Soluble E-cadherin: more than a symptom of disease

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1. ABSTRACT

Epithelial (E)-cadherin is a homophilic adhesion molecule which is responsible for maintenance of baso-lateral cell adhesion and polarity. E-cadherin can be lost from the cell surface by proteolytic cleavage, resulting in the generation of an 80kDa fragment referred to a soluble E-cadherin (sE-cad). Although originally discovered in the conditioned media of breast cancer cells and later verified in the fluids of cancer patients, today sE-cad has been reported in patients with viral and bacterial infections, organ failure, and benign disease. The proteases implicated in this cleavage event include members of the disintegrin family (ADAM10 and 15), bacterial proteases (gingipains and BFT), cathepsins (B, L, S), matrix metalloproteases (MMP-2, 3, 7, 9, and 14), Kallikrein-7 (KLK7), and plasmin. Stimulus that induces sE-cad generation by ADAMs, MMPs, KLK7, and plasmin *in vitro* ranges from serum withdrawal to pro-inflammatory cytokines to growth factors. The cellular or physiologic consequences of sE-cad accumulation include the disruption of adherens junctions, cellular migration and invasion, induction of MMPs, as well as cell signaling, suggesting that sE-cad may contribute to disease progression.

2. INTRODUCTION

2.1. Epithelial cadherin

E-cadherin is a homophilic, calcium-dependent, adhesion protein, which is expressed at adherens junctions between epithelial cells. E-cadherin has an extracellular region comprised of 5 domain repeats, each domain containing a set of seven beta-sheets arranged in an immunoglobulin fold (1). Adhesion is achieved by lateral dimerization between E-cadherin molecules on the same cell, creating a homodimer which can then interact with an

adjacent E-cadherin homodimer on a neighboring cell via cadherin repeat 1 (EC1) (2).

Although E-cadherin is predominantly a homophilic adhesion molecule, it also exhibits heterophilic interactions. Of particular interest, E-cadherin can associate with CD103 and killer cell lectin receptor G1 (KLRG1) (3-5). The E-cadherin receptor, CD103 or alpha$_E$beta$_7$, is located on T lymphocytes and helps to target T cells to epithelial cells, where it binds extra cellular domains EC1 and EC2 of E-cadherin (3). Another receptor for E-cadherin, KLRG1, is expressed on T cells and natural killer (NK) cells, where the binding of KLRG1 to E-cadherin-expressing cells prevents the lysis of epithelial targets (5). The interaction between KLRG1 and E-cadherin is mediated by the homodimeric interface on EC1, suggesting that monomeric E-cadherin at epithelial cell surfaces is responsible for controlling the activation threshold of NK and T cells and thereby suppressing immune response (6). E-cadherin, therefore, can serve as an adhesion molecule or a targeting molecule, depending on binding partner.

Within the cell, E-cadherin interactions support adhesion established through homodimerization. The cytoplasmic tail of E-cadherin associates directly with beta-catenin, p120, and indirectly with alpha-catenin. Beta-catenin binding to E-cadherin provides structural support and aids in transport of E-cadherin to the baso-lateral plasma membrane (7). The formation of this ordered structure allows for binding of alpha-catenin, which can bind actin, stabilizing and coordinating actin dynamics at the adherens junction (8, 9). p120 binding to E-cadherin also stabilizes the complex and maintains high E-cadherin levels (10). From the cytoplasmic side, regulation of E-cadherin adhesion can occur by altering the composition of the cadherin-catenin complex, the presence growth factors, tyrosine phosphorylation of the cadherin-catenin complex, p120 binding to E-cadherin, and the activity of small GTPases and proteins which aid in cell polarity determination (11). Additionally, E-cadherin adhesion can be disrupted by E-cadherin cleavage in the extracellular or cytosolic domains.

2.2. E-cadherin cleavage

The disruption of E-cadherin function by proteolytic cleavage can occur in the extracellular domain or cytosolic tail of E-cadherin. The initial observation of E-cadherin cleavage was reported by Wheelock *et al* who observed an 80 kDa soluble E-cadherin in the conditioned media of MCF-7 breast cancer cells (12). This fragment, containing the five extracellular domains of E-cadherin and referred to as sE-cad, could disrupt adherens junctions of mouse mammary tumor cells (12). To date, members of the A Disintegrin and Metalloprotease (ADAM) family (ADAM10 and 15), bacterial proteases (gingipains and BFT/fragilysin), cathepsins (B, L, S), matrix metalloprotease (MMP) family (MMP-2, 3, 7, 9, and 14), KLK7, and plasmin have all been implicated in the generation of sE-cad (13-31).

The extracellular cleavage of E-cadherin can also have implications for the intracellular domains. The generation of sE-cad allows the remaining membrane-bound fragment to undergo further processing by presenilin-1/ gamma-secretase complex at the membrane/cytosol interface, which results in the disassembly of the adherens junction (32). Additionally, p120, which is critical for the association between E-cadherin and gamma-secretase (33), can aid the C-terminal fragment of E-cadherin in entering the nucleus and binding DNA to promote gene transcription (34). This tightly regulated sequential degradation of E-cadherin and resulting disassembly of adherens junctions and nuclear translocation of the C-terminal fragment, however, is not required for all E-cadherin cytoplasmic domain processing.

Apoptosis-induced cleavage of the intracellular domain of E-cadherin by calpain and caspase-3 occurs independently of extracellular processing. Calpain cleavage of E-cadherin results in a 100kDa E-cadherin fragment which can no longer bind beta-catenin and diminishes the survival of prostate cancer cells (35). Conversely, caspase-3 can generate a 24kDa cytosolic fragment of E-cadherin, but requires other metalloproteases to generate the 29kDa fragment and sE-cad (36). The generation of sE-cad, however, is not limited to apoptosis, and there are a variety of stimuli which can induce E-cadherin cleavage.

3. GENERATION OF SE-CAD

3.1. Membrane sheddases

The proteases capable of extracellular E-cadherin cleavage, referred to as sheddases, are a diverse group. While many of these sheddases are misregulated or overexpressed in disease, the authors have limited their descriptions to cell systems and diseases where sE-cad is present in conditioned media of cells or patient fluids. To date, no sheddase unique to E-cadherin has been identified, and the sheddases described below are also responsible for cleavage and shedding events beyond sE-cad generation (Table 1).

3.1.1. ADAM family

The human ADAM family consists of 25 members whose expression varies across tissues. Functionally, the ADAMs play roles in adhesion and substrate cleavage. To that end, ADAMs are made up of an inhibitory prodomain, a zinc-dependent metalloprotease domain, a disintegrin domain, a cysteine-rich domain, an EGF-like domain, a transmembrane domain and a cytoplasmic tail. Within the ADAM family, ADAM10 and 15 have been implicated in E-cadherin shedding (13-16).

