Presenilin-dependent “γ-Secretase” Processing of Deleted in Colorectal Cancer (DCC)*

Received for publication, June 2, 2003, and in revised form, July 2, 2003
Published, JBC Papers in Press, July 2, 2003
DOI 10.1074/jbc.C300239200

Yoshihito Taniguchi†, Seong-Hun Kim§, and Sangram S. Sisodia¶
From the Department of Neurobiology, Pharmacology, and Physiology, The University of Chicago, Chicago, Illinois 60637

The presenilin-γ-secretase complex plays a critical role in mediating intramembranous proteolysis of several type I membrane proteins, including β-amyloid precursor protein (APP) and Notch. We now show that deleted in colorectal cancer (DCC) is subject to proteolysis within the ectodomain segment both in cultured cells and in vivo and that the residual membrane-tethered DCC “stub” is subsequently processed by γ-secretase to generate a derivative termed DCC-intracellular domain (ICD). The production of DCC-ICD is inhibited by selective γ-secretase inhibitors, and by the expression of the dominant negative PS1 D385A variant. Moreover, the membrane-tethered DCC “stubs” accumulate to high levels in PS1-deficient embryos. We also demonstrate that expression of a DCC-Gal4 chimera is capable of activating transcription in a luciferase-based reporter assay and this activity is dependent on γ-secretase activity. Our findings offer the proposal that DCC performs dual roles both as a cell surface receptor that modulates intracellular signaling pathways and as a transcriptional coactivator that relies on γ-secretase-dependent production and nuclear translocation of the cytoplasmic domain.

Presenilin 1 and 2 (PS1 and PS2)† are polytopic membrane proteins that are mutated in the vast majority of pedigrees with early-onset familial Alzheimer’s disease (1). PS plays an essential role in intramembranous, “γ-secretase” processing of several type I membrane proteins, including the β-amyloid precursor protein (APP)(2, 3), Notch1 (4, 5), ErbB-4 (6), N- and E-cadherins (7), low density lipoprotein receptor-related protein (8), CD44 (9), and nectin-1α (10). It is now well established that γ-secretase processing of transmembrane proteins is preceded by proteolysis near the interface of the ectodomain and transmembrane segments that generates a soluble ectodomain that is “shed” and one, or more, membrane-tethered derivatives. In the case of APP, a set of membrane-tethered APP derivatives, termed APP-CTFs, are the substrates for γ-secretase, and intramembranous processing leads to the production of Aβ peptides. Similarly, γ-secretase is responsible for processing of a membrane-tethered Notch1 derivative, termed S2/NEXT, resulting in the generation of a Notch derivative, termed S3/NICD that translocates to the nucleus and activates transcription of target genes (4, 11).

Intrigued by the observation that deleted in colorectal cancer (DCC) is a substrate for cleavage by a metalloprotease that leads to “shedding” of the ectodomain segment (12), we asked whether the resulting membrane-tethered “stub” might be processed by γ-secretase. In the present report, we show that in cultured mammalian cells, the truncated DCC stub is subject to a PS-dependent processing event that is inhibited by a highly potent γ-secretase inhibitor. Moreover, the DCC stub accumulates to high levels in spinal cords of PS1-deficient mice, leading us to postulate that this species is also a substrate for γ-secretase processing in vivo. Finally, we demonstrate that the γ-secretase-generated intracellular domain of DCC, termed DCC-ICD, can translocate to the nucleus and that this fragment has an intrinsic transcriptional activation domain.

EXPERIMENTAL PROCEDURES

Construct—pSecTag/A/DCC encodes c-myc-epitope tagged rat full-length DCC expression plasmid (Fig. 1B). To generate pCMVneo/DCC-GIC, pCMVneo/DCC (13) was partially digested with BstXI and the fragment encoding Gal4 DNA-binding domain was inserted into the BstXI site located between the transmembrane region and the conserved P1 domain of DCC intracellular domain. The V1117A and V1119A mutant DCC constructs were generated by overlapping PCR-based mutagenesis (14) using wild type pSecTag/A/DCC as a template. The sequences of the constructs were verified by DNA sequencing.

