**Dishonorable Discharge: The Oncogenic Roles of Cleaved E-Cadherin Fragments**

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### Abstract

Strong cell–cell interactions represent a major barrier against cancer cell mobility, and loss of intercellular adhesion by E-cadherin is a fundamental change that occurs during the progression of cancer to invasive disease. However, some aggressive carcinomas retain characteristics of differentiated epithelial cells, including E-cadherin expression. Emerging evidence indicates that proteolysis of E-cadherin generates fragments that promote tumor growth, survival, and motility, suggesting that E-cadherin cleavage converts this tumor suppressor into an oncogenic factor. In this review we discuss the emerging roles of cleaved E-cadherin fragments as modulators of cancer progression, and explore the translational and clinical implications of this research. Cancer Res; 72(12); 1–7. ©2012 AACR.

### Introduction

The overwhelming majority of cancers (>90%) are classified as carcinomas that are derived from the epithelial cells that line the exterior surfaces and internal cavities of the body. These highly specialized cells exhibit unique morphologic characteristics that include extensive intercellular junctional complexes, stable cell–cell and cell–matrix adhesions, and distinct apical and basolateral plasma membrane domains. The properties of a well-differentiated epithelium allow individual cells to function collectively as a single organized unit.

A pivotal hallmark of cancer is the ability of cancer cells to break free from the primary tumor and migrate to distant sites within the body to form new tumors. This metastatic capability is generally thought to arise through the progressive loss of epithelial characteristics as cancer cells adopt a more mesenchymal phenotype in a process termed epithelial-to-mesenchymal transition [EMT (1)]. In contrast to the well-differentiated epithelium, mesenchymal cells exhibit an elongated cell morphology with leading-edge/trailing-edge asymmetry, form only transient adhesions to neighboring cells and the extracellular matrix, and produce a variety of matrix-degrading enzymes. These alterations in cell phenotype simultaneously break up the integrity of the tissue and support increased motility and invasion as the tumor cells seek to penetrate into the vasculature for hematogenous dissemination.

Changes in the levels of cell–cell adhesion molecules are crucial for the development of aggressive carcinomas, and chief among these are alterations in E-cadherin expression. This protein forms cell–cell adhesions via calcium-dependent homophilic interactions, and downregulation of its expression permits the separation of individual cells from the primary mass. Although downregulation of E-cadherin was once thought to be a necessity, several reports have documented invasive and aggressive tumors that nonetheless maintain E-cadherin expression (1). In this review, we focus on the proteolytic cleavage and release of E-cadherin fragments from the plasma membrane as a mechanism that enhances tumor growth, survival, and motility. We discuss several reports that showed the oncogenic functions of these E-cadherin fragments in human cancer and then explore the wider clinical implications of this research.

### E-Cadherin Structure and Functions

E-cadherin is a single-pass type-I transmembrane glycoprotein that is localized to the adherens junction and basolateral membrane in epithelial cells and consists of a large extracellular domain, a transmembrane segment, and a conserved cytoplasmic domain. The extracellular portion contains 5 extracellular cadherin domains. These domains repeat that bind calcium ions to form a stiffened linear molecule (Fig. 1A). Homophilic interactions between cadherin molecules occur first between adjacent cells (trans-interaction) and then within the same cell by lateral association (cis-interaction) to form a zipper-like structure that strengthens the adhesion between the neighboring cells. The cytoplasmic domain interacts with the catenins and a variety of actin-binding proteins to anchor the cadherin–catenin complex to the actin cytoskeleton (2).

The adhesive function of E-cadherin plays a vital role in epithelial physiology. The fully formed cadherin–catenin complex, with its associated actin filaments, forms the core of the adherens junction, which brings together 2 apposed plasma membranes with an intercellular gap of only ~25 nm (2). This extremely close association halts the movement of individual epithelial cells (known as contact inhibition of motility) and...
Figure 1. Generation and functions of E-cadherin fragments. A, cleavage of full-length E-cadherin by α-secretase, γ-secretase, and caspase-3 generates sE-cad and E-cad/CTF1, E-cad/CTF2, and E-cad/CTF3 fragments, respectively. B, oncogenic functions of the sE-cad and E-cad/CTF2 fragments.

