

# Cancer Cells Cut Homophilic Cell Adhesion Molecules and Run

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## Abstract

The term contact inhibition (CI) encompasses the cellular changes that result in cessation of cell migration and of proliferation due to signals transduced when one cell comes into physical contact with another cell. Cancer cells, however, do not contact inhibit. A molecular understanding of the loss of CI in cancer cells is important for understanding tumor progression. In this *Perspective*, we propose that the loss of CI observed in cancer cells is the result of extracellular proteolysis of transmembrane cell–cell adhesion molecules (CAM) in the tumor microenvironment. Proteolysis of homophilic cell–cell CAMs results in a shed extracellular fragment and released cytoplasmic fragment(s) that disrupts adhesion and induces signals that promote proliferation and/or migration. The importance of this observation in tumor progression is supported by the presence of the shed extracellular fragments of homophilic cell–cell CAMs in serum and tumor tissue of cancer patients suggesting that instead of acting as tumor suppressors, the shed CAM extracellular and cytoplasmic fragments actually function as oncogenes. The study of cell–cell CAM cleavage will provide important and novel means of diagnosing, imaging, and treating tumor progression. *Cancer Res*; 71(2); 303–9. ©2010 AACR.

## Introduction

Normal cells grown in culture are inhibited or restricted from migration and/or proliferation by adhering to neighboring cells creating monolayers in culture (1). This phenomenon, known as contact inhibition (CI), was first observed in cells taken from chick embryo ventricles in 1953 (2). As early as 1957, it was recognized that cells derived from tumor tissue did not contact inhibit (3). CI of migration (CIM) describes the cessation of cell movement when a cell comes into contact with another cell. CI of proliferation (CIP) is a separate phenomenon that describes the cessation of cell division following cell–cell contact (1). The observation that tumor-derived cells do not contact inhibit (neither CIP nor CIM) led to the hypothesis that one step in tumor progression is the loss of cell–cell adhesion. Because loss of CI is one of the first observable steps in cancer progression, it is likely to be a key event. In the years since these observations, a number of proteins involved in promoting cell adhesion, known as cell adhesion molecules (CAM; ref. 1) have been identified.

CAMs are subdivided into 4 superfamilies: cadherin, immunoglobulin (Ig), integrin, and selectin. Members of all CAM superfamilies can mediate cell–cell interactions, whereas

integrins alone mediate cell–matrix interactions. CAMs bind to either the exact same proteins, known as homophilic binding, or different proteins, called heterophilic binding.

Many CAMs, including E-cadherin, cell–cell adhesion molecule 1 (C-CAM1) and deleted in colorectal cancer (DCC) are putative tumor suppressor genes (4). Genetic loss or DNA hypermethylation results in reduced CAM expression, and is one possible explanation for the loss of CI in cancer (4).

Posttranslational changes, such as proteolysis, of CAMs has been observed and may also result in the loss of CI observed in cancer. Cleavage of many CAMs resembles that of the Notch receptor following ligand binding (5). Three cleavage events occur in the processing of Notch, identified as S1, S2, and S3 (Fig. 1). S1 cleavage is attributed to a furin-like convertase in the *trans*-Golgi. S2 occurs in response to ligand binding and is mediated by a disintegrin and metalloprotease (ADAM). ADAM cleavage results in the shedding of the extracellular domain (ECD) of the Notch receptor. S3 is mediated by the  $\gamma$ -secretase complex and cleaves Notch within its transmembrane domain, thereby releasing its cytoplasmic fragment to translocate to the nucleus (5).  $\gamma$ -Secretase consists of 4 proteins at any one time: the protease, either presenilin-1 (PS-1) or presenilin-2 (PS-2), and 3 other essential members of the complex, nicastrin, Aph-1, and Pen-2 (5). This same cleavage paradigm has been observed for other transmembrane proteins, including cell–cell CAMs (6).