Cell Culture and Transfection—HEK293 or N2a cells were transfected using LipofectAMINE Plus (Invitrogen). 24 h after transfection, the cells were treated with vehicle, 50 μM compound E, 10 μM lactacystin or both compound E and lactacystin for additional 24 h. Detergent lysates were prepared as described previously (14) and analyzed by immunoblotting with the 9E10 antibody. For luciferase assays, cells were transfected with 0.5 μg of expression plasmid, 167 ng of pGSE1B-luc (15), and 17 ng of pRL-SV40 Renilla luciferase reporter gene (Promega). Transcriptional activities were determined 24 h after transfection using dual luciferase reporter assay system (Promega).

RESULTS

PS-dependent Cleavage of DCC Protein—DCC, first isolated as a gene deleted in colorectal cancer (13), encodes a type I integral membrane protein of 1,445 amino acids that is a receptor for the axonal chemotractant, termed netrin-1 (16). DCC is subject to a metalloprotease-dependent ectodomain shedding event (12). Like DCC, Notch1 is also subject to ectodomain shedding (17, 18), and in this case, the residual membrane-tethered stub, termed S2/NEXT, is subject to an intramembranous, presenilin-dependent, γ-secretase processing reaction that leads to the production of cytosolic derivatives termed S3/NICD (11). A valine residue at the P1 site is essential for γ-secretase-dependent processing of Notch (11), and a valine residue occurs at a similar position within the trans-
membrane domains of other γ-secretase substrates (Fig. 1A). Intrigued by the finding that DCC is subject to proteolysis in the ectodomain, coupled with the presence of several valine residues within the transmembrane domain proximal to the cytoplasmic segment, we asked whether DCC might also serve as a γ-secretase substrate.

We transiently expressed a myc epitope-tagged full-length rat DCC (pSecTag-DCC; Fig. 1B) into HEK293 cells, then treated cells with 0.5% MeSO (vehicle), or compound E (19), a highly potent inhibitor of γ-secretase. Treatment of the cells with 50 μM compound E resulted in the accumulation of ~56–58 kDa, myc antibody-reactive fragments that we refer to as "α" fragments (Fig. 2A, lane 3). As the DCC cytoplasmic tail is degraded by the proteasome (20), we treated cells with the proteasome inhibitor, lactacystin. Lactacystin treatment of DCC-expressing cells resulted in the accumulation of closely spaced fragments with an apparent molecular weight of between 48–52 kDa (Fig. 2A, lane 4). By virtue of the fact that these fragments accumulate to high levels with lactacystin treatment and are not present in cells treated with the γ-secretase inhibitor, we refer to these γ-secretase-generated, DCC derivatives as DCC-ICD fragments. Treatment with both compound E and lactacystin resulted in the accumulation of an α but not DCC-ICD fragments, as expected (Fig. 2A, lane 5). Lactacystin treatment also resulted in the accumulation of an ~20-kDa myc antibody-reactive fragment (Fig. 2A, lanes 4 and 5), the production of which was fully abolished upon treatment of cells with the broad spectrum caspase inhibitor, z-Val-Ala-Asp-fluoromethylketone (data not shown).

To determine whether γ-secretase cleavage of DCC occurs in an in vivo setting, we performed Western blot analysis of detergent extracts prepared from the spinal cords of E14.5 wild type and PS1-deficient (22) mouse embryos. We observed a prominent species of ~180 kDa, representing full-length DCC in the spinal cords from wild type embryos (Fig. 2B, lanes 1 and 2). In contrast, we observed ~54–56-kDa fragments that accumulated in extracts from PS1-deficient mice (Fig. 2B, lanes 3 and 4) that are reminiscent of the α fragments that accumulate in cultured cells treated with the γ-secretase inhibitor (Fig. 2A, lanes 3 and 5). Although we failed to detect a band correspond-
the transcriptional stimulatory activity of DCC is likely mediated by limiting levels of DCC-ICD in the nucleus.