Generation of sE-cad by E-cadherin cleavage reduces the amount of full-length E-cadherin on the plasma membrane (step 1), disrupts existing adherens junctions (step 2), activates the expression of matrix metalloproteinases (MMP) to augment ectodomain shedding (step 3), and activates EGFR pathway signaling by different mechanisms (step 4). Intracellular cleavage of E-cadherin activates Wnt/β-catenin pathway signaling (step 5). CD, cytoplasmic domain; EC, extracellular cadherin domain; EGFR, epidermal growth factor receptor; TM, transmembrane domain.
E-cadherin can in integrity and preserving tissue function (3). Furthermore, (i.e., contact inhibition of growth), thereby maintaining tissue expression of E-cadherin fails to inhibit motility and invasion remains to be investigated.

Although E-cadherin is expressed along the length of the basolateral plasma membrane of epithelial cells, it is unlikely that molecules below the adherens junction can participate in trans-homophilic interaction because the intercellular space is larger between adjacent cells in these regions. The potential role of these non-adherens-junction–associated molecules remains to be investigated.

E-Cadherin Expression in Cancer

Loss of E-cadherin

In stark contrast to the epithelial cells from which they are derived, carcinoma cells typically do not exhibit contact inhibition of either motility or growth. Given its prominence in promoting contact inhibition, E-cadherin represents a formidable molecular barrier that must be overcome if tumor cells are to proliferate, detach, and spread. The functional loss of E-cadherin is a frequent and well-characterized molecular alteration that occurs during tumor progression. It results from a variety of mechanisms, including transcriptional repression, epigenetic silencing, inactivating mutation, enhanced degradation, endocytosis, and proteolytic cleavage (5). Downregulation of E-cadherin is a standard feature of developmental EMT, and its loss in cancer plays a causal role in developmental EMT, and its loss in cancer plays a causal role in enhancing breast cancer, the most aggressive form of breast cancer, and blockage of its function inhibits inflammatory breast cancer cell invasion (12). Likewise, E-cadherin is detected in early-stage ovarian carcinoma (13) and enhances proliferation and survival by inducing ligand-independent activation of the receptor tyrosine kinase (RTK) epidermal growth factor receptor [EGFR (14)].

In both inflammatory breast cancer and epithelial ovarian cancer, E-cadherin promotes the formation of multicellular aggregates that are able to metastasize collectively (15, 16). This so-called cohort migration occurs as a normal developmental process, but tumors can also use this method of migration to permit the movement of carcinoma cells that have not lost their well-differentiated epithelial morphology. Additional tumor types, including breast and colorectal tumor cells, have also been reported to spread by cohort migration (1).

Retained E-cadherin expression in cancer

Although the prevailing belief is that induction of EMT and loss of E-cadherin is a prerequisite for progression to metastatic disease, tumors are actually remarkably heterogeneous and E-cadherin loss does not always correlate with invasion. EMT is a multifaceted process, and cancer cells may undergo only a partial transition that enhances invasion while still retaining certain epithelial characteristics, such as E-cadherin (1). In support of this notion, in vitro studies showed that forced expression of E-cadherin fails to inhibit motility and invasion in certain carcinoma cell lines (8, 9). Moreover, analysis of patient tumor samples from multiple cancers revealed an unexpectedly high frequency of E-cadherin–positive tumors that had invaded into the surrounding tissue (1). Surprisingly, high E-cadherin expression was actually associated with aggressive growth in prostate cancer (10) and with growth, invasion, and unfavorable outcome in a rare form of glioblastoma (11). E-cadherin is also highly overexpressed in inflammatory breast cancer, the most aggressive form of breast cancer, and blockage of its function inhibits inflammatory breast cancer cell invasion (12). Likewise, E-cadherin is detected in early-stage ovarian carcinoma (13) and enhances proliferation and survival by inducing ligand-independent activation of the receptor tyrosine kinase (RTK) epidermal growth factor receptor [EGFR (14)].

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Cleaved E-Cadherin Fragments in Cancer