ADAM10 can be found in mesenchymal stem cells, placenta, blood, myeloid cells, bladder, and bone marrow myeloid cells, where it is predominantly a sheddase with a substrate list featuring 27 proteins, including E-cadherin (37). In terms of E-cadherin cleavage in the skin, ADAM10 has been implicated in generating sE-cad in normal keratinocytes as well as melanoma cell lines. In keratinocytes, Maretzky *et al* examined the soluble 80kDa form and 37kDa C-terminal fragment (CTF) associated with the membrane and determined that ADAM10 constitutively sheds sE-cad (14). ADAM10-

Table 1. E-cadherin sheddases

Sheddase	Stimulus	System	Studies	Ref
ADAM10	IL-1 beta, TNF-alpha, IFN-gamma, TGF-beta, Lipopolysaccharide (LPS)	Normal keratinocytes	In, si	(14)[A]
	None (growing cultures)	Melanoma cell line	Exp.	(15)
	Helicobacter pylori infection	Gastric carcinoma cell line	si, In	(13)
	EGF	Benign prostatic hyperplasia cell line	In, sh	[B]
ADAM15	Serum withdrawal	Breast cancer cell line	OE, sh,	(16)
		Prostate cancer cell line	OE, sh, KO	[C]
		Bladder cancer cell line	OE, sh	[C]
BFT fragilysin	*B. fragilis* infection	Colorectal cancer cell line	PP	(18)[A]
Cathepsins (B, L, S)		Ms pancreatic cancer model	RP	(19)
Gingipains	*P. gingivalis* infection	Canine kidney cell line	PP	(17)
KLK7	?	Pancreatic cancer cell line	RP	(20)
MMP-2	Protein Kinase D1 (PKD1)	Prostate cancer cell line	In	(21)
MMP-3 Stromelysin	?	Breast cancer cell line	RP	(22)
	? (Activated mutant)	Ms mammary cell line	aOE, In	(23)
MMP-7 Matrilysin	HGF	Gastric cancer cell line	Ind, sh	(24)
	HGF	Prostate cancer cell line	RP, si	(25)
	Lung injury (bleomycin)	Lung cancer cell line, mouse lung injury	aOE, In, KO	(26)
	?	Breast cancer cell line	RP	(22)
MMP-9	Collagen binding integrins (alpha$_2$beta$_1$, alpha$_3$beta$_1$) interaction	Ovarian carcinoma cell line	Ind, In, FBA Ind, si	(27)
	EGF	Head and neck cancer cell line		(28)
		Prostate cancer cell line	In	
	PKD1			(21)
MT1-MMP MMP-14	Ischemia (mineral oil overlay)	Normal rat kidney cell line	In, FAB, sh	(29)
Plasmin	Lysophosphatidic acid (LPA)	Ovarian carcinoma cell line	In	(30)
	None (growing cultures)	Canine kidney cell line	RP, In	
?	Calcium influx, ionomycin	Lung tumor cell line	In	(65)
?	Ischemia (lung transplant)	Rat lung transplantation		(68)
TIMP-2 sensitive	Phorbol ester (PMA)	Breast cancer cell line	In	(22)
?	Serum withdrawal	Breast cancer cell line	In	(12)[D]
TAPI[E] sensitive	Apoptosis (staurosporine, camptothecin)	Canine kidney cell line	In	(36)

ARead-out is the C-terminal fragment, not sE-cad. B Manuscript submitted C Unpublished observations D Original report of sE-cad EADAM17 inhibitor which can block other metalloproteases. In: inhibitor. si: siRNA. sh: shRNA. Exp: expression. OE: Over-expression. RP: recombinant protein. PP: purified protein. Ind: Induction of metalloprotease. aOE: auto-activating mutant metalloprotease. KO: knockout mouse. FBA: function blocking antibody

dependet shedding of E-cadherin could be induced by the pro-inflammatory cytokines IL-1 beta, TNF-alpha, IFN-gamma, TGF-beta, and lipopolysaccharide (LPS). Additionally, biopsies from eczema patient lesions revealed elevated levels of ADAM10 and CTF (14). ADAM10 also exists in its active form in melanoma cell lines (15), but to date, no inhibitor or loss of function studies of ADAM10 in melanoma cell lines in terms of sE-cad generation have been undertaken. However, ADAM10 is up-regulated in metastatic melanoma compared to primary melanoma (38), and it is possible that it is the sheddase responsible for E-cadherin cleavage.

ADAM10 has also been implicated in shedding E-cadherin in response to *Helicobacter (H.) pylori* infection of gastric cancer cell lines (13). Previously, *H. pylori* infection was found to correlate with increased ADAM10 expression in gastric cancer patient samples and to induce ADAM10 expression in gastric cancer cell lines (39). Indeed, chemical inhibition or shRNA mediated knockdown of ADAM10 resulted in decreased sE-cad generation in response to *H. pylori* infection, suggesting that induction of ADAM10 by *H. pylori* in the gut promotes E-cadherin cleavage (13).

Recent work in our lab has determined that in benign prostatic hyperplasia (BPH) cells, epidermal growth factor (EGF) can induce sE-cad generation in an ADAM10-dependent manner (manuscript submitted to Cellular Signaling). In a paper by Arima *et al*, the authors demonstrated that ADAM10 is highly expressed on the cell surface of BPH patient samples versus cancer samples where ADAM10 resided predominantly within the nucleus (40). This suggests that based on location alone, ADAM10 is likely a major sheddase of E-cadherin in BPH, but not in prostate cancer.

Unlike ADAM10, ADAM15 has only four reported substrates, but it also has three integrin binding partners (37). The expression of ADAM15 is widespread in the human tissues, but it is the highest in mesenchymal stem cells and the urogenital system (37). It is also significantly over-expressed in breast, prostate, and lung cancer (16). Najy *et al* demonstrated that serum withdrawal from breast cancer cells induced E-cadherin cleavage by ADAM15, and the generation of sE-cad could be abrogated by shRNA knockdown of ADAM15 or increased by ADAM15 over-expression (16). Our unpublished observations also suggest that prostate cancer and bladder

cancer cells shed sE-cad in response to serum withdrawal in an ADAM15- dependent manner as well. Additionally, in untransformed mouse cells, ADAM15 knockout prostate cell lines fail to shed appreciable amounts of sE-cad as compared to wild-type control cell lines, suggesting ADAM15 can also cleave E-cadherin in mouse prostate epithelial cells (unpublished observations).

3.1.2. Bacterial proteases (Gingipains, BFT/fragilysin)

Gingipains (HRgpA, RgpB, and Kgp) are secreted cysteine proteases which are encoded in the genome of *Porphyromonas gingivalis* (*P. gingivalis*). *P. gingivalis* has been reported to contribute to adult periodontitis in two ways. First, by infecting epithelial cells, *P. gingivalis* can influence signal transduction and innate immune response (41). Independent of epithelial cell infection, *P. gingivalis* can disrupt adherens junctions, allowing for infection of underlying tissues (42). The disruption of the adherens junction is believed to be mediated by Kgp cleavage of E-cadherin (17). Although HRgpA and RgpB can also cleave immunoprecipitated E-cadherin, they are unable to process E-cadherin from the cell surface of Madin-Darby canine kidney (MDCK) cells (17).

Another bacterial protease which has been implicated in E-cadherin cleavage is *Bacteroides fragilis* (*B. fragilis*). *B. fragilis* produces an enterotoxin referred to as *B. fragilis* toxin (BFT) or fragilysin. Treatment of HT29/C1 colorectal adenocarcinoma cells with BFT results in the generation of the 33kDa and 28kDa cytoplasmic E-cadherin fragments (18). Although the authors demonstrated that BFT did not enter the cells and hence could not generate the cytoplasmic fragments, they were unable to observe BFT-mediated cleavage of their recombinant E-cadherin and generation of sE-cad (18). These studies suggest that E-cadherin cleavage may be an important step in bacterial infection.

3.1.3. Cathepsins

Cysteine cathepsins are intracellular proteases which are responsible for protein degradation in the lysosome and play critical roles in apoptosis, autophagy, and necrosis (43). Although located within the lysosome under normal conditions, an emerging body of evidence suggests cathepsins can be miss-localized or released from the cell. For example, release of active cathepsin B has been demonstrated in mechanically injured mouse gut (44), and pro-cathepsin B can interact and localize with annexin II tetramer on the extracellular surface of human breast cancer and glioblastoma cells (45). These studies suggest that in the context of disease, cathepsins may be a viable candidate for extracellular cleavage of E-cadherin.