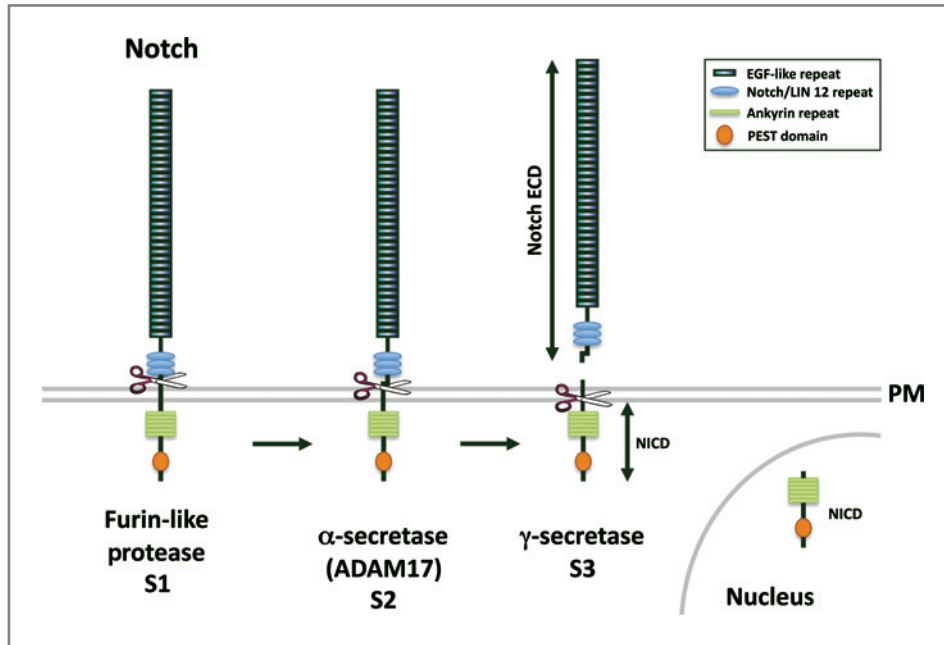
We suggest 4 potential mechanisms whereby cleavage of CAMs could alter their biological function and result in the loss of CI. First, the cleavage and shedding of the ectodomain (ECD) of cell–cell CAMs can reduce cell–cell adhesion and promote proliferation and/or migration due to its release from the plasma membrane, potentially antagonizing cell–cell adhesion by "occupying" the transmembrane receptor (7).

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**doi:** 10.1158/0008-5472.CAN-10-2301

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**Figure 1.** Notch cleavage. The Notch receptor is cleaved in a 3-step process to yield a functional signaling protein. S1 cleavage occurs in the Golgi by a furin-like protease. S2, or  $\alpha$ -secretase, cleavage by ADAM17, occurs on the extracellular face of the plasma membrane (PM), and produces the shed ECD of Notch. S3 cleavage by the  $\gamma$ -secretase complex occurs in the transmembrane domain that releases the Notch ICD (NICD), which is able to translocate to the nucleus.

Second, the shed fragment may associate with integrins and/or components of the extracellular matrix and form a new molecular substrate for cell migration (8, 9). Third, the shed ECD may bind to different receptors on other cells and activate distinct signals that regulate either proliferation or migration (9, 10). Finally, the released cytoplasmic fragment (ICD) may not associate with its normal signaling partners and may instead activate novel signals or potentiate normal signals that regulate either proliferation or migration when not anchored to the plasma membrane (11).

We propose a new theory to explain the loss of CI in tumor progression, in which the cleavage of homophilic cell-cell CAMs deregulates CI by interfering with stable cell-cell adhesion and/or activating signals that promote cell proliferation and/or migration. We only consider homophilic cell-cell CAMs in this *Perspective* because cell-extracellular matrix CAMs are not involved in mediating inhibition of growth and migration initiated by cell-cell contact. We will review the 4 best examples of homophilic cell-cell CAM cleavage in cancer: E-cadherin, N-cadherin, epithelial cell adhesion molecule (EpCAM), and PTP $\mu$  subfamily receptor protein tyrosine phosphatases (RPTP). Table 1 lists what is known about the cleavage and shedding of other homophilic cell-cell CAMs that may also disrupt CI. When considered altogether, there is a large body of evidence that supports our theory that disruption of stable cell-cell adhesion and adhesive signaling through homophilic cell-cell CAM proteolysis may be the key to explaining the loss of CI observed in cancer.

### E-cadherin

Cadherins are calcium-dependent cell-cell adhesion molecules. All classical or type I cadherins share the same domain structure: 5 extracellular cadherin domains linked to conserved cytoplasmic tails. Like other classical cadherins, E-

cadherin stabilizes cell-cell adhesions following homotypic binding by interacting with catenins and the actin cytoskeleton.

