble through their coiled-coil domains resulting in the aggregation and activation of BCL10. Signaling complexes at the plasma membrane (e.g., T and B cell receptors) may recruit and activate the CARD/MAGUK proteins (CARD10, 11, and 14) through their C-terminal PDZ/SH3/GUK tripartite structure found at the C terminus. This tripartite structure may recruit and activate the CARD/MAGUK proteins (CARD9 and 11) through their SH3 domain, and a GUK domain with homology to guanylate kinase. Although the CARD domain of CARD10 (residues 31–117) shows significant similarity to those found in other CARD proteins (CARD11, 9, 11, and 14), whose N-terminal CARD domains also interact selectively with the CARD activation domain of BCL10 (10, 11; see Figs. 1B and 2A). CARD10 is comprised of at least five putative functional domains, an N-terminal CARD domain, a central coiled-coil domain, and a C-terminal tripartite structure consisting of a PDZ domain, an SH3 domain, and a GUK domain with homology to guanylate kinase. Although the CARD domain of CARD10 (residues 31–117) shows significant similarity to those found in other CARD family members (Fig. 2B), it is most similar to the CARD domains of CARD11 (58% identity), CARD14 (46% identity), and CARD9 (46% identity). Adjacent to the N-terminal CARD domain are coiled-coil structures with extensive regions of heptad repeats that function in protein oligomerization and activation (27). The COILS2 program (28) predicts with a probability of greater than 70% at least four coiled-coil structures in CARD10 (residues 138–206, 210–256, 263–307, and 326–456) that are interrupted by regions with a lower coiled-coil potential. The PDZ/SH3/GUK tripartite structure found at the C terminus (Fig. 2, A, C, D, and E) classifies CARD10 as a novel member of the MAGUK family of proteins that function to organize signaling complexes at plasma membranes (29). Northern blot analysis revealed that CARD10 is expressed as a 4.4-kilobase transcript in a variety of adult tissues, including heart, kidney, and liver (Fig. 2F). CARD10 was also expressed abundantly in fetal lung, liver, and kidney tissues, as well as in multiple cancer cell lines, including HeLa S3, chronic myelogenous leukemia K562 cells, colorectal adenocarcinoma SW480 cells, and lung carcinoma A549 cells (data not shown).
downstream CARD binding partners. A unique feature of CARD10, CARD11, and CARD14 is the presence of C-terminal PDZ/SH3/GUK domains that suggest a role for these proteins in signal transduction by receptors at the plasma membrane. A recent study implicating BCL10 as a mediator of antigen receptor signaling in B and T cells (24) suggests that CARD10 and the other CARD/MAGUK family members might function to recruit BCL10 to receptor complexes.