Interest in cathepsin cleavage of E-cadherin originated with the observation that pancreatic tumors from cathepsin B, L, or S knockout mice on the background of the RT2 pancreatic cancer mouse model retained expression of E-cadherin, suggesting E-cadherin processing was deficient (19). Indeed, when Gocheva *et al* combined recombinant E-cadherin with active cathepsins B, L, or S, E-cadherin was cleaved to a 64kDa extracellular fragment (19). Because cathepsins B and L are upregulated during pancreatic cancer progression (19) and high cathepsin B expression is an independent prognostic marker for pancreatic cancer recurrence (46), it is likely that the miss-localized cathepsins play a role in E-cadherin cleavage in pancreatic cancer.

3.1.4. Kallikrein-7

Kallikrein-7 (KLK7) is serine protease which is normally expressed in the salivary gland, nervous system, kidney, mammary gland and skin and to a lesser extent in the uterus, thymus, thyroid, placenta, and trachea (47). It is not expressed in the normal pancreas, but it is dramatically over-expressed in pancreatic cancer (20). In pancreatic cancer cell line cultures, recombinant KLK7 was capable of cleaving E-cadherin *in vitro* and from the cell surface of pancreatic cancer cell lines (20). Because of its dramatic up-regulation in pancreatic cancer, it is likely that KLK7 is a responsible protease for E-cadherin cleavage in pancreatic cancer patients.

3.1.5. MMP family

Like the ADAM disintegrins, the MMPs are zinc dependent proteases (37, 48). While the majority of the MMP family are secreted, a subset of the MMP family, the membrane type (MT) MMPs remain associated with the cell membrane (48). MMP activity is tightly controlled by MMP gene transcription, pro-enzyme activation, and MMP inhibition (48). In E-cadherin cleavage, five MMPs have been implicated: MMP-2, MMP-3, MMP-7, MMP-9 and MT1-MMP (MMP-14).

In prostate cancer, MMP-2 is an independent predictor of patient survival. Early immunohistochemical studies of MMP-2 in prostate cancer patients demonstrated that epithelial expression of MMP-2 in prostate tumors correlated with a decrease in patient survival (49). *In vitro,* MMP-2 has been implicated in sE-cad generation in prostate cancer cells which have been transfected with protein kinase D1 (PKD1) (21). The *in vitro* data and the over-expression of MMP-2 in prostate cancer suggest MMP-2 is a possible candidate for E-cadherin shedding in prostate cancer.

MMP-3 shedding of E-cadherin has been reported in mouse and human mammary cells. Early studies by Lochter *et al* revealed that an auto-activating MMP-3 mutant transfected into mouse mammary cells resulted in the shedding of sE-cad from their cell surface (22, 23). Later analysis of MMP-3 and 7 by Noe *et al* demonstrated that E-cadherin can be cleaved *in vitro* by these metalloproteases in breast cancer cells as well (22). In patients, MMP-3 is over-expressed and activated in breast cancer samples versus normal tissue (50, 51), suggesting MMP-3 could be an E-cadherin sheddase in breast cancer.

MMP-7 generation of sE-cad has been reported in prostate, gastric, and breast cancer cells, as well as in a mouse model of lung injury (22, 24-26). In prostate cancer and gastric cancer cell lines, treatment of cells with hepatocyte growth factor/scatter factor (HGF/SF), results in the release of MMP-7 and cleavage of E-cadherin (24, 25).

When MMP-7 levels are decreased by short hairpin (sh) RNA against MMP-7, sE-cad generation is lost (24, 25). Prostate cancer patients with advanced disease have more active MMP-7 in their serum (52), while for gastric patients, expression of MMP-7 correlates with a decrease in patient survival and more advanced stage (53) . In breast cancer cell lines, E-cadherin can be cleaved *in vitro* by MMP-7 (22), and in patients MMP-7 positive tumors by immunohistochemistry correlate with a worse prognosis (54). Because MMP-7 is over-expressed in breast, prostate, and gastric cancer, it is possible that MMP-7 is major sheddase of E-cadherin in these cancers.

MMP-7 over-expression is not unique to cancer and can occur in response to injury. In a mouse lung injury model using bleomycin, MMP-7 is dramatically upregulated in injured lung epithelium (26, 55). McGuire *et al* also demonstrated that MMP-7 knockout mice did not generate sE-cad from wounded trachea explants, unlike their wild-type controls, implicating that the up-regulation of MMP-7 in response to wounding is responsible for sE-cad generation in this model (55). MMP-7 up-regulation occurs in pulmonary fibrosis patients (56), so MMP-7 generation of sE-cad may play a role in disease progression. Interestingly, in these studies only the lung cancer cells that were transfected with an auto-activating MMP-7 produced the active form of the enzyme; native full length cDNA for MMP-7 did not produce active enzyme (26), suggesting that there is a missing mediator required for MMP-7 activation in these epithelial lung cancer cells.

MMP-9 shedding of E-cadherin appears in ovarian, head and neck, and prostate cancer cell lines. MMP-9 expression is a negative predictor for survival in ovarian cancer, head and neck cancer, as well as prostate cancer (57-59). In ovarian cancer cell lines, aggregation of collagen binding integrins $alpha_2beta_1$ and $alpha_3beta_1$ induces MMP-9 expression which promotes E-cadherin shedding (27). Early studies of MMP-9 in head and neck cancer patients demonstrated that serum levels of MMP-9 were highest in patients with more advanced disease (59) and *in vitro*, stimulation of head and neck cell lines with EGF demonstrated increased expression of MMP-9 and increased sE-cad (28). Finally, the re-expression of PKD1 in prostate cancer cells also resulted in increased MMP-9 expression, which correlates with increased sE-cad generation, and could be abrogated by the addition of MMP-9 inhibitors (21). Based on the high expression of MMP-9 in ovarian, head and neck, and prostate cancer patients, it is likely that MMP-9 contributes to sE-cad levels in these patients.

MT1-MMP (MMP-14) has been implicated in sE-cad generation in a model of kidney ischemia. Normal rat kidney cells under ischemic conditions generated sE-cad which, by inhibitor studies, was not mediated by MMP -1, -3, -8, or -9 (29). Covington *et al* did, however, observe an increase in MT1-MMP expression in response to ischemia, and determined that loss of MT1-MMP by shRNA decreased sE-cad accumulation, confirming that sE-cad can be generated by MT1-MMP under these conditions (29). In a mouse model of hind-limb ischemia, active MT1-

MMP was up-regulated in the ischemic limb as compared to the control, sham operated limb (60), suggesting that MT1-MMP may be the sheddase of E-cadherin under ischemic conditions.

3.1.6. Plasmin
Plasmin is a serine protease with limited specificity, which can act on fibrin, fibrinogen, extracellular matrix components, and pro-forms of growth factors either directly or by activating metalloproteases (61). It is also a downstream component of the urokinase-type plasminogen activator (uPA) system, which can be activated in ovarian cancer cells by Lysophosphatidic acid (LPA) (62). LPA is found in high concentration in ovarian cancer ascites and promotes growth in ovarian cancer cell lines *in vitro* and *in vivo* (63). In ovarian cancer cell lines, LPA activates the urokinase-type plasminogen activator (uPA) which activates plasmin, resulting in E-cadherin cleavage (30). Although the authors could not rule out metalloproteases downstream of plasmin, they do note that in their studies, LPA only increased pro-MMP-9 slightly, suggesting that plasmin may be directly acting upon E-cadherin (30). Other work in MDCK cells demonstrated that treating cells with plasmin can generate sE-cad and this process can be inhibited by the addition of aprotinin, a serine protease inhibitor (31). Since uPA system disregulation correlates with worse outcome in ovarian cancer patients (64), it is likely that an elevated level of plasmin in these patients generates sE-cad.