**DISCUSSION**

Presenilins play an essential role in the intramembranous "γ-secretase" processing of membrane-tethered stubs of several type I membrane proteins, including the APP/APLP (2, 3), Notch receptors (4, 5), ErbB-4 (6), E-cadherin (7), low density lipoprotein receptor-related protein (8), CD44 (9), and nectin-1α (10). The finding that the netrin 1 receptor, DCC, is subject to a metalloprotease-mediated ectodomain shedding event (12), and the presence of several valine residues in the DCC transmembrane domain, led us to hypothesize that this molecule might also serve as substrate for γ-secretase. In the present report, we confirm this prediction and offer several insights relevant to the molecular apparatus responsible for intramembranous processing of DCC and the potential functional significance of this processing event.

First, we demonstrate that in cultured mammalian cells, the production of the γ-secretase-generated cytoplasmic derivative of DCC, termed DCC-ICD, is inhibited by a highly potent inhibitor of γ-secretase activity, or by the expression of the dominant-negative D385A PS1 mutant, but not by mutations at the conserved valine residues in the DCC transmembrane domain of DCC. The PS-dependent γ-secretase generated DCC-ICD fragments that are highly labile and only detected by the addition of the proteosomal inhibitor, lactacystin. The identity of the amino termini of these fragments is not known. However, it seems somewhat unlikely that the fragments are all generated by intramembranous cleavage events as the difference in molecular weights of the fragments are too broad. Thus, we sus-

![Presenilin-dependent Cleavage of DCC](image)

**FIG. 2.** PS-dependent cleavage of DCC protein. A, HEK293 cells were transfected with pSecTagA/DCC. 24 h later, cells were treated with 10 μM lactacystin and/or 50 nM compound E for an additional 24 h. Detergent lysates were analyzed by immunoblotting with myc-specific 9E10 antibody. B, protein extracts from the spinal cords of PS1-deficient embryos (KO, lanes 3 and 4) or their wild type littermates (WT, lanes 1 and 2) were analyzed by immunoblotting with the DCC intracellular domain-specific G97-449 monoclonal antibody. Each lane represents protein extracts from different embryos. High levels of a fragment of DCC are apparent in PS1-deficient embryos. C, N2a neuroblastoma cells stably expressing wild type (lanes 1–5) or D385A mutant PS1 (lanes 6–10) were transiently transfected with pSecTagA/DCC. The cells were incubated with 10 μM lactacystin and/or 50 nM compound E, and DCC-related polypeptides were analyzed as described in A. D, HEK293 cells were transiently transfected with wild type (lanes 1–4), V1117A (lanes 5–8), or V1119A mutant DCC (lanes 9–12) 24 h after transfection, the cells were treated for additional 24 h with vehicle (lanes 1, 5, and 9), 50 nM compound E (lanes 2, 6, and 10), 10 μM lactacystin (lanes 3, 7, and 11), or both compound E and lactacystin (lanes 4, 8, and 12). Detergent lysates were analyzed by immunoblotting with the 9E10 antibody. FL, full-length DCC; α, membrane-tethered α fragment; γ, DCC-ICD fragments generated by γ-secretase; caspase, COOH-terminal half of DCC-ICD fragment generated by caspase. α and DCC-ICD fragments are also marked by asterisks and circles, respectively.

![γ-Secretase-generated DCC-ICD modulates nuclear transcriptional activity](image)

**FIG. 3.** γ-Secretase-generated DCC-ICD modulates nuclear transcriptional activity. A, HEK293 cells were transfected with pCMVneo, pCMVneo/DCC, or pCMVneo/DCC-GIC and pG5E1B-luc reporter plasmid. The pRL-SV40 Renilla luciferase vector was included as a transfection efficiency control. 3 h after transfection, 500 μM compound 1, 50 nM compound E, or 0.1% (v/v) Me₂SO were added to the medium. Relative luciferase activity was detected 24 h after transfection and expressed as fold induction over vector control. Experiments were carried out in triplicate and the standard errors are shown. B, Western blot analysis of detergent lysates from HEK293 cells expressing DCC-GIC treated with vehicle (lane 1), lactacystin (lane 2), or compound E (lane 3). FL, full-length DCC-GIC; α, a fragment of DCC-GIC; γ, the intracellular domain of DCC-GIC generated by γ-secretase.
pect that differing levels of posttranslational modifications such as phosphorylation or ubiquitination contribute to the differing mobilities of the fragments.