Proteolysis of E-cadherin

Given the occurrence of E-cadherin–positive yet invasive tumors, it stands to reason that there are additional means by which E-cadherin contributes to tumor development. Proteolytic processing and release of membrane protein fragments (termed ectodomain shedding) is a common way in which cells modify the functional properties of membrane proteins. It is now known that cleavage of E-cadherin generates protein fragments that possess distinct oncogenic properties. An α-secretase cleavage occurs on the extracellular face of the plasma membrane and is catalyzed by several proteases, including matrix metalloproteinases (MMP-3, MMP-7, MMP-9, and MT1-MMP), A-disintegrin-and-metalloproteinases (ADAM10 and ADAM15), plasmin, and kallikrein 7 (Fig. 1A; refs. 5, 17). This converts the mature 120-kDa E-cadherin into an extracellular N-terminal 80-kDa fragment and an intracellular C-terminal 38-kDa fragment. The ectodomain fragment [termed soluble E-cadherin (sE-cad)] is released from the plasma membrane and diffuses into the extracellular environment and even the bloodstream to serve as a paracrine/autocrine signaling molecule. In contrast, the intracellular C-terminal fragment (E-cad/CTF1) remains embedded within the plasma membrane until cleavage occurs at the intracellular face by means of a γ-secretase (presenilin-1/2). This disassembles the adherens junction and releases an intracellular 33-kDa fragment (E-cad/CTF2) into the cytosol that functions in intracellular signaling (Fig. 1A; refs. 18, 19). This fragment can also be further proteolyzed by caspase-3 to yield a 29-kDa fragment (E-cad/CTF3) of unknown function (20).

An important question to consider is whether the adherens junction–associated E-cadherin is cleaved or whether molecules located along the basolateral plasma membrane are preferentially cleaved. We speculate that the non-adherens-junction–associated E-cadherin is the predominant source of
sE-cad because the larger intercellular distances below the adherens junction may facilitate E-cadherin cleavage by soluble proteases. This supposition remains to be addressed experimentally.

**sE-cad enhances motility and invasion**

Shedding of the ectodomain fragment of E-cadherin was first discovered in the conditioned media of MCF-7 mammary carcinoma cells (21). In several subsequent studies, investigators observed significantly elevated levels of sE-cad in cancer patients’ sera from a variety of tumor types and reported that elevated sE-cad was associated with invasive disease and/or poor prognosis (5). The first function attributed to sE-cad was the disruption of cell–cell adhesion, based on the observation that treatment of cells with sE-cad in vitro decreased cell aggregation and increased migration and invasion (13, 17, 21–26). The mechanism by which sE-cad effects these changes involves at least 4 steps (Fig. 1B). First, cleavage of E-cadherin degrades the full-length molecule, reducing the available pool of adhesion-competent E-cadherin in the plasma membrane. Second, because sE-cad retains the ability to form homophilic bonds, it may obstruct full-length E-cadherin homodimers between adjacent cells. Third, sE-cad displays chemotactic properties and may become trapped within the extracellular matrix to serve as anchoring points for full-length E-cadherin molecules on migrating cells (5, 24). Fourth, sE-cad causes upregulation of major MMPs (MMP-2, MMP-9, and MT1-MMP) that degrade the basement membrane to allow tumor invasion into the stroma (26). Of note, given that MMP-9 was also shown to cleave E-cadherin (13), it is tempting to speculate that protease induction by sE-cad also constitutes a positive-feedback loop to continuously generate sE-cad.

**sE-cad induces EGFR-dependent growth and survival signaling**

It is well known that E-cadherin regulates cell signaling. RTK activation occurs through receptor dimerization and autophosphorylation, and the clustering of E-cadherin molecules in cis following intercellular homophilic interaction can promote ligand-independent dimerization and activation of EGFR (14, 27, 28). Because the extracellular domain of E-cadherin physically interacts with EGFR (29), cleavage and shedding of this domain into the extracellular environment converts this molecule into a soluble growth factor.

This novel function of sE-cad was first shown in breast cancer cells. The EGFR (ErbB) family of RTKs consists of 4 members: EGFR (HER1, ErbB1), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). These receptors can both homo- and heterodimerize in response to ligand binding. Najy and colleagues (25) observed that sE-cad could complex with both HER2 and HER3, stabilize their interaction, and activate signaling to enhance proliferation and migration (Fig. 1B). Because HER2 overexpression is a relatively common event in breast cancer that portends a poor prognosis (30), the proteolytic release of sE-cad may serve to stimulate this pathway to promote the aggressive growth and spread of these tumors.

Signaling by sE-cad can also interfere with apoptosis. Our laboratory showed that treatment of Madin–Darby canine kidney (MDCK) cells with 10 μg/mL sE-cad inhibited apoptosis in response to serum withdrawal without altering cell–cell adhesion (31). We further showed that sE-cad prevented the formation of multicellular hollow cysts when these cells were cultured in a 3-dimensional collagen matrix. Cyst formation requires the apoptosis of centrally located cells, and we found that sE-cad blocked cyst hollowing via ligand-independent activation of EGFR (Fig. 1B; ref. 31). Our studies collectively show that sE-cad acts as an antiapoptotic factor, which suggests that it may play a critical role in the survival and initiation of a preneoplastic condition in epithelial tissues.

