Published in final edited form as:

Cell Signal. 2012 February ; 24(2): 532–538. doi:10.1016/j.cellsig.2011.10.004.

EGF Promotes the Shedding of Soluble E-cadherin in an ADAM10-dependent Manner in Prostate Epithelial Cells

Magdalena M. Grabowska^{a,b}, Brindar Sandhu^{a,c}, and Mark L. Day^{a,b,d}

^aDepartment of Urology, University of Michigan, 6131 Cancer Center, 1500 E. Medical Center Drive, Ann Arbor, MI 48109, USA

^bProgram in Cell and Molecular Biology, 6131 Cancer Center, 1500 E. Medical Center Drive, Ann Arbor, MI 48109, USA

Abstract

During the progression of prostate cancer, the epithelial adhesion molecule (E)-cadherin is cleaved from the cell surface by ADAM15 proteolytic processing, generating an extracellular 80kDa fragment referred to as soluble E-cadherin (sE-cad). Contrary to observations in cancer, the generation of sE-cad appears to correlate with ADAM10 activity in benign prostatic epithelium. The ADAM10-specific inhibitor INCB8765 and the ADAM10 prodomain inhibit the generation of sE-cad, as well as downstream signaling and cell proliferation. Addition of EGF or amphiregulin (AREG) to these untransformed cell lines increases the amount of sE-cad shed into the conditioned media, as well as sE-cad bound to EGFR. EGF-associated shedding appears to be mediated by ADAM10 as shRNA knockdown of ADAM10 results in reduced shedding of sE-cad. To examine the physiologic role of sE-cad on benign prostatic epithelium, we treated BPH-1 and large T immortalized prostate epithelial cells (PrEC) with an sE-cad chimera comprised of the human Fc domain of IgG₁, fused to the extracellular domains of E-cadherin (Fc-Ecad). The treatment of untransformed prostate epithelial cells with Fc-Ecad resulted in phosphorylation of EGFR and downstream signaling through ERK and increased cell proliferation. Pre-treating BPH-1 and PrEC cells with cetuximab, a therapeutic monoclonal antibody against EGFR, decreased the ability of Fc-Ecad to induce EGFR phosphorylation, downstream signaling, and proliferation. These data suggest that ADAM10-generated sE-cad may have a role in EGFR signaling independent of traditional EGFR ligands.

Keywords

ADAM10; EGFR; soluble E-cadherin

© 2011 Elsevier Inc. All rights reserved.

^dCorresponding author: 6306 CCGC, E. Medical Center Drive, Ann Arbor, MI 48109, USA. mday@umich.edu Phone: 1-734-763-9968. Fax: 1-734-647-9271.

^cPresent address: Genetics and Molecular Biology Graduate Program, Emory University, Atlanta, GA 30322, USA

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Epithelial (E)-cadherin is a homophilic adhesion molecule, which is expressed at the basolateral membrane of epithelial tissues. Within the adherens junction, cellular adhesion requires E-cadherin homodimerization with an adjacent E-cadherin and then further complex formation with a homodimer on an adjacent cell [1]. Intracellular interactions of E-cadherin with beta-catenin, p120, and alpha catenin also support adhesion and stabilization of the adherens junction [2–5]. E-cadherin can be lost from the cell surface by promoter hypermethylation, gene deletion and mutation or proteolytic cleavage [6, 7]. Previously, we have observed that E-cadherin can be shed into the serum of benign prostatic hyperplasia (BPH) and prostate cancer patients [8]. One of the sheddases implicated in E-cadherin cleavage is ADAM10.

ADAM10 is a member of the ADAM (A Disintegrin And Metalloprotease) family of zinc-dependent metalloproteases which is composed of 40 members, of which 12 members (including ADAM10) are catalytically active [9]. Family members are characterized by five extracellular domains: prodomain, metalloprotease, disintegrin, cysteine-rich, and EGF-like. The multiple domains of ADAMs allow for a myriad of functions including proteolysis, integrin binding, and signal transduction [9]. ADAM10 dysregulation in inflammation and disease has made the protein's catalytic domain a target for therapy [10–12], but ADAM10 also appears to play significant roles in normal biology. The *Adam10* $-/-$ mouse is embryonic lethal at embryonic day 9.5, with defective central nervous system and heart development [13], and studies of tissue specific *Adam10* $-/-$ mice have also indicated a critical role for ADAM10 in brain, cardiovascular, thymocyte, skin, marginal zone B cell development and intestinal cell sorting [14–19], suggesting ADAM10 may also play critical roles in development and adult tissues. *In vitro*, ADAM10 has been implicated in E-cadherin cleavage in keratinocytes and gastric cancer cell lines [20, 21]. In the prostate, membranous ADAM10 expression is high in benign prostatic hyperplasia (BPH) patient samples [22], and E-cadherin and ADAM10 co-localizes at the adherens junction [23]. Because ADAM10 is predominantly a sheddase, which is known to cleave epidermal growth factor (EGF)-like ligands from the cell-surface, thus promoting epidermal growth factor receptor (EGFR) family signaling [24], we hypothesized that ADAM10 generated sE-cad contributes to EGFR signaling by binding EGFR.