Second, DCC appears to be a *bona fide* substrate for an ectodomain shedding activity in vivo by virtue of the fact that a membrane tethered α derivative accumulates to high levels in spinal cords of mice with genetic ablations of PS1. Because we have failed to detect DCC-ICD in spinal cords of wild type embryos, we can only hypothesize that the α fragment is a substrate for γ-secretase. Indeed, the absence of detectable DCC-ICD in wild type embryos and accumulation of DCC α derivatives in spinal cords from PS1-deficient embryos is very similar to our earlier findings that APP-derived AICD is undetectable in brains of wild type embryos, while α- and β-secretase-generated APP-CTFs accumulate in brains of PS1-deficient embryos (3). Interestingly, our preliminary immuno-cytochemical studies have revealed that commissural axon projections in the developing spinal cord are highly disorganized. It is not clear whether the disorganization of commissural axons is a reflection of defects in γ-secretase processing of DCC, alone, or deficits in intramembranous processing of other axonal molecules involved in migration and cell adhesion, including nectin-1α and N-cadherins.

Third, we examined the hypothesis that the γ-secretase-generated DCC-ICD fragments are translocated to the nucleus and activate transcription of a reporter gene. We show that a chimera in which the Gal4 DNA-binding domain is inserted between the DCC transmembrane and cytoplasmic domains is competent to activate transcription of a promoter containing Gal4 binding sites and that the transcriptional activation by DCC was largely blocked by γ-secretase inhibitors. These results indicate that the intracellular domain of DCC acts as a transcriptional activator in mammalian cells and that γ-secretase plays an essential role in the regulation of DCC-dependent transcription. At present, the identity of the factors that regulate translocation and transcriptional activity of DCC-ICD is not known. Future efforts to characterize these factors and the downstream target genes may offer new insights into the signaling pathways responsible for mediating DCC-regulated axonal guidance during development and cell cycle regulation in neoplasias.

Acknowledgments—We are grateful to Drs. Yimin Zou and Lora Hedrick for providing pCSETagADCC and pCMVneo/DCC vectors, respectively. We also thank Dr. Todd Golde (Mayo Clinic, Jacksonville, FL) for providing compound E.