3.1.7. Unattributed sheddase activity
In addition to the studies which successfully define sE-cad sheddases, other studies demonstrate the existence of sE-cad as a consequence of stimuli, but do not identify the responsible protease. In these studies, the protease could be one of the aforementioned sheddases or it could be a novel sheddase. Ito *et al* demonstrated that calcium influx by serum withdrawal or ionomycin treatment allowed for sE-cad to accumulate in the conditioned media of cancer cells (65). Although they never identified a responsible sheddase, they did report only the membrane fractions and not cell supernatants were capable of cleaving E-cadherin *in vitro*, suggesting a membrane bound metalloprotease was required and ruled out the classical MMPs in direct cleavage (65).

In the breast cancer MCF-7/AZ cell line, the phorbol ester PMA can induce the shedding of E-cadherin by a metalloprotease that is sensitive to tissue inhibitor of metalloprotease-2 (TIMP-2) inhibition (22). In the same study, immunopurified MMP-3 and MMP-7 are shown to cleave E-cadherin directly and from the cell surface of MDCK cells (22), but a direct sheddase from PMA induction was not demonstrated. Other studies of apoptotic MDCK cells also implicated a metalloprotease which was sensitive to TAPI, an ADAM17 inhibitor which can inhibit other metalloproteases (36).

Several studies have determined that the accumulation of sE-cad may be a biomarker for tissue damage and predict surgical outcome (66, 67). Goto *et al* applied this to a model of lung transplantation in rats and

observed that rats with transplanted lungs had a higher level of sE-cad than the sham operated rats (68). Again, no direct evidence implicates a sheddase, but other studies have implicated MMP-7 in rodent lung damage studies (26, 55).

3.2. Proteolytic cascades

The study of sE-cad shedding is greatly complicated by the existence of proteolytic cascades, particularly those involving the MMPs and the uPA system. Synthesized as zymogens, MMPs require proteolytic processing to become active, and this process can be mediated by other MMP family members. As summarized in reviews by Egeblad and McCawley: MMP-2 can generate active MMP-1, 2, 9, and 13; MMP-3 can generate active MMP 1, 3, 7, 8, 9, and 13; MMP-7 can generate active MMP-1, 2, 7 and 9; MMP-9 can generate active MMP-2; finally, MMP-14 can generate active MMP-2 and 13 (69, 70). Based on the ability of MMPs to activate other family members, studies examining upstream MMPs may have difficulty distinguishing effects due to catalytic activity on a substrate by the upstream metalloprotease versus a catalytic activation of a downstream mediator and subsequent substrate cleavage. In the uPA system, the proteolytic cascade is more manageable. Here, uPA converts plasminogen to plasmin, which can then cleave proMMP-2 and 9 to active MMP-2 and 9 (71). Because plasmin is a serine protease which can activate zinc-dependent metalloproteases, these different enzyme classes allow for specific inhibitors and easier determination of the responsible sheddase. The existence of proteolytic cascades coupled with the redundancy observed in E-cadherin cleavage may explain why sE-cad is observed in multiple patient conditions.

4. SE-CAD IS PRESENT IN PATIENT FLUIDS IN A VARIETY OF CONDITIONS

sE-cad was first observed in the conditioned media of MCF-7 cells by Wheelock *et al* (12), and since then, many studies have been conducted on patient fluids to determine whether sE-cad could serve as a biomarker for disease. The initial report by Katayama *et al* determined that levels of sE-cad do not vary significantly between men and women or different age groups (72). Although the initial focus on sE-cad was as a cancer biomarker for disease, progression, or recurrence, today there are several studies which describe the presence of sE-cad in other disease states, such as HIV infection and benign prostatic disease (73, 74). In order to be included, studies must have reported on the presence of sE-cad in more than one patient. For example, the initial report of sE-cad in patient samples by Katayama *et al* included a report for one pancreatic and one ovarian cancer patient (72), which was not sufficient for inclusion in this review (Table 2).

4.1. Cancer
4.1.1. Bladder

Sera from newly diagnosed bladder cancer patients have significantly (*P* equals 0.017) higher levels of sE-cad than normal controls (1,013 ng/ml v. 3,955 ng/ml) (75). Additionally, high levels of sE-cad correlate

with higher grade, number of tumors, and recurrence but not tumor bulk (75). In the urine, healthy controls exhibited 582 ng/ml of sE-cad in the urine, while bladder cancer patients averaged 1,272 ng/ml across all stages and grades (*P* less than 0.001) (76). The authors suggested, however, that using total protein in urine is equally effective at this determination (76). In a later study, Shi *et al* found that urine levels of sE-cad normalized to creatinine were significantly (*P* less than 0 .01) lower in normal (1.306 mg/mol) versus cancer samples (3.724 mg/mol), and that samples from recurrent patients (10.497 mg/mol) had significantly (*P* less than 0.01) higher levels of sE-cad than primary tumors (77). Additionally, they found that sE-cad correlated well with tumor grade, but not with stage, size, and the number of tumors (77). Based on these data, both serum and urine concentrations of sE-cad can be used to stratify bladder cancer patients.

4.1.2. Colorectal

In colorectal cancer, the initial report determined that there was no statistically significant difference between sE-cad levels from healthy controls and colorectal cancer patients (78). Later, Willmanns *et al* found that sE-cad levels were statistically different between healthy controls versus benign disease (*P* equals 0.005) versus cancer (*P* equals 0.009) (3,476 ng/ml; 5,248 ng/ml; 5,495 ng/ml) and that the highest levels were found in metastatic patients (79). They also observed that in this cancer cohort, patients with renal or hepatic failure had high levels of sE-cad and that patients who had been treated with chemotherapy had lower sE-cad levels compared to untreated patients (79). From these data, it is apparent that while sE-cad serum levels may be useful in determining cancer spread in untreated patients, it would be important to rule out organ failure or dysfunction.

4.1.3. Esophageal squamous cell carcinoma

The recent study in squamous cell carcinoma compared the pre-operative levels of sE-cad for patients who had surgery alone to those patients who had chemoradiation therapy (CRT) before surgery. Patients in the surgery alone arm had significantly (*P* equals 0.032) higher (5,108 ng/ml) levels of sE-cad than patients who had already received chemoradiation therapy (3,688 ng/ml) (80). This decrease of sE-cad levels after chemotherapy agrees with data from colorectal cancer patients (79), and suggests sE-cad could be used to monitor patient response to therapy. For the patients who received surgery, levels of sE-cad higher than the median pre-surgery sE-cad concentration correlated with a decrease in survival; however, there was no prognostic significance for patients who had undergone neoadjuvant CRT (80). Therefore, sE-cad as prognostic marker for esophageal squamous cell carcinoma would be limited to patients who have yet to undergo any treatment (80).