A cleaved soluble form of the ECD of E-cadherin, termed soluble E-cadherin (sE-cad) is generated by an  $\alpha$ -secretase cleavage mechanism on the extracellular face of the plasma membrane (Fig. 2). A handful of proteases are capable of mediating this cleavage event, including plasmin, MMP-3, MMP-7, ADAM-10, and ADAM-15 (7, 12–16). At least 3 cytoplasmic fragments of E-cadherin are also generated by separate proteolytic events (Fig. 2; refs. 11, 17–19). The membrane-associated fragment, E-cad/CTF1, is produced by the  $\alpha$ -secretase cleavage that generates sE-cad (15, 17, 19). The 33-kDa E-cad/CTF2 is produced by  $\gamma$ -secretase cleavage. E-cad/CTF2 is released into the cytosol (17, 19). E-cad/CTF3 is produced by caspase-3 cleavage to yield a 29-kDa fragment that is also found in the cytoplasm (17, 18).

Cleavage of E-cadherin to yield sE-cad produces a fragment capable of homophilic binding that is no longer associated with the membrane. This fragment prevents cell-cell aggregation (7, 12, 20), induces cell invasion (7, 12, 14, 21), and promotes epithelial to mesenchymal transition (14). Stimulation of cells with an sE-cad-Fc chimera and/or the presence of sE-cad may promote cell proliferation (10, 15). The mechanism by which sE-cad is capable of disrupting cell-cell adhesion may be due to antagonizing cell-surface-bound E-cadherin in a paracrine manner (7, 20). Recent data suggests that sE-cad may also antagonize CIM and CIP by stimulating the phosphorylation of ERK through heterodimerization and activation of the ErbB receptors HER2 and HER3 (10). Increased ERK activity may account for the increased motility and proliferation observed in cells with elevated sE-cad. In addition, stimulation of cells with sE-cad conditioned media can also induce MMP expression in the same cells (22), thereby promoting migration. To

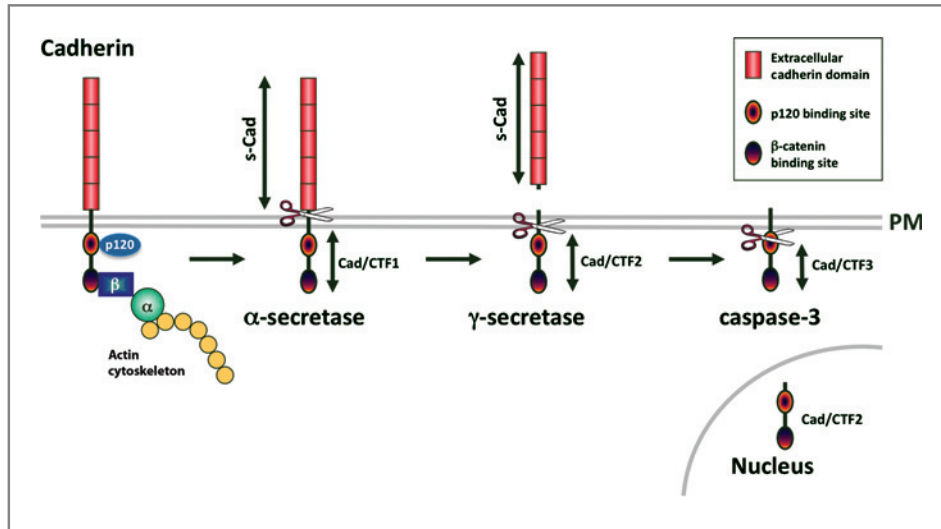
Table 1. Summary of Cell-Cell CAM Cleavage in Cancer