REFERENCES

1. Price, D. L., and Sisodia, S. S. (1998) *Annu. Rev. Neurosci.* 21, 479–505
2. De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Sudhof, T. C., Annauert, W., Van Figura, K., and Van Leuven, F. (1998) *Nature* 391, 387–390
3. Naruse, S., Thinakaran, G., Luo, J. J., Kusiak, J. W., Tomita, T., Iwatsubo, T., Qian, X., Ginty, D. D., Price, D. L., Borchelt, D. R., Wong, P. C., and Sisodia, S. S. (1998) *Neuron* 21, 1213–1221
4. De Strooper, B., Annauert, W., Cuper, P., Saftig, P., Craessaerts, K., Mumm, J. S., Schroeter, E. H., Schrijvers, W., Welle, M. S., Ray, W. J., Geate, A., and Kopan, R. (1999) *Nature* 398, 518–522
5. Struhl, G., and Greenwald, I. (1999) *Nature* 386, 522–525
6. Ni, C. Y., Murphy, M. P., Golde, T. E., and Carpenter, G. (2001) *Science* 294, 2179–2181
7. Marambaud, P., Shioz, J., Serhan, G., Georgakopoulos, A., Serger, N., Nagy, V., Baki, L., Wen, P.,Efthimiopoulos, S., Shao, Z., Wiesiewski, T., and Robakis, N. K. (2002) *EMBO J.* 21, 1948–1956
8. May, P., Reddy, Y. K., and Herz, J. (2002) *J. Biol. Chem.* 277, 18736–18743
9. Lammich, S., Okochi, M., Takeda, M., Kaether, C., Capell, A., Zimmer, A. K., Edsauer, D., Walter, J., Steiner, H., and Haass, C. (2002) *J. Biol. Chem.* 277, 44764–44779
10. Kim, D. Y., Ingano, L. A., and Kuvacs, D. M. (2002) *J. Biol. Chem.* 277, 49976–49981
11. Schroeter, E. H., Kisslinger, J. A., and Kopan, R. (1998) *Nature* 393, 382–386
12. Galko, M. J., and Tessier-Lavigne, M. (2000) *Science* 289, 1365–1367
13. Hedrick, L., Cho, K. R., Fearon, E. R., Wu, T. C., Kinzler, K. W., and Vagelstein, B. (1994) *Genes Dev.* 8, 1174–1183
14. Kim, S. H., Leem, J. Y., Lab, J. J., Shunt, H. L., Levey, A. I., Thinakaran, G., and Sisodia, S. S. (2001) *J. Biol. Chem.* 276, 43343–43350
15. Cao, X., and Sudhof, T. C. (2001) *Science* 293, 115–120
16. Keino-Masu, K., Maus, M., Hinck, L., Leonardo, E. D., Chan, S. N., Culotti, J. G., and Tessier-Lavigne, M. (1996) *Cell* 87, 175–185
17. Mumm, J. S., Schroeter, E. H., Saxena, M. T., Griesemer, A., Tian, X., Pan, D. J., Ray, W. J., and Kopan, R. (2000) *Mol. Cell* 5, 197–206
18. Brou, C., Logeat, F., Gupta, N., Bessia, C., LeBail, O., Daoen, J. R., Cunamo, A., Roux, P., Black, R. A., and Israel, A. (2000) *Mol. Cell* 5, 207–216
19. Seiffert, D., Bradley, J. D., Rominger, C. M., Rominger, D. H., Yang, F., Meredith, J. E., Jr., Wang, Q., Roach, A. H., Thompson, I. A., Spitz, S. M., Higaki, J. N., Prakash, S., Combs, A. P., Copeland, R. A., Arneric, S. P., Hartig, P. R., Robertson, D. W., Cordell, B., Stern, A. M., Olson, R. E., and Zaczek, R. (2000) *J. Biol. Chem.* 275, 34086–34091
20. Hu, G., Zhang, S., Vidal, M., Baer, J. L., Xu, T., and Fearon, E. R. (1997) *Genes Dev.* 11, 2701–2714
21. Huppert, S. S., Le, A., Schroeter, E. H., Mumm, J. S., Saxena, M. T., Milner, L. A., and Kopan, R. (2000) *Nature* 405, 966–970
22. Wong, P. C., Zheng, H., Chen, H., Becher, M. W., Sirinathsinghji, D. J., Trumbauer, M. E., Chen, H. Y., Price, D. L., Van der Ploeg, L. H., and Sisodia, S. S. (1997) *Nature* 387, 288–292
23. Baeke, S. H., Ohg, K. A., Rose, D. W., Koo, E. H., Glass, C. K., and Rosenfeld, M. G. (2002) *Cell* 110, 55–67
24. Tian, G., Sobotka-Briner, C. D., Zyk, J., Liu, X., BIRR, C., Sylvestre, M. A., Edwards, P. D., Scott, C. D., and Greenberg, B. D. (2002) *J. Biol. Chem.* 277, 31499–31505
Presenilin-dependent "γ-Secretase" Processing of Deleted in Colorectal Cancer (DCC)
Yoshihito Taniguchi, Seong-Hun Kim and Sangram S. Sisodia

J. Biol. Chem. 2003, 278:30425-30428.
doi: 10.1074/jbc.C300239200 originally published online July 2, 2003

Access the most updated version of this article at doi: 10.1074/jbc.C300239200

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 24 references, 12 of which can be accessed free at http://www.jbc.org/content/278/33/30425.full.html#ref-list-1