The EGFR family (EGFR/HER1/ErbB1, HER2/ErbB2/Neu, HER3/ErbB3, and HER4/ErbB4) is a prominent family of receptor tyrosine kinases. Comprised of an extracellular ligand binding domain, a transmembrane region, and a cytoplasmic tail containing the tyrosine kinase domain, these receptors play critical roles in development and cancer [25]. In the normal adult prostate, EGFR is expressed in basal cells and is localized in the lateral membrane junctions between the basal and luminal epithelium, while in BPH patients, EGFR staining expands to include moderate staining in a portion of luminal epithelium [26]. 36% of BPH samples over-express EGFR [27], and increased levels of EGFR expression correlate with BPH grade [28]. Some studies have even reported that by immunohistochemistry, BPH samples express more EGFR than prostate cancer samples [26, 29]. These results suggest that EGFR may be a component of a regulatory pathway involved in aberrant epithelial hyperproliferation and disease in the prostate gland [28, 30].

We have previously published on the interaction between sE-cad and HER2/HER3 [31], and now present new evidence demonstrating a functional interaction between sE-cad and EGFR using benign prostatic epithelial models. This study also describes a novel signaling axis involving sE-cad shedding and EGFR binding. Characterization of this signaling mechanism in the prostate would establish the sE-cad/EGFR axis as a potentially important mechanism of prostatic epithelial proliferation and possibly disease.

2. Materials and Methods

2.1. Cell culture

Benign prostatic hyperplasia -1 (BPH-1) and prostate epithelial cells immortalized with large T antigen (PrEC) were cultured in RPMI 1640 (Lonza) with 8% fetal bovine serum (HyClone) and 2mmol/L L-glutamine (Invitrogen) and Pen/Strep Amphotericin B (Pen/Strep: 10,000U/mL, Ampho 25µg/mL; Bio Whittaker). Knockdown cell lines were additionally supplemented with 100µg/µl Zeocin (Invitrogen). Cells were incubated at 37°C.

2.2. Cell treatments

Cells were pretreated with or treated in serum free, phenol free RPMI (Gibco). Stock solutions of cell treatments: 10ng/µL EGF in PBS, 100ng/µL AREG in PBS, 100ng/µL Fc-E-cadherin in PBS (R&D Systems), 100ng/µL Fc in PBS (R&D Systems); .05M 1,10 phenanthroline in methanol (Sigma); 10mM INCB8765 in DMSO [12] (Incyte); prodomain of ADAM10 in 10% glycerol/PBS [11] (Biozyme); 2mg/mL cetuximab (ImClone).

2.3. Protein isolation, Western blotting and immunoprecipitation

Cells were harvested by scraping and lysed as previously reported [32]. Lysates were pelleted at 12,000rpm for 8 minutes at 4°C. The supernatants were collected and quantitated using a Bradford (BioRad) assay with each sample being run in triplicate. For western blotting, equal amounts of protein were loaded into precast Tris-glycine SDS gels (Invitrogen) and transferred to nitrocellulose membranes (Millipore). Blots were blocked with 10% milk in TBST buffer, probed with antibodies diluted in 2.5% milk in TBST, and developed using ECL (Pierce; high sensitivity Millipore). Antibodies: E-cadherin (HECD-1, Invitrogen); EGFR (Ab-15, Neomarkers); ADAM10, tubulin (Millipore); phosphoERK, ERK, phosphoEGFR Y992, phosphoEGFR Y1068 (Cell Signaling).