Cell-cell CAM	ECD protease	ICD protease	Location of ICD	Evidence for role of cleavage in cancer	References
E-cadherin	Plasmin, MMP-3, MMP-7, ADAM10, ADAM17	PS-1/2	Cytoplasm, nucleus	High levels in serum and urine of cancer patients; cleavage promotes cell migration	7, 10–25
N-cadherin	ADAM 10, MT1-MMP, MT5-MMP	PS-1	Cytoplasm, not nucleus	High levels in serum of cancer patients; cleavage promotes migration of GBM cells, stimulates angiogenesis <i>in vivo</i>	26–33
P-cadherin	MMP-1, MMP-2	Unknown	Unknown	Stimulation with sP-cad promotes breast cancer cell invasion	42
Protocadherin-γ3	MMP	PS-1/2	Cytoplasm, nucleus	Shedding reduces cell–cell aggregation	43, 44
PTPμ	ADAM/MMP	PS-1/2	Cytoplasm, nucleus	Present in glioblastoma multiforme tissue not in normal brain tissue; PTPμ-ICD promotes cell migration, growth factor independent survival and anchorage independent cell growth	39, 40
PTPκ	ADAM10	PS-1	Cytoplasm, nucleus	Cleaved in breast cancer cells, results in dissolution of Adherens junctions	38
LAR	ADAM17	PS-1/2	Cytoplasm, nucleus	Shed in breast cancer cells, disrupts Adherens junctions	45, 46
L1	ADAM10	PS-1	Cytoplasm, nucleus	Soluble L1 in uterine and ovarian carcinoma tissue; shed L1 promotes adhesion to ECM and migration	8, 47, 48
EpCAM	ADAM17	PS-2	Cytoplasm, nucleus	EpCAM-ICD promotes cell proliferation, subcutaneously injected HEK293 cells expressing EpCAM-ICD induces tumor formation in mouse flank model	36
NrCAM	ADAM/MMP	Unknown	Unknown	NrCAM-Fc promotes proliferation and migration; NrCAM ECD is capable of inducing tumor formation in mouse flank model.	9

summarize, the literature supports our assertion that sE-cad can interfere with CIM and CIP.

Cleavage of E-cadherin also results in the dissolution of cadherin-based adherens junctions and, in the case of E-cad/CTF1 and CTF2 production, nuclear translocation of  $\beta$ -catenin (15, 17, 19), thereby potentiating Wnt signaling. Unlike full-length E-cadherin, E-cad/CTF2 itself is capable of translocating to the nucleus in association with p120 (11). p120 binds Kaiso and blocks its ability to repress transcription. When in a complex with p120, E-cad/CTF2 enhances the ability of p120 to block Kaiso-mediated gene repression (11). Enhanced transcription of

the *matrilysin (MMP7)* gene results from the nuclear translocation of the p120-E-cad/CTF2 complex, which can further promote E-cadherin cleavage. Nuclear translocation of p120-E-cad/CTF2 also prevents staurosporine-induced apoptosis (11). Therefore, the sum of the literature on E-cadherin proteolysis suggests that E-cadherin cleavage may interfere with CIM by antagonizing stable cell–cell adhesion via its shed sE-cad (7, 12, 20) and by the dissolution of cadherin-catenin–based adherens junction as a result of E-cad/CTF fragment generation. E-cadherin cleavage may also disrupt CIP via the activation of signals downstream of receptor tyrosine kinases, ERK, and  $\beta$ -catenin.



**Figure 2. Cadherin cleavage.** Classical cadherins are linked to the actin cytoskeleton via  $\beta$ -catenin ( $\beta$ ) and  $\alpha$ -catenin ( $\alpha$ ). E- and N-cadherin cleavage by an  $\alpha$ -secretase produces the shed s-Cad subunit and a membrane tethered Cad/CTF1. Subsequent  $\gamma$ -secretase cleavage produces Cad/CTF2 that is released from the plasma membrane (PM) and is capable of translocating to the nucleus. Caspase 3 cleavage generates an even smaller fragment of E-cadherin, Cad/CTF3.

The presence of sE-cad in human tumor cell lines and in the serum of cancer patients has been frequently observed (e.g., ref. 23, 24). Circulating sE-cad is correlated with the development of distant metastases (25). Although the presence of sE-cad in human serum has not yet been shown to be causative in antagonizing CIM, it is supportive of our hypothesis that increased cell-cell CAM cleavage occurs during tumor progression. Cytoplasmic fragments of E-cadherin have also been observed in breast, colon, esophageal, and epidermal carcinoma cell lines (11, 18, 19).

**N-cadherin**

As with E-cadherin, N-cadherin is first cleaved by an extracellular metalloprotease to yield a 95-kDa soluble N-cadherin fragment (sN-cad) and a 40-kDa membrane-tethered N-cad/CTF1 fragment (Fig. 2; ref. 26). Proteases capable of sN-cad production are ADAM10 (27), ADAM15 (28), MT1-MMP (29), and/or MT5-MMP (30). ECD shedding and PS-1 activity are required to produce the 35-kDa N-cad/CTF2 fragment (26). N-cad/CTF2 binds to the CREB transcription factor and promotes its degradation, thereby decreasing CREB-mediated transcription (26). In addition, the cleavage of the cytoplasmic domain of N-cadherin increases  $\beta$ -catenin-mediated transcription, which could be linked to CIP (27).