4.1.4. Gastric

Initials reports of elevated sE-cad in gastric cancer came from Katayama *et al* (2,000 ng/ml vs. 3,515 ng/ml; *P* less than 0.0001) (72), which were confirmed by Gofuku *et al* (81). Later studies by Chan *et al* demonstrated that not only was sE-cad elevated in gastric cancer patients

Table 2. sE-cad can be found in the fluids of patients with multiple conditions

Patient diagnosis	Type	sE-cad correlates with:	Source	sE-cad Levels (ng/mL)	Ref #
Cancer	Bladder	Cancer, grade, number, recurrence	Serum	N: 1013	(75)
		Cancer		C: 3955	
			Urine	N: .516 mg/mol	(76)
		Cancer, grade		C: 1.536 mg/mol	
			Urine	N: 1.306 +/- 1.249 mg/mol	(77)
		Recurrence		C: 3.724 +/- 1.892 mg/mol	
				R: 10.497 +/- 7.47.1 mg/mol	
	Colorectal	Cancer, Progression	Serum	N[1]: 3467	(79)
				B[1]: 5248	
				C[1]: 5495	
		Not significant	Serum	N: 3.53	(78)
				C: 3.17	
	Esophageal squamous cell carcinoma	Survival (surgery only)	Serum	PreOp: 5108.96	(80)
				PreCRT: 3688.932	
				PostCRT: 3981.029	
	Gastric	Cancer	Serum	N: 2515 +/- 744	(81)
				C: 4735 +/- 2310	
		Cancer	Serum	N: 5616	(82)
				C: 9344	
		Recurrence	Serum	sE-cad above 10000	(83)
		Survival	Serum	sE-cad above 10000	(84)
		Cancer	Serum	N: 2000	(72)
				C: 3510 +/- 1790	
	Liver	Cancer	Serum	N: 2000	(72)
				C: 5550 +/- 3110	
		Cancer	Serum	N: 5798	(87)
				C: 10759	
		Recurrence (early)		sE-cad above 8000	(87)
	Non-epithelial Leimyosarcoma	Cancer	Serum	N: 2000	(72)
				C: 3280 +/- 720	
	Leukemia	Cancer	Serum	C: 2520 +/- 1000	(72)
	Multiple Myeloma	Cancer	Serum	N: 622.9	(88)
				C: 3291.4	
		Survival	Serum	s-Ecad above 3000	(88)
	Non-small cell lung	Cancer	Serum	N: 1015	(89)
				C: 3455	
		Metastasis	Serum	L[2]: 2487.8	(89)
				M[2]: 4422.2	
		Cancer	Serum	N: 1015 +/- 125	(90)
				NSCL: 3455 +/- 1082.4	
				SCLC: 3428.3 +/- 1198.8	
		Metastasis	Serum	L NSCL: 2460 +/- 388.2	(90)
				M NSCL: 4579.5 +/- 279.3	
				L SCLC: 3035 +/- 586.9	
				M SCLC: 3871 +/- 77.7	
	Ovarian	Not significant	Serum	Luteal cyst: 3677	(92)
				Dermoid tumor: 2325	
				Cystadenoma: 2200	
				C: 2250	
		Cancer	Cyst	Luteal cyst: 2035	(92)
				Dermoid tumor: ND	
				Cystadenoma: 2000	
				C: 14500	
		Malignant ascites	Ascites	N: 2061 +/-1968	(27)
				C: 12241 +/-5314	
		Present in ascites	Ascites	C: 89.96 (ug/ul)/ug total protein	(30)
	Prostate	Cancer, metastasis	Serum	N: 6.270 ug/l	(74)
				L: 9.460 ug/l	
				M: 27.490 ug/l	
	Skin Basal cell	Not significant	Serum	N: 808 +/- 272	(94)
				C: 879 +/-485	
	Melanoma	Cancer, rising S100	Serum	N: 3198	(15)
		Cancer, metastasis		C: 4975	
			Serum	N: 808 +/- 272	(94)
		Invasion		M: Values not reported	
	Paget's disease	Not significant	Serum	C: Not reported	(94)
	Squamous cell		Serum	C: 838 +/- 374	(94)
Non-cancer	Acute pancreatitis	Severe cases	Serum	N: 5181 +/- 1350	(95)
				D: 17780 +/- 7853	
	Benign prostatic hyperplasia (BPH)	BPH	Serum	N: 6.27 ug/l	(74)
				B: 7.26 ug/l	
	Diabetes	Not significant	Serum	N: 2000	(72)
				D: 2330 +/- 1580	
		Not significant	Urine	N: 652.7 +/-87	(96)

				Diabetic: 721.9 +/-93	
	Diabetic nephropathy (DN)	Nephropathy	Urine	N: 652.7 +/-87 DN0: 721.9 +/-93 DN1: 2751.5 +/- 164 DN2: 5839.6 +/- 428	(96)

	Inflammatory skin diseases
	  Psoriasis
	  Dermatitis
		
		Severe cases
		Severe cases
			
			Serum
			Serum
				
				Values not reported
				Values not reported
					(94)

Infection	HIV	Viral load	Plasma	Values not reported	(73)
	Hepatitis	Not significant	Serum	N: 2000 D: 2340 +/- 520	(72)
Organ dysfunction	Multi-organ	Sepsis, organ dysfunction	Serum	N: 3280 D: 6000	(66)
	Cholesystectomy	Inflammation	Serum	Lap: 1850 +/- 250 Open: 3110 +/- 330	(67)

N: normal, C: cancer, M: metastatic D: disease, R: recurrence, NR: No recurrence, PreOp: Preoperative . CRT: neoadjuvant chemoradiation therapy. L: localized, M: metastatic, ND: not detected, NSCL: non-small cell lung cancer.   SCLC: small cell lung cancer. DN0: diabetic, no nephropathy, DN1: diabetic nephropathy, microalbuminuria, DN2: diabetic nephropathy, macroalbuminuria, Lap: laparoscopic 1Value conversion from log (sE-cad ng/ml) to sE-cad ng/ml by Grabowska and Day. 2Observation reported by study's author, but numbers generated by Grabowska and Day.

(5,616 ng/ml vs. 9,344 ng/ml; P equals 0.001) but also that this correlated with tumor size and poor outcome markers (82). When Chan et al determined the optimal sensitivity and specificity of sE-cad following tumor resection by ROC analysis, they determined 10,000 ng/ml as a point of elevated sE-cad. Once a patient's serum concentration of sE-cad exceeded 10,000 ng/ml, the patient would eventually recur (83). On average, elevated sE-cad levels predated the recurrence by 13 months (83). Most importantly, the sensitivity of this test was similar in patients with more and less advanced disease (83). In another study, Chan et al determined that a pre-therapeutic 10,000 ng/ml sE-cad concentration was also a predictor of survival, where 90% of patients whose sE-cad levels were above the cutoff had a survival time of less than three years (P equals 0.009) (84). Chan et al acknowledged the substantial differences between normal sE-cad levels in the Katayama study (72) and theirs (82) by suggesting that the differences may be attributed to the different populations used in the study. Later studies by Pedrazzani et al determined that while sE-cad is indeed elevated in gastric cancer patients as previously reported, there was a linear increase with sE-cad levels in normal controls and gastric tumor patients over time (85), which is inconsistent with previous observations (72).

sE-cad levels in gastric cancer patients can be decreased by resection or therapy. Resection alone can significantly (P less than 0.0001) reduce sE-cad levels (81). Later studies by Zhou et al demonstrated that if surgery was coupled with neoadjuvant Celecoxib therapy, patients showed a significant (P less than 0.01) decrease in sE-cad levels during therapy and a significant (P less than 0.01) decrease in sE-cad post surgery (86). Based on the data, the use of sE-cad in gastric cancer would be quite informative for patient survival and recurrence, particularly since there is a significant amount of time between elevated sE-cad levels and actual recurrence, which would allow for appropriate therapeutic intervention (83). Additionally, future studies of sE-cad in gastric patient response could provide an early indication of failed therapy and appropriate therapeutic intervention.

4.1.5. Liver
Patients with liver cancer have an increased level of sE-cad (P less than 0.0001; P less than 0.05) (72, 87). The levels reported, however, are quite disparate with normal controls being reported as 2000 ng/ml or 5,798 ng/ml and cancer levels at 5550 ng/ml versus 10,759 ng/ml (72, 87). Soyama et al also demonstrated that levels of sE-cad in the serum of patients did not correlate with levels of E-cadherin in hepatic lesions, tumor markers, size, number, vascular invasion, stage, gender, age, or viral status (87). However, patients with levels higher than 8,000ng/ml were more likely to recur and metastasize (P less than 0.05) (87). It appears that sE-cad could be a useful biomarker for disease spread and recurrence in liver cancer.