For immunoprecipitation (IP), equal amounts of protein were pre-cleared with 100µl of a 50/50 mix of Sepharose A beads (Invitrogen) and 2.5% milk in TBST containing antibody-specific animal IgG for 30 minutes with end over end rotation. Lysates were then spun down for 3min at 8,000rpm and supernatants were transferred to new tubes containing 1µg of antibody and rotated end over end for 1hr at 4°C. Beads were then added and after another hour of rotation, IPs were spun down for 3min at 8,000rpm and supernatants aspirated. Beads were washed three times and spun down. After final wash, supernatant was aspirated off and 35µL of β-mercaptoethanol (βME)-containing loading buffer was added. After 5min at 100°C, IPs were again spun down and supernatants collected for Western blotting.

2.4. In vitro cleavage assay

The *in vitro* cleavage assay has been previously described [31]. Briefly, immunopurified ADAM10 and E-cadherin were combined in Eppendorf tubes in PBS for 8hrs at 37°C. After incubation, 15µl of βME-containing loading buffer were added and samples were boiled for 5min, spun down, and supernatants collected.

2.5. Proliferation assays

5,000 BPH-1 or 10,000 PrEC cells were plated in each well of a 96 well dish and allowed to grow up 24hrs. Cells were then washed and placed in serum free media and allowed to recover for 1hr. After 1hr in serum free media, cells were supplemented with treatments in quadruplicate for 24hr and 48hrs, at which point, CellTiter-Blue (Promega) was added and incubated for 1–4hrs. Plates were read on a Gemini Microplate Reader (Molecular Devices). Experimental values were normalized by dividing experimental values by the control values of each time point. With the exception of Figure 6, which is a representative experiment analyzed using the Kruskal-Wallis and Dunn's multiple comparison tests, three independent experiments were combined, and statistical analysis was performed by Graphpad Prism utilizing the one-way ANOVA and Tukey's multiple comparison test or unpaired, two-tailed t-test, as appropriate. Results were graphed as the mean with the standard error of the mean (SEM) for error bars. Values were considered significant if $p < 0.05$.

2.6. Generation of shADAM10 and shEGFP constructs

Knockdown cell lines for BPH-1 and PrEC were generated by lentiviral transduction of short hairpin constructs for ADAM10 (shA10) (forward: CAC CGC AGG TTC TAT CTG TGA GAA ACT CGA GTT TCT CAC AGA TAG AAC CTG C; reverse: AAA AGC AGG TTC TAT CTG TGA GAA ACT CGA GTT TCT CAC AGA TAG AAC CTG C) and EGFP (forward: CAC CGC CAC AAC GTC TATA TCA TGG CGA ACC ATG ATA TAG ACG TTG TGG; reverse: AAA AGC CAC AAC GTC TAT ATC ATG GTT CGC CAT GAT ATA GAC GTT GTG GC) to serve as the non-specific scrambled (scram) shRNA control. Constructs also encoded Zeocin (Invitrogen) antibiotic resistance, and culture media was supplemented with 100µg/mL Zeocin.

3. Results

3.1. Proteolytic activation of ADAM10 correlates with generation of sE-cad in immortalized prostate epithelial cells

Previously, we demonstrated that ADAM15-mediated shedding of sE-cad supported signaling through HER2 in human breast cancer cells [31]. To determine whether this mechanism plays a role in normal prostate biology, we evaluated sE-cad in prostate epithelial cells immortalized with large T antigen (PrEC) and benign prostatic hyperplasia cells (BPH-1). Under serum free conditions, sE-cad is generated in normal (PrEC) and hyperplastic (BPH-1) cells and shed into the culture media (Figure 1A). Unlike our previous findings, active ADAM15 does not correlate with sE-cad; instead the presence of active ADAM10 correlates with increased sE-cad, suggesting that ADAM10 plays a role in the cleavage event of E-cadherin in untransformed epithelial cells. Indeed, ADAM10

immunopurified from BPH-1 cells is capable of cleaving E-cadherin to sE-cad *in vitro*, which can be inhibited by the addition of the ADAM10-specific inhibitors INCB8765 (INC, Incyte) and the prodomain of ADAM10 (BIO, Biozyme). The broad-spectrum metalloprotease inhibitor 1, 10-phenanthroline (OPT) can also inhibit the generation of soluble E-cadherin (Figure 1B).