Although both of these studies (25, 31) reported the activation of EGFR signaling by sE-cad, they differed in terms of the requirement for full-length E-cadherin expression. Najy and colleagues (25) showed direct binding of sE-cad to HER2 and HER3, and subsequent signaling in the absence of full-length E-cadherin, whereas we found that interaction between sE-cad and full-length E-cadherin was essential to mediate survival signaling (Fig. 1B; ref. 31). The binding of sE-cad to cell surface E-cadherin may mimic the intercellular homophilic binding between neighboring cells that results in downstream signaling (14, 27, 28). However, cell–cell adhesion was unaffected in our study, suggesting that sE-cad interacts with non-adherens-junction–associated E-cadherin molecules that are expressed along the length of the basolateral plasma membrane.

The link between sE-cad and EGFR also seems to be a reciprocal relationship in which EGFR activation promotes MMP- and ADAM-dependent generation of sE-cad (32, 33). This suggests a feedback mechanism whereby EGFR activation produces sE-cad, which can then further activate EGFR signaling to continuously promote oncogenic proliferation and invasion (Fig. 1B).

**E-cadherin cleavage activates Wnt/β-catenin pathway signaling**

A central component of the cadherin–catenin complex is β-catenin, which also functions as the primary mediator of the Wnt signaling pathway. In the classical Wnt pathway, the binding of Wnt ligand triggers the accumulation of β-catenin, which translocates into the nucleus and binds to the transcription factor T-cell factor/lymphocyte enhancer factor-1 to activate growth-stimulatory target genes (34). By complexing with β-catenin, E-cadherin effectively sequesters this protein at the plasma membrane and prevents its transcriptional activities (35). However, cleavage of E-cadherin disassembles this complex and releases β-catenin to potentiate oncogenic Wnt pathway signaling (Fig. 1B; refs. 17–19). This pathway is also enhanced directly by the intracellular E-cad/CTF2 fragment. In addition to freeing β-catenin, E-cadherin cleavage also liberates p120 catenin, which translocates into the nucleus and binds to the transcriptional repressor Kaiso to relieve its repression of various Wnt/β-catenin pathway gene targets (36). Of importance, the E-cad/CTF2 fragment can remain bound to p120 to enhance its inhibitory effects upon Kaiso-mediated transcriptional repression (Fig. 1B; ref. 37).
These studies suggest a dual mechanism by which E-cadherin cleavage facilitates Wnt/β-catenin pathway signaling. The resulting aberrant activation of the Wnt/β-catenin pathway may serve to support rapid tumor cell proliferation and also augment tumor cell survival, as the expression of nuclear E-cad/CTF2 has also been shown to suppress the induction of apoptosis (37). Additionally, E-cadherin cleavage may promote the acquisition of a cancer stem cell phenotype, because Wnt/β-catenin pathway signaling is involved in stem cell regeneration (34). Furthermore, because MMP-7 is a Wnt/β-catenin target (38) that has been shown to cleave full-length E-cadherin (23), activation of this gene may establish a positive-feedback loop of continuous E-cadherin cleavage to ensure sustained oncogenic signaling.

Clinical Implications and Future Directions

Preclinical studies of sE-cad function

The presence of sE-cad in the sera of healthy patients indicates that it may play a role in normal physiologic processes. Given its ability to enhance motility (promoting epithelial cell movement) and simultaneously block apoptosis (to prevent anoikis of detached epithelial cells), sE-cad production may be a key feature in normal wound healing. Tantalizing pieces of evidence point to this possibility, because calcium influx is a critical event in both wound healing and E-cadherin cleavage (17, 18). Additionally, sE-cad may play a role in somatic cell turnover by disrupting cell–cell adhesion to promote the expulsion and replacement of older cells in continuously renewing tissues.

Whether circulating sE-cad contributes directly to tumor growth, survival, and motility in vivo remains to be confirmed experimentally. The wealth of in vitro experimental data and the clinical association of invasive disease with high serum sE-cad suggest that sE-cad plays a pathologic role in vivo. For instance, circulating sE-cad may aid in the preparation of metastatic sites by disrupting epithelial junctions at distant locations to ease tumor cell invasion and growth into these secondary sites. Furthermore, the induction of lumen filling in 3-dimensional cultures may translate in vivo: lumen filling in epithelial tissues adjacent to a tumor mass may transform these neighboring tissues into preneoplastic cells that secrete tumor-promoting factors into the local microenvironment. By blocking the normal apoptotic removal of genetically damaged cells, sE-cad–induced lumen filling may also permit the propagation and even the transformation of mutated cells within these adjacent tissues into separate tumors. Future investigations will be necessary to expand our understanding of the consequences of E-cadherin ectodomain shedding in cancer progression in vivo.