4.1.6. Non-epithelial
sE-cad levels can also be found in patients with non-epithelial tumors such as leukemia, multiple myeloma, and leiomyosarcoma. Patients with leukemia (myelogenous, monocytic, lymphatic) have increased levels of sE-cad (P less than 0.01), as do patients with leiomyosarcoma (P less than 0.05) (68). Multiple myeloma patients have five times higher levels of serum sE-cad than control samples (P less than 0.0001) (88). sE-cad is also a survival predictor, where patients with levels of sE-cad below 3,000 ng/ml lived longer (P equals 0.0015), and an increase in sE-cad of 100ng/ml increased their risk of death from multiple myeloma by 6% (P equals 0.013) (88). In a non-epithelial setting, the source of sE-cad cannot come from the tumor cells themselves; instead it is more likely due to the tumors invasion into epithelial tissue. For example, leiomyosarcomas can occur in the smooth muscles cells of the stomach and grow into the stomach proper, resulting in epithelial tissue damage and shed sE-cad. In new multiple myeloma patients, sE-cad levels could be used as a prognostic marker because high levels correlate with poor disease outcome (88).

4.1.7. Non-small cell lung (NSCLC)
In NSCLC patients, sE-cad serum levels are much higher than in healthy volunteers (3,455 ng/ml vs. 1,015 ng/ml; P less than 0.001) and the highest levels of sE-cad also correlate with metastasis (P less than 0.001)

(89). There was, however, no statistically significant difference between the levels of sE-cad and histological type of cancer (adenocarcinoma, squamous cell carcinoma, or large cell carcinoma), sex, or smoking habit (89). Later studies confirmed these observations in NSCL, but also demonstrated elevated levels of sE-cad in small cell lung cancer (SCLC) and that increasing levels of sE-cad correlated with metastasis in SCLC (90). This suggests that while sE-cad might be useful in determining whether a patient has metastatic disease, it would not be useful in disease classification.

As in gastric cancer, sE-cad levels in NSCLC can decrease following therapy. Reckamp *et al* evaluated serum sE-cad levels in NSCLC patients before and after Celecoxib and Erlotinib treatment (91). Although there was no difference between sE-cad levels between patients with partial response, stable disease, and progressive disease initially, after 8 weeks of therapy, patients who achieved a partial response had significantly lower levels of sE-cad than those with stable or progressive disease (P equals 0.021) (91), suggesting sE-cad may be a useful marker for therapeutic response.

4.1.8. Ovarian
In an early report of sE-cad levels in ovarian cancer, Darai *et al* determined that the levels of serum sE-cad between luteal cyst, dermoid tumor, cystadenoma and malignant tumors did not vary significantly (92). Conversely, when the cyst fluid was examined, the levels of sE-cad were significantly (P equals 0.001) higher in patients with malignant versus benign disease (92). Other researchers examined the ascites of benign ovarian disease or ovarian cancer patients and determined that patients with ovarian cancer had very high levels of sE-cad (P less than 0.000005) (27). Gil *et al* later confirmed the presence of sE-cad in malignant ascites of women with advanced ovarian cancer (30). Because serum sE-cad levels fail to distinguish between benign and malignant disease, the use of sE-cad as a biomarker in ovarian cancer would have to be limited to cyst fluid or ascites.

4.1.9. Prostate
The initial report of sE-cad in prostate cancer by Kuefer *et al* demonstrated the presence of sE-cad in prostate cancer tissues, with increased expression in metastatic deposits and significantly elevated serum levels in patients with metastatic disease (P less than 0.001) (93). Later studies comparing BPH, localized and metastatic prostate cancer sE-cad concentrations to healthy controls demonstrated significant differences (normal v. BPH P equals 0.023; BPH v. localized prostate cancer P equals 0.011; localized v. metastatic P less than 0.001) (74). In this study, Kuefer *et al* also evaluated sE-cad as a biomarker to predict outcome. Surprisingly, sE-cad at the time of diagnosis could predict biochemical failure, mainly that localized disease with high levels of sE-cad (above 7.9ug/l) would likely result in late biochemical failure (P less than 0.05) (74). In prostate cancer, therefore, sE-cad may be useful in stratifying patients, but the greatest use might be in categorizing high-risk for recurrence patients.

4.1.10. Skin
Levels of sE-cad in different types of skin cancer vary according to type. In basal or squamous cell carcinoma, the levels of serum sE-cad did not vary significantly from the healthy controls (94). In Paget's disease, the levels of sE-cad were significantly elevated above control samples, but only once the disease became invasive (94). In melanoma, early reports suggested that levels of sE-cad were elevated once patients had metastatic disease (94). Later studies confirmed that levels of sE-cad in melanoma patients were higher than in normal controls (3,198 ng/ml v. 4,975 ug/ml; P less than 0.05), and the expression of sE-cad correlated with rising S100 values, indicating melanoma progression (P less than 0.05) (15). Interestingly, high sE-cad levels were observed in some patients with low levels of S100, which the authors suggest, may be an indication that generation of sE-cad may serve as an early marker of progression for a subgroup of patients (15).

4.2. Non-cancer
Although sE-cad has been extensively studied as a biomarker in cancer, it has also been observed and evaluated as a biomarker for non-cancer conditions such as BPH, dermatitis, psoriasis, acute pancreatitis, diabetes, and diabetic nephropathy. In BPH patients, the levels of sE-cad were significantly higher than in normal control patients (P equals 0.023) , but not as high as those patients with prostate cancer (74). Similarly, patients suffering from acute psoriasis and dermatitis had elevated levels of sE-cad in their serum, but unlike skin cancers where sE-cad levels correlated with invasion (15, 94), in the non-cancer setting, sE-cad correlated with the severity of the disease (94).

Serum concentrations of sE-cad are also predictive of acute pancreatitis. As of 2009, when the study was conducted, the standard tests for acute pancreatitis were poor predictors of severity (95), so Sewpaul *et al* hypothesized that because patients with systemic inflammatory response shed sE-cad (66), sE-cad could be a used as a marker for acute pancreatitis. Indeed, sE-cad levels were elevated in patients with mild acute pancreatitis (7,358 ng/ml) versus acute severe pancreatitis (17,789 ng/ml) versus healthy controls (5,181 ng/ml) (P equals 0.0166; P equals 0.0039) (95). The levels of sE-cad in severe acute pancreatitis were also significantly higher (P equals 0.0073) than other abdominal inflammatory pathologies (acute diverticulitis, perforated duodenal ulcer, cholangitis, acute appendicitis, and acute cholecystitis), suggesting that sE-cad levels could be a specific predictor for acute severe pancreatitis (95). Most importantly, this study demonstrated that sE-cad levels at 12 hours from onset of pain could predict the severity of pancreatitis, allowing for appropriate intervention (95).