3.2. ADAM10 supports downstream signaling and proliferation in immortalized prostate epithelial cells

Because ADAM10 is a major sheddase of the pro-form of several growth factors, we hypothesized that ADAM10 supports cell signaling and proliferation in prostate epithelial cells through the release of these growth factors. Unsurprisingly, inhibition of ADAM10 with the small molecule inhibitors (INC) or its prodomain (BIO) in BPH-1 cells reduces signaling through ERK (Figure 2A). OPT treatment of BPH-1 cells inhibits all metalloproteases and completely inhibits ERK signaling, suggesting there are metalloproteases beyond ADAM10 that contribute to ERK signaling. Inhibition of ADAM10 by INC also reduces proliferation in BPH-1 cells as compared to vehicle controls (Figure 2B). ADAM10 knockdown in PrEC cells also results in a loss of ERK signaling and a decrease in proliferation (Figure 2C, D).

3.3. sE-cad generation is promoted by EGF and mediated by ADAM10

In untransformed cells, the shedding of sE-cad into conditioned media is promoted by the addition of EGF or AREG, and increasing concentrations of these EGFR ligands results in increasing levels of sE-cad as compared to serum free controls (Figure 3A). We theorized that this EGFR ligand promoted generation of sE-cad was mediated by ADAM10 and we generated knockdown cell lines to test this hypothesis. Knockdown of ADAM10 in BPH-1 cells did not affect ADAM15 expression following EGF treatment (Supplemental Figure 1). As hypothesized, ADAM10 knockdown in BPH-1 and PrEC cells reduced sE-cad generation (Figure 3B, C), suggesting that ADAM10 is responsible for E-cadherin cleavage in normal prostate epithelium.

3.4. sE-cad binds EGFR

Based on our previous studies in breast cancer cells [31], we theorized that sE-cad could play a role in downstream signaling in normal prostate epithelium. In order to determine whether traditional ligands could compete with sE-cad for binding to EGFR, we pre-treated BPH-1 and PrEC cells with the high-affinity ligand EGF or the low affinity ligand AREG [33]. We were surprised to observe that in the presence of these ligands, there is more sE-cad bound to the receptor, suggesting that EGFR ligands promote the interaction between EGFR and sE-cad in BPH-1 and PrEC cells (Figure 4A, B).

3.5. Fc-E-cadherin binds the extracellular domain of EGFR and induces receptor phosphorylation and downstream signaling

In order to determine the effect of sE-cad on non-transformed epithelial cells, a commercially available sE-cad analog was used. Fc-Ecadherin (Fc-Ecad) is a chimeric protein of human IgG₁ Fc domain and the five extracellular domains of E-cadherin, which is

the same as sE-cad fused to human IgG₁. To account for off-target Fc effects, Fc alone treatments were included in the analysis. Treatment of BPH-1 and PrEC cells with .1nM or 1nM Fc-Ecad, respectively, results in the phosphorylation of EGFR at tyrosine residues 992 and 1068, which correspond to activation of the ERK pathway [34, 35], and results in ERK phosphorylation as compared to treatment with Fc alone (Figure 4C, D). Fc-Ecad treatment did not induce phosphorylation of any other receptors, as evaluated by the Human Phospho-Receptor Tyrosine Kinase Array Kit (R&D) (Supplemental Figure 2).

The addition of Fc-E-cad to the untransformed cell lines also supports an increase in proliferation (Figure 5A, B). The increased proliferation observed with Fc-Ecad can partially rescue the proliferation defect observed in the BPH-1 and PrEC shADAM10 cell lines, suggesting that sE-cad signaling may be a component of ADAM10-mediated proliferation and signaling (Figure 6A, B). Because this proliferative effect is mediated by EGFR, we theorized that pre-treatment of BPH-1 and PrEC cells with cetuximab, a therapeutic monoclonal antibody against the extracellular domain of EGFR, would prevent Fc-Ecad induced signaling. Indeed, pre-treatment of BPH-1 and PrEC cells with cetuximab is enough to reduce the amount of ERK signaling induced by Fc-Ecad and EGF (Figure 7A, B). 20nM or 10nM Cetuximab pre-treatment is also sufficient to reduce the proliferative effect of Fc-Ecad for BPH-1 and PrEC cells, respectively (Figure 7C, D).

4. Discussion

E-cadherin plays critical roles in epithelial cell maintenance, and its loss from the cell surface during tumor progression has been well documented. Previous work in the lab has focused on the accumulated 80kDa fragment known as sE-cad and the metalloprotease responsible during breast and prostate cancer progression [8, 32, 36, 37]. While ADAM15 appears to be the predominant metalloprotease responsible for sE-cad shedding in breast and prostate cancer, it may not play a significant role in normal prostate biology.