Pathologic analysis of E-cadherin in tumor tissues

It is now widely accepted that EMT in cancer is not an "all or none" phenomenon. Although it is clear that loss of E-cadherin plays a pivotal role in cancer-associated EMT, there are numerous cases in which invasive carcinomas have been shown to retain differentiated epithelial markers such as E-cadherin (1). Furthermore, it is likely that immunohistochemical studies of tumor samples overestimated the proportion of E-cadherin loss, because many of these studies used antibodies that could not detect the shedding of ectodomain fragments. This was documented in an analysis of ovarian carcinoma in which focal areas of high MMP-9 staining were found to colocalize with low or absent E-cadherin staining in otherwise E-cadherin–positive tumors, indicating active ectodomain shedding in these focal areas (13). The evident heterogeneity of human tumors suggests that accurate profiling of E-cadherin expression requires more-sophisticated methods. First, immunohistochemical analysis should be used to compare the staining of extracellular- and intracellular-specific antibodies from the same sample. Intracellular staining without concurrent membrane staining may indicate ectodomain shedding in the tumor. This could be further validated by comparing E-cadherin mRNA levels in the tumor and matched normal tissue to confirm that alterations in the immunohistochemical staining are not due to transcriptional repression of E-cadherin. Laser-capture microdissection has made it practical to isolate pure populations of tumor cells for RNA extraction, and this technology is also compatible with various tissues, staining methods, and preservation techniques, allowing its use for fresh and stored specimens alike. Future studies using these methods may provide a more precise assessment of E-cadherin loss and sE-cad production in human cancer, as well as additional insights into the pathophysiologic relevance of this molecule.

sE-cad as a clinical biomarker for diagnosis and treatment

The clinical detection of elevated serum sE-cad in a wide variety of cancer types, coupled with its frequent association with invasion and poor prognosis, may designate sE-cad as a general diagnostic and prognostic biomarker for invasive and/or metastatic disease. For example, initial measurement of serum sE-cad at clinical presentation may aid in the discrimination of slow-growing tumors that do not necessitate immediate intervention versus those that are actively progressing and require more urgent treatment. Continuous monitoring of sE-cad levels may also be a relatively inexpensive method for tracking therapeutic outcome, as treatment efficacy was associated with decreasing sE-cad levels in separate studies of gastric and lung cancers (39, 40). In the future, sE-cad may become useful for guiding the decision to continue a given treatment or replace it early in favor of another option. Furthermore, the newly ascribed roles for cleaved E-cadherin fragments in cellular signaling suggest that elevated sE-cad is indicative of EGFR and/or Wnt/β-catenin pathway activation and may predict sensitivity to inhibitors of these pathways.

Validation of sE-cad as a pathologically significant molecule in cancer progression raises the question as to whether it can be targeted to slow cancer growth and invasion. Inhibition of relevant proteases may prevent sE-cad generation; however, inhibitors of these enzymes have yet to yield satisfying clinical results (41). The development of methods to improve the specificity of these inhibitors and deliver them in a targeted manner is an area of ongoing research, and such methods may become viable in the future. An alternative target is the EGFR
signaling pathway, which promotes the shedding of sE-cad (32, 33). Blockade of this pathway may reduce ectodomain shedding and diminish the pathologic effects of sE-cad; however, sE-cad production independent of EGFR signaling will necessitate the targeting of other pathways to obtain clinical benefit. It may also be feasible to develop agents that remove sE-cad from the blood and/or tumor microenvironment, such as neutralizing antibodies or small molecules that bind specifically to sE-cad and clear it from the body.

Conclusions

It is apparent from multiple investigations that E-cadherin plays a much more complex role in cancer biology than solely that of a tumor-suppressive cell adhesion molecule. The existence of invasive yet E-cadherin--expressing tumors presents an apparent paradox that can be reconciled if one considers that maintaining E-cadherin allows for the production of oncogenic sE-cad and E-cad/CTF2 fragments. Because these fragments promote tumor cell growth, survival, and motility, the cleavage and release of these molecules represents a novel method of subverting contact inhibition imposed by full-length E-cadherin. Although further investigation into the pathophysiologic consequences of E-cadherin cleavage remains to be done, a better understanding of its diverse functions may yield important advances in the diagnosis and management of numerous cancers.

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