In diabetes, levels of sE-cad in the sera or urine of healthy controls versus diabetic patients do not show significant difference (72, 96). However, sE-cad levels in the urine may be useful in determining which diabetic patients are suffering from diabetic nephropathy (96). The urine levels of diabetic patients with normoalbuminuria, microalbuminuria, and macroalbuminuria vary significantly

Table 3. Consequences of sE-cad presence

Result	System	sE-cad source	Mechanism (if known)	Ref.
Disruption of adherens junctions	Ovarian cancer cell line Mouse mammary tumor cells	Recombinant, Fc fusion Antibody affinity chromatography		(27) (12)
Disruption of anti-viral function	Peripheral blood mononuclear cells from HIV patients	Recombinant sE-cad	Abrogation of IFN-gamma response	(73)
Disruption of cell aggregation	Pancreatic cancer cell line Canine kidney cell line	Immunodepletion Immunodepletion		(20) (31)
Invasion	Ovarian cancer cell line	Recombinant, Fc fusion		(30)
	Pancreatic cancer cell line	Immunodepletion		(20)
	Lung cancer cells	Immunodepletion; HAV peptide	Induction of MMP-2, 9, 14	(98)
	Canine kidney cells	Immunodepletion		(31)
Proliferation	Breast cancer cells	Recombinant, Fc fusion	HER2/HER3 phosphorylation	(16)
Signaling	Breast cancer cells	Endogenous; Recombinant, Fc fusion	Binding to HER2/HER3 ERK signaling	(16)
	Canine kidney cells	Recombinant, myc tag	pERK/pAKT via EGFR	(99)
	Peripheral blood mononuclear cell from HIV patients	Recombinant sE-cad	KLRG1 ligation	(73)
Survival	Canine kidney cells	Recombinant, myc tag	EGFR signaling	(99)

(P less than 0.001) , suggesting that sE-cad might be a biomarker for diabetic nephropathy (96).

4.3. Infection

For HIV infection, the levels of sE-cad correlate with viral load in patients. Interest in sE-cad in HIV infection arose from the observation that the intestine is a site of increased permeability in infected patients, suggesting a disruption in E-cadherin function (73). Indeed, high HIV viral titers significantly (*P* equals 0.004) correlate with high levels of serum sE-cad, suggesting that sE-cad is a marker for severity of infection (73). Conversely, acute hepatitis does not elevate the levels of sE-cad above controls (72).

4.4. Organ dysfunction

The levels of sE-cad are significantly (*P* equals 0.0019) higher in patients with sepsis and organ dysfunction as compared to normal controls, and tend to increase with the amount of organ dysfunction and sepsis in the patient (66). In surgery, the levels of sE-cad can be used as a biomarker of tissue injury and inflammation. For example, a comparison of open to laparoscopic cholecystectomy demonstrated that the less invasive laparoscopic procedure resulted in less sE-cad generated (*P* equals 0.04) (67). These studies suggest that sE-cad levels in patient serum can be used to determine the extent of tissue damage and systemic inflammatory response.

5. CONSEQUENCES OF SE-CAD PRESENCE

5.1. Disruption of cell-cell interactions

The initial report by Wheelock *et al* demonstrated that sE-cad purified from MCF-7 cells was capable of disrupting cell-cell adhesion between mouse mammary tumor cells which were already growing in clusters (12) (Table 3). Later work demonstrated that treatment of ovarian cancer cell lines with a recombinant human ectodomain of E-cadherin fused to Fc, resulted in disruption of established cell junctions (27). In re-aggregation assays, pancreatic cancer cells and MDCK

cells treated with sE-cad immunodepleted media, were more efficient at re-aggregating than the cells which were re-aggregating in the presence of conditioned media with sE-cad present (20, 31). The presence of sE-cad can, therefore, not only disrupt established adherens junctions, but can also interfere with establishing adherence junctions in cell re-aggregation assays.

sE-cad can also interfere with immune cell interactions by serving as a dummy ligand for KLRG1 and interfering with anti-viral functions. In HIV-infected peripheral blood mononuclear cells, the presence of a recombinant sE-cad interfered with the ability of T cells to secrete IFN-gamma in response to HIV-1 Gag stimulation (73). Because KLRG1 on the CD8^{+} T cells bound sE-cad, the HIV infected CD4^{+} T cells were not targeted, resulting in an increase in survival of infected cells (73). These data suggest that sE-cad is sufficient to disrupt cell-cell interactions which has implications for epithelial tissue stability and immune response.

5.2. Migration and invasion

The presence of sE-cad can also induce cells to invade. Ovarian cancer cells exposed to Fc-E-cadherin invade through a modified Boyden chamber (30). Similarly, pancreatic cancer and MDCK cells exposed to conditioned media containing sE-cad versus conditioned media immunodepleted of sE-cad, show much greater inclination toward migration in the presence of sE-cad (20, 97). In the case of lung cancer, the presence of sE-cad in the conditioned media or in the form of an activating HAV peptide based on EC1 of E-cadherin, can induce MMP-2, 9, and 14 transcription and activity as evaluated by zymography and increased invasion (98). sE-cad, therefore, can promote migration and invasion, which may be due to its ability to induce additional metalloprotease activity to aid in these processes.

5.3. Signaling, proliferation, and survival

There have been several studies that have examined the specific effects of sE-cad presence on cells.

In breast cancer cells, endogenous sE-cad can be observed bound to HER2 and HER3 by immunoprecipitation (16). Stimulation with exogenous Fc-E-cadherin results in phosphorylation of HER2 and HER3, as well as downstream ERK signaling (16). Work by Najy *et al* also demonstrated that using a recombinant sE-cad resulted in a proliferative response in breast cancer cells, which was not mediated by full length E-cadherin since the line has a homozygous deletion for CDH1 (16). In our studies, sE-cad can also bind EGFR and signal downstream through ERK (manuscript submitted to Cellular Signaling).

Conversely, in MDCK cells under serum free conditions, the anti-apoptotic signals provided by a myc-tagged sE-cad required E-cadherin expression (99). Treatment of these MDCK cells with a myc-tagged sE-cad resulted in signaling through EGFR and ERK (99). These studies suggest that sE-cad signaling is mediated by EGFR family members and, depending on the cellular context, may or may not be dependent on full length E-cadherin. Moreover, these studies suggest that sE-cad can stimulate proliferation and survival in non-transformed and transformed cells.

6. DISCUSSION

Although the accumulation of sE-cad was initially believed to be solely related to tumorigenesis, cell culture studies have revealed that the generation of sE-cad can be mediated by several mechanisms in a variety of pathological states. E-cadherin cleavage can be induced by pro-inflammatory cytokines, bacterial infection, serum withdrawal, apoptosis, and growth factors (13-31). To date, ADAMs (10 and 15), bacterial proteases (gingipains and BFT), cathepsins (B, L, S), MMPs (2, 3, 7, 9, and 14), KLK7, and plasmin have all been implicated in the generation of sE-cad (13-31), but the study of E-cadherin processing is complicated by redundancy and the presence of proteolytic cascades.

Proteolytic cascades, much like signal transduction cascades, allow for the amplification of a stimulus. Mainly, when protease A is activated, it can activate protease B or C. The problem lies in determining whether it is protease A which is acting on the substrate or protease B or C, particularly when both protease belong to the same family, for example MMP-2, 9, and 13. One definitive measure of protease and substrate specificity is to use recombinant and purified proteins or their catalytic domains in an *in vitro* cleavage assay. This method requires the protease to be able to act upon the substrate without activating another mediator. The downside to this approach, however, is that it removes the protease and substrate from physiologically relevant situations, such as activation of the protease, presence of protease inhibitors, cell membrane interactions, as well as proteins associated with the substrate. Therefore, while an *in vitro* cleavage assay will demonstrate cleavage in a best case scenario, it does not prove that the protease can act on the substrate in the context of a cell or biological system. The most thoroughly researched proteases, therefore, have extensive studies into their *in vitro* cleavage capabilities as well as

complementary studies utilizing genetic knockout strategies, protease targeting shRNAs, as well as other specific targeting agents.