This work has demonstrated that ADAM10 plays a significant role in the proliferation of prostate epithelium, and that by blocking ADAM10 activity or reducing its expression, there is decreased cell signaling and division. We also demonstrated that non-transformed prostate epithelial cells can be induced to generate sE-cad by the addition of EGF or AREG and that this process is dependent upon ADAM10. While the cleavage of E-cadherin by ADAM10 is not a novel finding [20], this is the first report of it in prostate epithelial cells.

Additionally, the promotion of sE-cad generation by EGF suggests that shedding of E-cadherin may contribute to epithelial to mesenchymal transition in pathologies such as BPH and prostate cancer. The loss of differentiated epithelial phenotypes and the acquisition of motility and invasiveness which are the hallmarks of EMT, play critical roles in tumor progression [38, 39]. In prostate cancer, EMT plays a critical role in bone metastasis [40], and emerging evidence suggests EMT may play a role in BPH as well. Work by Alonso-Magdalena *et al* suggested that in human BPH samples, the disease did not arise from stroma, but from mesenchymal cells derived from the epithelium, which implicates the process of EMT [41]. These data suggest that the cleavage of E-cadherin induced by a potent EMT activator such as EGF may contribute to EMT progression.

While previous publications from our group have demonstrated that sE-cad can bind to HER2 and HER3 [31], this is the first report of the sE-cad bound to EGFR in a non-transformed prostate cell line model. Other studies have reported the interaction of full length E-cadherin and EGFR, which depends on the extracellular domain of E-cadherin and not on cytoplasmic betacatenin or p120 binding [42]. Our studies have also demonstrated that Fc-Ecad can induce phosphorylation of EGFR, which induces downstream signaling and culminates in increased proliferation. These experiments suggest that sE-cad binding to EGFR may play a role in aberrant proliferation of prostate epithelial cells as described in BPH. Other studies have demonstrated that sE-cad can disrupt cell adhesion, anti-viral function, cell aggregation [7, 43–46], and support invasion, migration, proliferation, and survival [31, 43, 44, 46–49]. Taken together, these results suggest that EGF promoted-ADAM10 cleavage of E-cadherin may contribute to proliferative disorders by allowing sE-cad to bind EGFR and alter downstream signaling and proliferation in prostate epithelium.

5. Conclusions

Based on these data, we conclude that ADAM10 plays a role in prostate epithelial cell signaling. We have also determined that in prostate epithelial cells, EGF can induce E-cadherin cleavage in an ADAM10-dependent manner. sE-cad can then bind to EGFR and stimulate EGFR phosphorylation, downstream signaling through ERK, and proliferation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Dr. Stefan Stoll for providing the knockdown constructs, Dr. Marcia Moss (Biozyme) for providing the prodomain of ADAM10, and Dr. Peggy Scherle (Incyte) for providing the INCB8765. This work was supported by 1 R01 CA154252 (MLD), NIH T 32 GM007315 (MMG), and 2 T32 CA009676 (MMG).

Abbreviations

sE-cad	soluble E-cadherin
pA10	proform of ADAM10
mA10	mature form of ADAM10
INC	ADAM10 inhibitor, INCB8765
BIO	ADAM10 prodomain
OPT	1, 10-Phenanthroline
AREG	amphiregulin

References

1. Boggon TJ, Murray J, Chappuis-Flament S, Wong E, Gumbiner BM, Shapiro L. Science. 2002; 296:1308–1313. [PubMed: 11964443]
2. Chen Y-T, Stewart DB, Nelson WJ. J. Cell Biol. 1999; 144:687–699. [PubMed: 10037790]