REFERENCES

1. Pawson, T., and Nash, P. (2000) Genes Dev. 14, 1027–1047
2. Hofmann, K. (1999) Cell. Mol. Life Sci. 55, 1113–1128
3. Bertin, J., and DiStefano, P. S. (2000) Cell Death Differ. 7, 1273–1274
4. Staub, E., Dahl, E., and Rosenthal, A. (2001) Trends Biochem. Sci. 26, 83–85
5. Pawlowski, K., Pio, F., Chu, Z., Redd, J., and Godzik, A. (2000) Trends Biochem. Sci. 26, 85–87
6. Reed, J. C. (2000) Am. J. Path. 157, 1415–1430
7. Masumoto, J., Taniguchi, S., Sarvotham, H., Kishino, T., Niikawa, N., Hidaka, E., Katsuyama, T., Higuchi, T., and Sagara, J. (1999) J. Biol. Chem. 274, 9955–9961
8. Humke, E. W., Shriver, S. K., Starovasnik, M. A., Fairbrother, W. J., and Dixit, V. M. (2000) Cell 103, 99–111
9. Chu, Z. L., Pio, F., Walsh, K., Krajewska, M., Krajewski, S., Godzik, A., and Reed, J. C. (2001) J. Biol. Chem. 276, 9239–9245
10. Bertin, J., Guo, Y., Wang, L., Srinivasula, S. M., Jacobson, M. D., Poyet, J.-L., Melam, S., Du, M.-Q., Dyer, M. J. S., Robison, K. E., Distefano, P. S., and Alnemri, E. S. (2000) J. Biol. Chem. 275, 41082–41086
11. Bertin, J., Wang, L., Guo, Y., Jacobson, M. D., Poyet, J.-L., Srinivasula, S. M., Melam, S., Distefano, P. S., and Alnemri, E. S. (2001) J. Biol. Chem. 276, 11877–11882
12. Hliang, T., Guo, R. F., Dilley, K. A., Loussia, J. M., Morrish, T. A., Shi, M. M., and Ward, P. A. (2001) J. Biol. Chem. 276, 9230–9238
13. Ogura, Y., Inohara, N., Benito, A., Chen, F. F., Yamaoka, S., and Nunez, G. (2001) J. Biol. Chem. 276, 4812–4818
14. Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S. M., Ahmad, M., Alnemri, E. S., and Wang, X. (1997) Cell 91, 479–489
15. Bertin, J., Nir, W.-J., Fischer, C. M., Tayber, O. V., Errada, P. R., Grant, J. R., Keilty, J. J., Gosselin, M. L., Robison, K. E., Wong, G. H. W., Glucksmann, M. A., and DiStefano, P. S. (1999) J. Biol. Chem. 274, 12855–12858
16. Inohara, N., Koseki, T., del Peso, L., Hu, Y., Yee, C., Chen, S., Carrio, R., Merino, J., Liu, D., Ni, J., and Nunez, G. (1999) J. Biol. Chem. 274, 14560–14567
17. Inohara, N., Koseki, T., Lin, J., del Peso, L., Lucas, P. C., Chen, F. F., Ogura, Y., and Nunez, G. (2000) J. Biol. Chem. 275, 27293–27301
18. Willis, T. G., Jadayel, D. M., Du, M. Q., Peng, H., Perry, A. R., Abdul-Rauf, M., Price, H., Karran, L., Majekodunmi, O., Wlodarska, I., Pan, L., Crook, T., Hamoudi, R., Isaacson, P. G., and Dyer, M. J. (1999) Cell 96, 33–45
19. Srinivasula, S. M., Ahmad, M., Lin, J. H., Poyet, J. L., Fernandes-Alnemri, T., Tsichlis, P. N., and Alnemri, E. S. (1999) J. Biol. Chem. 274, 17946–17954
20. Koseki, T., Inohara, N., Chen, S., Carrio, R., Merino, J., Hottiger, M. O., Nabel, G. J., and Nunez, G. (1999) J. Biol. Chem. 274, 9955–9961
21. Costanza, A., Guiet, C., and Vito, P. (1999) J. Biol. Chem. 274, 20127–20132
22. Thome, M., Martinon, F., Hofmann, K., Rubio, V., Steiner, V., Schneider, P., Mattmann, C., and Tschopp, J. (1999) J. Biol. Chem. 274, 9962–9968
23. Zhang, Q., Siebert, R., Yan, M., Hinzmann, B., Cui, X., Xue, L., Rakewstraw, K. M., Naeve, C. W., Beckmann, G., Weisenburger, D. D., Sanger, W. G., Nowotny, H., Vesely, M., Callet-bauzhu, E., Salles, G., Dixit, V. M., Rosenthal, A., Schlegelberger, B., and Morris, S. W. (1999) Nat. Genet. 22, 63–68
24. Ruland, J., Duncan, G. S., Elia, A., Barrantes, I., Nguyen, L., Plyte, S., Millar, D. G., Bouchard, D., Wakeham, A., Ohashi, P. S., and Mak, T. W. (2001) Cell 104, 33–42
25. Ye, H., Dogan, A., Karran, L., Willis, T. G., Chen, L., Wlodarska, I., Dyer, M. J., Isaacson, P. G., and Du, M.-Q. (2000) Am. J. Pathol. 157, 1147–1154
26. Ahmad, M., Srinivasula, S. M., Wang, L., Talanian, R. V., Litwarska, G., Fernandes-Alnemri, T., and Alnemri, E. S. (1999) Cancer Res. 59, 615–619
27. Lupas, A. (1996) Trends Biochem. Sci. 21, 375–382
28. Lupas, A., Van Dyke, M., and Stock, J. (1991) Science 252, 1162–1164
29. Fanning, A. S., and Anderson, J. M. (1999) Curr. Opin. Cell Biol. 11, 432–439
30. Poyet, J.-L., Srinivasula, S. M., Lin, J. H., Fernandes-Alnemri, T., Yamaoka, S., Tsichlis, P. N., and Alnemri, E. S. (2000) J. Biol. Chem. 275, 37966–37977
CARD10 Is a Novel Caspase Recruitment Domain/Membrane-associated Guanylate Kinase Family Member That Interacts with BCL10 and Activates NF-κB

Lin Wang, Yin Guo, Waan-Jeng Huang, Xiaoling Ke, Jean-Luc Poyet, Gulam A. Manji, Sarah Merriam, M. Alexandra Glucksmann, Peter S. DiStefano, Emad S. Alnemri and John Bertin

J. Biol. Chem. 2001, 276:21405-21409.
doi: 10.1074/jbc.M102488200 originally published online March 20, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M102488200

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 30 references, 17 of which can be accessed free at http://www.jbc.org/content/276/24/21405.full.html#ref-list-1