Assuming all reports of E-cadherin sheddases are accurate, there is a significant redundancy in mechanisms that generate sE-cad. Because cell culture evidence suggests that sE-cad can disrupt cell adhesion, immune response, as well as induce signaling and invasion (Table 3) and is associated with disease severity in patients (Table 2), sE-cad may be more than a symptom of protease disregulation and may actually be contributing to the progression or severity of disease. Inhibiting E-cadherin cleavage, particularly in cancer, could be beneficial to patients and accomplished either by specific targeting of proteases implicated in a patient's disease or using broad-spectrum inhibitors. Due to the poorly understood complex role of protease families such as the MMPs in early clinical trials, unintentional targeting of the entire zinc metalloprotease family (MMPs and ADAMs), showed little efficacy in cancer patients with advanced disease (48), and has prompted the development of more specific inhibitors for specific proteases and families, such as inhibitors for ADAM10 and 17 and the MMPs (48, 100, 101). Although these specific inhibitors have not been designed for inhibiting E-cadherin cleavage per se, preventing the generation of sE-cad could provided an additional, albeit unintended, benefit to patients undergoing cancer therapy.

Regardless of the source of sE-cad, based on the published data, there are several observations that come to mind. First, sE-cad is present in a myriad of conditions from cancer to infection to organ failure, which suggests sE-cad will not be a singular biomarker for any specific disease or type. Instead, the use of sE-cad in a clinical setting would, most likely, be a prognostic marker and be used in conjunction with other biomarkers. Another issue with sE-cad as a biomarker is the ranges of sE-cad reported vary greatly for normal controls, and whether this has to do with race (82), healthy volunteers with unknown medical problems, or technical differences between laboratories executing the ELISA remains unclear. Should sE-cad be used as a biomarker for severity of disease, then the ELISA would have to be standardized on a national or international level, and part of that would have to entail analyzing different populations for serum sE-cad concentrations. Another approach would be to divide the sE-cad values by the normal control values, generating fold change values, but even this method produces considerable by overlap between various disease states (Figure 1).

There are, however, several appealing aspects to using sE-cad in a clinical setting. For one, the majority of conditions use serum or urine samples, which are easy collection procedures versus needle biopsies, etc. Additionally, since sE-cad can be used to screen for many health issues, the ELISA could be run often in a diagnostic laboratory and contribute insights into disease severity, progression, recurrence, therapeutic response, and prognosis. In disease severity, sE-cad can predict cases of severe acute pancreatitis after 12 hours of pain (95) as well
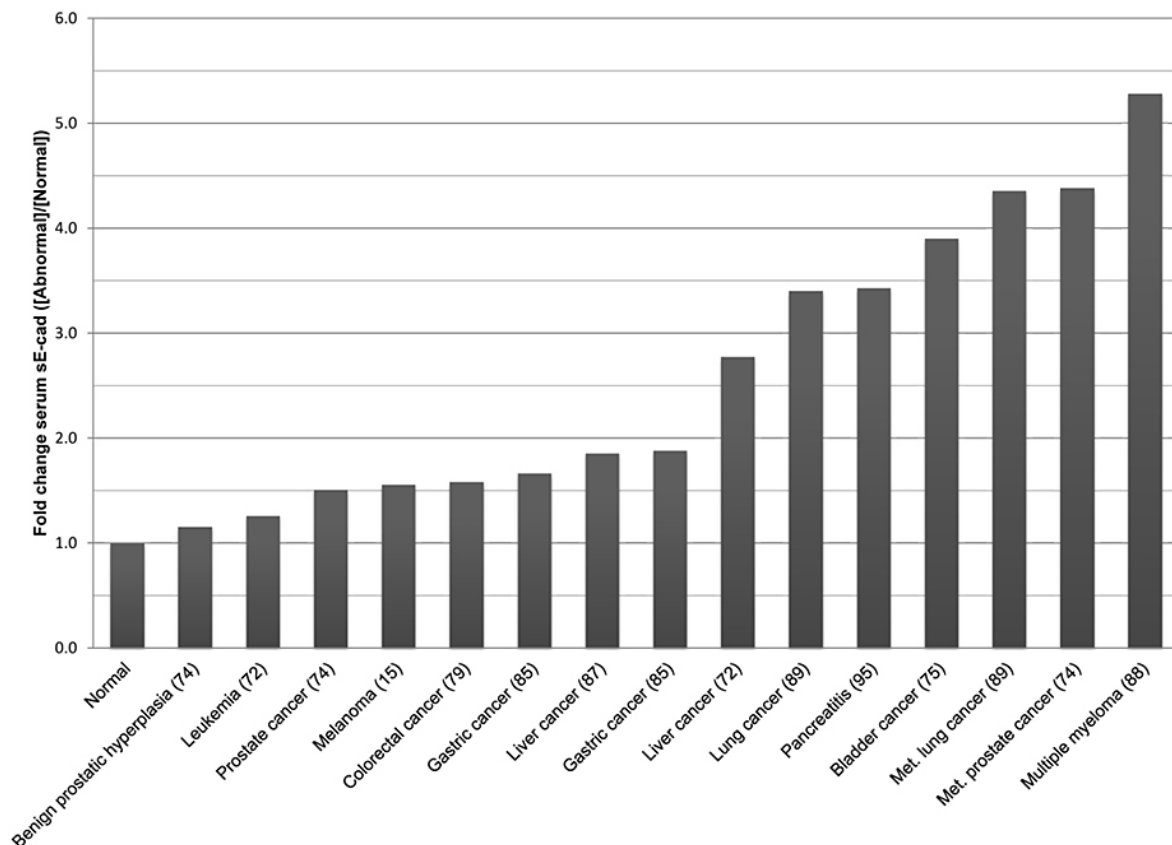
Figure 1. Comparison of serum sE-cad levels among various malignancies. Malignancy concentrations of sE-cad were divided by the average normal concentrations within each study to provide fold change values. Study reference in parenthesis.

as the levels of kidney dysfunction in diabetes patients (96). sE-cad levels can also predict disease progression and recurrence. On average, elevated sE-cad predicts gastric cancer recurrence an average 13 months before the recurrence (83), and higher levels of sE-cad in localized prostate cancer predict early recurrence (74). In melanoma, elevated levels of sE-cad in patients with low S100 values may also indicate patients likely to progress rapidly (15). Bladder, gastric, and liver cancer patients with high sE-cad levels are also more likely recur than patients with lower levels of sE-cad (75, 77, 83, 87). In terms of therapeutic response, NSCLC patients who have a partial response to Celecoxib and Erlotinib treatment show a decrease in sE-cad levels at 8 weeks (91). sE-cad is also an indicator of patient survival: esophageal squamous cell carcinoma, gastric cancer, and multiple myeloma patients with higher levels of sE-cad have a much shorter survival time than lower sE-cad patients (80, 84, 88). These studies provide convincing evidence that sE-cad may provide additional information on disease severity, progression, recurrence, and therapeutic response which could aid in determining appropriate therapeutic intervention.

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Abbreviations: E-cadherin: epithelial cadherin, sE-cad: soluble E-cadherin, ADAM: A Disintegrin and Metalloprotease, MMP: matrix metalloprotease, KLRG1: killer lectin receptor G1, EC: extracellular domain, NK: natural killer, BPH: benign prostatic hyperplasia, MDCK: Madin-Darby canine kidney, Il-1 beta: Interleukin-1 beta, TNF-alpha: tumor necrosis factor alpha, IFN-gamma: interferon gamma, LPS: lipopolysaccharide CTF: C-terminal fragment, H, pylori: Helicobacter pylori, EGF: epidermal growth factor, PKD1: protein kinase D1, shRNA: short hairpin RNA, uPA: urokinase-type plasminogen activator, LPA: Lysophosphatidic acid, TIMP: tissue inhibitor of metalloprotease, CRT: chemoradiation therapy

Key Words: Soluble E-cadherin, ADAM, MMP, Cancer, Disease, Biomarker, Review

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