3. Vasioukhin V, Bauer C, Yin M, Fuchs E. *Cell*. 2000; 100:209–219. [PubMed: 10660044]
4. Drees F, Pokutta S, Yamada S, Nelson WJ, Weis WI. *Cell*. 2005; 123:903–915. [PubMed: 16325583]
5. Ireton RC, Davis MA, van Hengel J, Mariner DJ, Barnes K, Thoreson MA, Anastasiadis PZ, Matrisian L, Bundy LM, Sealy L, Gilbert B, van Roy F, Reynolds AB. *J. Cell Biol.* 2002; 159:465–476. [PubMed: 12427869]
6. Hirohashi S. *Am. J. Pathol.* 1998; 153:333–339. [PubMed: 9708792]
7. Wheelock M, Buck C, Bechtol K, Damsky C. *J. Cell. Biochem.* 1987; 34:187–202. [PubMed: 3611200]
8. Kuefer R, Hofer MD, Zorn CSM, Engel O, Volkmer BG, Juarez-Brito MA, Eggel M, Gschwend JE, Rubin MA, Day ML. *Br. J. Cancer.* 2005; 92:2018–2023. [PubMed: 15870707]
9. Edwards DR, Handsley MM, Pennington CJ. *Mol. Aspects Med.* 2008; 29:258–289. [PubMed: 18762209]
10. Crawford HC, Dempsey PJ, Brown G, Adam L, Moss ML. *Curr. Pharm. Des.* 2009; 15:2288–2299. [PubMed: 19601831]
11. Moss ML, Bomar M, Liu Q, Sage H, Dempsey P, Lenhart PM, Gillispie PA, Stoeck A, Wildeboer D, Bartsch JW, Palmisano R, Zhou P. *J. Biol. Chem.* 2007; 282:35712–35721. [PubMed: 17895248]
12. Zhou B-BS, Peyton M, He B, Liu C, Girard L, Caudler E, Lo Y, Baribaud F, Mikami I, Reguart N, Yang G, Li Y, Yao W, Vaddi K, Gazdar AF, Friedman SM, Jablons DM, Newton RC, Fridman JS, Minna JD, Scherle PA. *Cancer Cell*. 2006; 10:39–50. [PubMed: 16843264]
13. Hartmann D, de Strooper B, Serneels L, Craessaerts K, Herreman A, Annaert W, Umans L, Lubke T, Lena Illert A, von Figura K, Saftig P. *Hum. Mol. Genet.* 2002; 11:2615–2624. [PubMed: 12354787]
14. Jorissen E, Prox J, Bernreuther C, Weber S, Schwanbeck R, Serneels L, Snellinx A, Craessaerts K, Thathiah A, Tesseur I, Bartsch U, Weskamp G, Blobel CP, Glatzel M, De Strooper B, Saftig P. *J. Neurosci.* 2010; 30:4833–4844. [PubMed: 20371803]
15. Weber S, Niessen MT, Prox J, Lüllmann-Rauch R, Schmitz A, Ralf Schwanbeck, Blobel CP, Jorissen E, Strooper Bd, Niessen CM, Safti P. *Development*. 2011; 138:495–505. [PubMed: 21205794]
16. Tian L, Wu X, Chi C, Han M, Xu T, Zhuang Y. *Int. Immunol.* 2008; 20:1181–1187. [PubMed: 18635581]
17. Zhang C, Tian L, Chi C, Wu X, Yang X, Han M, Xu T, Zhuang Y, Deng K. *Dev. Dyn.* 2010; 239:2594–2602. [PubMed: 20803506]
18. Solanas G, Cortina C, Sevillano M, Batlle E. *Nat Cell Biol.* 13:1100–1107. [PubMed: 21804545]
19. Gibb DR, Shikh ME, Kang D-J, Rowe WJ, Sayed RE, Cichy J, Yagita H, Tew JG, Dempsey PJ, Crawford HC, Conrad DH. *J. Exp. Med.* 2010; 207:623–635. [PubMed: 20156974]
20. Maretzky T, Scholz F, Koten B, Proksch E, Saftig P, Reiss K. *J. Invest. Dermatol.* 2008; 128:1737–1746. [PubMed: 18200054]
21. Schirrmester W, Gnad T, Wex T, Higashiyama S, Wolke C, Naumann M, Lendeckel U. *Exp. Cell Res.* 2009; 315:3500–3508. [PubMed: 19665015]
22. Arima T, Enokida H, Kubo H, Kagara I, Matsuda R, Toki K, Nishimura H, Chiyomaru T, Tatarano S, Idesako T, Nishiyama K, Nakagawa M. *Cancer Sci.* 2007; 98:1720–1726. [PubMed: 17727679]
23. Wild-Bode C, Fellerer K, Kugler J, Haass C, Capell A. *J. Biol. Chem.* 2006; 281:23824–23829. [PubMed: 16777847]
24. Blobel CP. *Nat Rev Mol Cell Biol.* 2005; 6:32–43. [PubMed: 15688065]
25. Olayioye MA, Neve RM, Lane HA, Hynes NE. *EMBO J.* 2000; 19:3159–3167. [PubMed: 10880430]
26. Leav I, McNeal JE, Ziar J, Alroy J. *Hum. Pathol.* 1998; 29:668–675. [PubMed: 9670822]
27. Schwartz S, Caceres C, De Torres I, Morote J, Rodriguez-Vallejo JM, Gonzalez J, Reventos J. *Int. J. Cancer.* 1998; 76:519–522. [PubMed: 9590128]
28. Kumar VL, Majumder PK, Kumar V. *Int. Urol. Nephrol.* 2000; 32:73–75. [PubMed: 11057777]