Studies of N-cadherin proteolysis suggest that the shed form of N-cadherin may also antagonize CIM. Using either a peptide containing the N-cadherin HAV motif necessary for N-cadherin-mediated adhesion or purified sN-cad, Derycke and colleagues (31) showed that sN-cad stimulates the migration of human endothelial pSV-1 cells and stimulates angiogenesis *in vivo*. Using siRNA directed against ADAM-10 or a cleavage site mutant of N-cadherin, Kohutek et al. (32) observed a significant reduction in the transwell migration of human glioblastoma cells. Clinical significance to N-cadherin cleavage in cancer is supported by the high levels of sN-cad found in the serum of cancer patients compared with normal controls (33).

**EpCAM**

EpCAM is an intriguing homophilic cell-cell CAM, as it mediates homophilic cell-cell aggregation, but can also interfere with cadherin-mediated adhesion (34). Structurally, EpCAM is a type I membrane protein composed of an extracellular epidermal growth factor-like domain and a thyroglobulin-like domain fused to a short cytoplasmic domain with an NPXY internalization motif and 2  $\alpha$ -actinin binding sites (35). It does not fit into a canonical CAM superfamily.

EpCAM is a marker of various carcinomas (35). Recently, it has been suggested that proteolysis of EpCAM may be responsible for its ability to promote cell proliferation (36). Like the cadherins, EpCAM is cleaved by an ADAM, ADAM17, to yield Ep-ECD, and then it is cleaved by the  $\gamma$ -secretase/PS-2 complex to yield Ep-ICD (36). Importantly, EpICD is localized to the cytoplasm and nucleus in colon carcinoma tissue and cells, whereas full-length EpCAM is localized to the plasma membrane at cell junctions (36). Ep-ICD, but not full-length EpCAM, promotes cell proliferation, as assayed by cell counts and Ki67 labeling. This likely occurs through the interaction of Ep-ICD in the nucleus with ‘four and a half LIM domain’ protein 2 (FHL2), the Lef1 transcription factor and  $\beta$ -catenin to induce transcription of the c-myc gene. siRNA of EpCAM reduces the levels of c-myc protein and cell proliferation. C-myc expression and cell proliferation can be fully rescued by the reexpression of Ep-ICD. Finally, subcutaneous injection of HEK293 cells expressing EpCAM-YFP or Ep-ICD-YFP into mouse flank is capable of inducing tumor formation (36). Together, these data show that EpICD can promote cell proliferation and tumorigenesis and may thus be involved in the deregulation of CIP in tumor progression.

**PTP $\mu$  subfamily of RPTPs**

RPTPs differ from most CAMs in that they have catalytic tyrosine phosphatase domains in their intracellular segments in addition to extracellular segments capable of mediating

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cell–cell adhesion. RPTPs are classified on the basis of their ECD structure (37). The greatest evidence exists for the proteolysis of the type IIb PTP $\mu$  subfamily members in the loss of CI, although there is evidence for cleavage and shedding of the type IIa subfamily member, LAR, in cancer progression (Table 1).

The PTP $\mu$  family of homophilic cell–cell CAMs is characterized by a meprin-A5 (neuropilin)-mu (MAM) domain, 1 Ig domain, and 4 fibronectin III (FNIII) repeats in its ECD. ECD shedding and ICD cleavage has been observed for PTP $\kappa$  (38) and PTP $\mu$  (39). Like the other CAMs discussed (Fig. 2), the ECDs of these RPTPs are cut by ADAM-like metalloproteases before being cleaved by the  $\gamma$ -secretase complex (38, 39). Both PTP $\kappa$ - and PTP $\mu$ -ICDs translocate to the nucleus (38, 39). Although both full-length PTP $\kappa$  and PTP $\kappa$ -ICD dephosphorylate  $\beta$ -catenin, dephosphorylation of  $\beta$ -catenin by PTP $\kappa$ -ICD in the nucleus promotes TCF-mediated transcription, whereas full-length, transmembrane PTP $\kappa$  does not (38). By promoting  $\beta$ -catenin/TCF signaling, we suggest that cleavage of PTP $\kappa$  may lead to the loss of CIP.