29. Ibrahim G, Kerns B, MacDonald J, Ibrahim S, Kinney R, Humphrey P, Robertson C. *J Urol.* 1993; 149:170–173. [PubMed: 7678041]
30. Kumar VL, Majumder PK, Gujral S, Kumar V. *Cancer Lett.* 1998; 134:177–180. [PubMed: 10025878]
31. Najy AJ, Day KC, Day ML. *J. Biol. Chem.* 2008; 283:18393–18401. [PubMed: 18434311]
32. Najy AJ, Day KC, Day ML. *Cancer Res.* 2008; 68:1092–1099. [PubMed: 18281484]
33. Shoyab M, Plowman GD, McDonald VL, Bradley JG, Todaro GJ. *Science.* 1989; 243:1074–1076. [PubMed: 2466334]
34. Rojas M, Yao S, Lin Y-Z. *J. Biol. Chem.* 1996; 271:27456–27461. [PubMed: 8910327]
35. Emlet DR, Moscatello DK, Ludlow LB, Wong AJ. *J. Biol. Chem.* 1997; 272:4079–4086. [PubMed: 9020117]
36. Kuefer R, Day KC, Kleer CG, Sabel MS, Hofer MD, Varambally S, Zorn CS, Chinnaiyan AM, Rubin MA, Day ML. *Neoplasia.* 2006; 8:319–329. [PubMed: 16756724]
37. Kuefer R, Hofer MD, Gschwend JE, Pienta KJ, Sanda MG, Chinnaiyan AM, Rubin MA, Day ML. *Clin. Cancer. Res.* 2003; 9:6447–6452. [PubMed: 14695147]
38. Thiery JP. *Nat Rev Cancer.* 2002; 2:442–454. [PubMed: 12189386]
39. Polyak K, Weinberg RA. *Nat Rev Cancer.* 2009; 9:265–273. [PubMed: 19262571]
40. Zhau H, Odero-Marah V, Lue H-W, Nomura T, Wang R, Chu G, Liu Z-R, Zhou B, Huang W-C, Chung L. *Clin. Exp. Metastasis.* 2008; 25:601–610. [PubMed: 18535913]
41. Alonso-Magdalena P, Brössner C, Reiner A, Cheng G, Sugiyama N, Warner M, Gustafsson J-A. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:2859–2863. [PubMed: 19196965]
42. Qian X, Karpova T, Sheppard AM, McNally J, Lowy DR. *EMBO J.* 2004; 23:1739–1784. [PubMed: 15057284]
43. Ryniers F, Stove C, Goethals M, Brackenier L, Noe V, Bracke M, Vandekerckhove J, Mareel M, Bruyneel E. *Biol. Chem.* 2002; 383:159–165. [PubMed: 11928810]
44. Streeck H, Kwon DS, Pyo A, Flanders M, Chevalier MF, Law K, Jülg B, Trocha K, Jolin JS, Anahtar MN, Lian J, Toth I, Brumme Z, Chang JJ, Caron T, Rodig SJ, Milner DA, Piechoka-Trocha A, Kaufmann DE, Walker BD, Altfeld M. *Blood.* 2011; 117:5112–5122. [PubMed: 21403126]
45. Symowicz J, Adley BP, Gleason KJ, Johnson JJ, Ghosh S, Fishman DA, Hudson LG, Stack MS. *Cancer Res.* 2007; 67:2030–2039. [PubMed: 17332331]
46. Johnson SK, Ramani VC, Hennings L, Haun RS. *Cancer.* 2007; 109:1811–1820. [PubMed: 17354228]
47. Gil OD, Lee C, Ariztia EV, Wang F-Q, Smith PJ, Hope JM, Fishman DA. *Gynecol. Oncol.* 2008; 108:361–369. [PubMed: 18073130]
48. Nawrocki-Raby B, Gilles C, Polette M, Bruyneel E, Laronze J-Y, Bonnet N, Foidart J-M, Mareel M, Birembaut P. *Int. J. Cancer.* 2003; 105:790–795. [PubMed: 12767064]
49. Inge LJ, Barwe SP, D'Ambrosio J, Gopal J, Lu K, Ryazantsev S, Rajasekaran SA, Rajasekaran AK. *Exp. Cell Res.* 2011; 317:838–848. [PubMed: 21211535]

Highlights

- We report ADAM10-dependent generation of soluble E-cadherin in prostate cells.
- ADAM10-dependent generation of soluble E-cadherin is potentiated by EGF treatment.
- Soluble E-cadherin binds EGFR and induces phosphorylation and signaling via ERK.