Cleavage of PTP $\mu$  occurs preferentially in tumor-derived tissue and cancer cell lines (refs. 39, 40; S.M.B.-K., unpublished observations). For example, full-length PTP $\mu$  is downregulated in glioblastoma multiforme (GBM; ref. 41) via proteolysis (39, 40). ECD and ICD fragments of PTP $\mu$  are present only in GBM tissue compared with the surrounding normal tissue. Notably, PTP $\mu$  cleavage to yield PTP $\mu$ -ICD promotes GBM cell migration, growth factor independent survival and anchorage independent growth (39). The ability of PTP $\mu$ -ICD to promote GBM cell migration depends on tyrosine phosphatase activity of the ICD fragment (39). Furthermore, studies of PTP $\mu$  cleavage show that PTP $\mu$  proteolysis and the presence of the PTP $\mu$ -ICD fragment directly result in more migratory cells, that is, the loss of CIM.

Importantly, the shed ECD of PTP $\mu$  remains in the vicinity of the GBM tumor (40). Fluorescently tagged peptides that bind to PTP $\mu$ -ECD injected intravenously in mouse xenograft tumor models were effective at demarcating the main tumor and its edges in both mouse flank tumors and intracranial glioma tumors (40). This shows that shedding of PTP $\mu$ -ECD occurs preferentially in the tumor microenvironment and can be utilized as a novel means of identifying the tumor and its margin.

## Conclusions

The ability of cells to recognize one another and cease growth and/or migration as a result of cell–cell contact is an important cellular mechanism, and the loss of CI of proliferation and migration is a primary step in cancer progression. Transcriptional changes in CAMs that mediate cell–cell adhesion may be one means of overcoming CI. We propose that proteolysis of homophilic cell–cell CAMs also contributes to the loss of CIM and CIP that promotes tumorigenesis. The upregulation of ADAM proteases in tumor tissue (6) is one explanation for increased cell–cell CAM cleavage observed in cancer. We suggest that this has been overlooked because proteolysis can only be detected

by examining protein size on immunoblots, and not through large-scale screening techniques. Although this method is labor intensive, evidence is accumulating that proteolysis of many cell–cell CAMs results in the disruption of cellular adhesion, growth and migration (Table 1). Cleavage of Nr-CAM (9) has been shown to promote cell proliferation, whereas proteolysis of P-cadherin (42) and L1 (8) increases cell migration.

Cell–cell CAM proteolysis results in increased motility of cells, increased cell proliferation, and resistance to apoptosis. How the cleaved cell–cell CAMs are able to affect these processes is not altogether understood. We know that the shed ECDs are found in the tumor microenvironment and/or circulating in bodily fluids. These shed ECDs are capable of antagonizing the adhesion of cell-surface-bound CAMs (20), stimulating CAM cleavage (36), activating additional receptors [such as HER2 and HER3, in the case of sE-cad (10); or integrin receptors, in the case of L1 and NrCAM (8, 9)], and stimulating MMP expression (11, 22). In each of these situations, cell migration and/or proliferation can be stimulated.

The cleaved ICDs potentiate Wnt and Tcf/Lef signaling pathways. For example, the PTP $\kappa$ -ICD, E-cad/CTF1, and N-cad/CTF2 all promote  $\beta$ -catenin nuclear translocation. Furthermore, PTP $\kappa$ -ICD and Ep-ICD stimulate TCF- and Lef1-mediated transcription (36, 38). Ultimately, increased expression of c-myc (36), cyclin-D1 (15), and/or MMP-7 (11) has been observed following the generation of cell–cell CAM ICDs. These proteins can regulate signaling pathways that affect both cell proliferation and migration.

The recognition that tumor cells overcome CI by proteolyzing cell–cell CAMs provides a target for therapeutic intervention. Although the use of ADAM/MMP inhibitors has been employed in treatment of cancer before (6), we suggest that they must be used in concert with  $\gamma$ -secretase inhibitors to limit the function of both the extracellular and intracellular fragments generated by cell–cell CAM cleavage. Given that the presenilin protease that cleaves cell–cell CAMs may differ (Table 1), it is possible that additional specificity in tumor treatment may be achieved using specific MMP and presenilin inhibitors in combination therapy.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Acknowledgment

We thank Dr. Polly Phillips-Mason for her help in editing this manuscript. This work is dedicated to Tabitha Yee-May Lou whose endowment supports research in the Brady-Kalnay lab.

## Grant Support

This research was supported by the following NIH grants: RO1-NS051520, RO1-NS063971, and P30-CA043703.

Received June 29, 2010; revised October 15, 2010; accepted October 26, 2010; published OnlineFirst November 17, 2010.

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