A. **B.**

Figure 1. Generation of sE-cad is associated with active ADAM10 expression in untransformed prostate epithelial cells

A. ADAM10 and ADAM15 profiles of BPH-1 and PrEC cells treated with serum free media for 24hrs. B. *In vitro* cleavage assay with ADAM10 and E-cadherin immunopurified from BPH-1 cells using ADAM10 specific inhibitors: Incyte inhibitor INCB008765 (INC), Biozyme ADAM10 prodomain (BIO), and broad spectrum metalloprotease inhibitor, 1,10-phenanthroline (OPT). CM: conditioned media. WCL: whole cell lysate. sE-cad: soluble E-cadherin. fE-cad: Full length E-cadherin. pA10: pro form of ADAM10. mA10: mature form of ADAM10. pA15 pro form of ADAM15. mA15: mature form of ADAM15.

Figure 2. ADAM10 contributes to downstream signaling and proliferation in untransformed prostate epithelial cells

A. BPH-1 cells treated with ADAM10 specific inhibitors (1 μ M, 10 μ M INC; 1 μ M BIO) for one hour show decreased pERK signaling, while the broad spectrum metalloprotease inhibitor OPT (1mM) abolishes all pERK signaling. B. BPH-1 cells treated with 1 μ M INC also show decreased proliferation as compared to vehicle (DMSO). C. Knockdown of ADAM10 in PrEC correlates with a decrease in pERK signaling in response to 5nM EGF as well as a decrease in proliferation (D). NT: no treatment. Scram: non-specific shRNA control. shA10: shADAM10. pERK: phosphoERK.

Figure 3. EGFR ligands promote the generation of sE-cadherin in an ADAM10-dependent manner

A. Treatment of BPH-1 cells with increasing concentrations (nM) of EGF and AREG results in increased sE-cad in the conditioned media at 24 hours (5nM EGF and AREG duplicate lanes are shown as controls). The loss of ADAM10 by shRNA knockdown reduces the amount of sE-cad shed in response to 24 hour 5nM EGF stimulation in BPH-1 (B) and PrEC (C) cells. CM: conditioned media. WCL: whole cell lysate. NT: no treatment.

Figure 4. sE-cad can bind EGFR and result in downstream signaling

The addition of 5nM EGF and AREG (AR due to space constraints) for 15 minutes also increases sE-cad association with EGFR in BPH-1 (A) and PrEC (B) cells. Treatment of BPH-1 (C) and PrEC (D) cells with 1nM Fc-Ecad for 15 minutes (following 1hr pre-treatment in serum free media) results in increased phosphorylation of EGFR at tyrosine residues 992 (Y992), 1068 (Y1068) and increased phosphorylation of ERK, as compared to untreated and Fc domain (Fc) treated cells. NT: No treatment. fE-cad: full length E-cadherin. sE-cad: soluble E-cadherin.

A. **B.**

Figure 5. Fc-Ecad induces proliferation

BPH-1 cells (A) and PrEC (B) cells respond to .1nM or 1nM Fc-Ecad, respectively, by increasing cell proliferation. Values were considered significant if $p < 0.05$. *: $p < 0.05$

Figure 6. FcE-cad can rescue the proliferation defect in shADAM10 cells

1nM Fc-Ecad stimulation of the BPH-1 shA10 (A) and PrEC shA10 (B) cells results in partial rescue of the knockdown proliferation defect. Values were considered significant if $p < 0.05$. *: $p < 0.05$

Figure 7. Cetuximab inhibits signaling and proliferation in response to Fc-Ecad
 BPH-1 (A) and PrEC (B) cells treated for one hour with 10nM cetuximab (Cet) show inhibition of phosphoERK signaling in response to 5nM EGF and Fc-Ecad (FcE). BPH-1 (C) and PrEC (D) Fc-Ecad induced cell proliferation is inhibited by 20nM or 10nM cetuximab treatment. Values were considered significant if $p < 0.05$. Asterisks denote comparison between Fc-Ecad and Fc-Ecad + Cetuximab. 48hr difference between

Cetuximab and Cetuximab+ Fc-Ecad is not statistically significant for either cell line. NT: no treatment. NS: not significant. ***: $p < 0.0001$