Posttranslational Truncation and Inactivation of Human E-Cadherin Distinguishes Prostate Cancer from Matched Normal Prostate

Michael G. Rashid, Martin G. Sanda, Christopher J. Vallorosi, Jonathan Rios-Doria, Mark A. Rubin, and Mark L. Day

Department of Surgery, Section of Urology [M. G. R., M. G. S., C. J. V., J. R.-D., M. A. R., M. L. D.], Cellular and Molecular Biology Graduate Program [J. R.-D.], Department of Pathology [M. A. R.], and the University of Michigan Comprehensive Cancer Center [M. G. R., M. G. S., C. J. V., J. R.-D., M. A. R., M. L. D.], University of Michigan, Ann Arbor, Michigan 48109-0944

Abstract

An essential event in the progression of adenocarcinoma is the loss of organized epithelial attachment (both to the basement membrane and to adjoining epithelial cells). The E-cadherin cell adhesion molecule has an established function in maintaining normal phenotype and tissue homeostasis, and loss of E-cadherin function has been implicated in tumorigenesis. Aberrations in E-cadherin are associated with prostate cancer progression; however, these aberrations are not simply a result of prodigious allelic loss. We have previously demonstrated a novel posttranslational truncation within the cytosolic domain of native Mr 120,000 E-cadherin to a membrane-bound Mr 97,000 species. We hypothesize that truncation of E-cadherin is an inactivating event that is significantly increased in localized prostate tumors and that it represents a novel molecular event that may distinguish prostate cancer from adjacent normal tissue. E-cadherin was characterized by Western blot analysis in matched normal and cancer tissue from 18 prostate cancer patients. Imaging and densitometry software were used to quantify the truncation of E-cadherin by measuring the ratio of Mr 97,000 E-cadherin to Mr 120,000 E-cadherin, which was significantly increased in the tumor aspect of the prostate gland. Herein, we report the first experiment comparing case-matched human normal and cancerous prostate tissue in the context of E-cadherin truncation.

Introduction

Aberrations in E-cadherin expression have been noted in malignant degeneration of prostate epithelium, metastasis, and decreased patient survival (1–6). Loss of E-cadherin function does not seem secondary to allelic loss, but rather to transcriptional repression of the gene (7–9). Homeostasis of the prostate gland is, in part, maintained by the interepithelial transmembrane adhesion glycoprotein E-cadherin. The mature form of E-cadherin is a Mr 120,000 protein (E-cad120) that consists of an extracellular portion of five tandem repeats, the most distal of which is required for calcium-mediated homotypic binding between E-cadherin molecules from neighboring epithelial cells (10–13). E-cadherin also contains an intracellular portion that is responsible for linking E-cadherin to the actin cytoskeleton through α, β, and γ catenin that is necessary for the adhesive function of E-cadherin (11, 14–17). Studies from our laboratory using cell culture and in vivo models have demonstrated the role of E-cadherin-dependent aggregation in prostate and mammary epithelial survival. We have also demonstrated that the posttranslational cleavage of the native E-cad120 results in a novel membrane-bound Mr 97,000 fragment (E-cad97) in cells destined to undergo apoptosis (18). This cleavage effectively removes the β-catenin binding domain, rendering the E-cadherin molecule functionless. This inactivating truncation of E-cadherin may then represent a novel mechanism by which E-cadherin function is abrogated in human prostate cancer. In this study, we evaluated whether this truncation occurs in human prostate tissue with subsequent loss of the β-catenin binding domain and to determine whether E-cadherin truncation relates to clinical prostate cancer progression.

Materials and Methods

Tissue Procurement. Patients with localized prostate cancer provided written informed consent in an Institutional Review Board approved study at the University of Michigan to have their prostate tissue evaluated in experimental studies. Fresh radical prostatectomy specimens were frozen in liquid nitrogen within 30 min after surgical excision. Histological confirmation of both tumor and normal regions of each prostate gland were confirmed by a genitourinary pathologist at the University of Michigan, and the corresponding tumor or normal region was excised before processing for protein analysis.

Protein Preparation and Western Blot Analysis. To prepare protein lysates from procured human tissue, the samples were homogenized and allowed to lyse for 1 h (on ice) in protein lysis buffer [50 mM Tris (pH 7.5), 120 mM NaCl, and 0.5% NP40] in combination with the following protease inhibitors: 40 μM phenylmethylsulfonyl fluoride, 50 μg/ml leupeptin, 50 μg/ml aprotinin, and 200 μM orthovanadate. Lysates were centrifuged, and the supernatants were collected and quantitated in triplicate in a 96-well microtiter Bradford assay. All experimental samples were quantitated on the same microtiter plate against the same BSA standard curve. The absorbance was read using a dual mode (test filter at 595 nm and reference filter at 405 nm on a Dynatech Laboratories MRX Microplate reader using End Point Program version 1.1). Curve fitting was done using a linear power fit with tails. Protein (150 μg) from each tumor and normal pair was loaded and separated on 6% Tris/Glycine precast NOVEX gels and analyzed using the NOVEX and enhanced chemiluminescence (Amersham) detection systems as described previously (19).

Bradford Assay for Microtiter Plate

Densitometry. One-minute exposures of the Western blots from the matched tumor and normal prostate specimens were scanned at 300 dpi in gray scale format into Vista Scan. These files were then imported into NIH imaging, and densitometry of each E-cad120 and E-cad97 band was then performed. Western blot densitometry results of matched prostate cancer and normal prostate clinical specimens were analyzed by the Wilcoxon matched pairs test using Statistica software (StatSoft, Tulsa, OK). Stratification by stage or progression status was not possible due to limited variance of these parameters in the study cohort.

Antibodies. To detect the E-cad120 and E-cad97 forms of human E-cad, the HEC1-1 monoclonal antibody (Zymed Laboratories, Inc.), which was raised against the extracellular domain of E-cad, was used. The E-cad cytoplasmic antibody 4A2 was a gift from Dr. Margaret Wheelock (University of Toledo, Toledo, OH). For β-catenin immunoprecipitations and Western blots, the CAT-5H10 antibody (Zymed Laboratories, Inc.) was used. The appropriate
Western Blot Analysis of E-cad	extsuperscript{97} and E-cad	extsuperscript{120} in Human Prostate Tissue. Prior studies have associated apoptosis with the truncation of E-cadherin (18), but clinical prostate cancer specimens have not been previously evaluated for such E-cadherin processing. For this purpose, consecutive radical prostatectomy specimens were identified that had sufficient amounts of both normal and cancerous prostate tissue for protein analysis. Eighteen patients with Gleason scores 6–8 and pathologic stage T2 and T3 prostate cancer, whose preoperative prostate-specific antigen ranged from 5–24 ng/ml, constituted the cohort for this analysis. To confirm that the accumulation of E-cad	extsuperscript{97} did not result from protein degradation during lystate preparation, the human prostate cell line LNCaP, which expresses only E-cad	extsuperscript{120}, was subjected to the same protein isolation protocol, with no visible generation of the truncated species (data not shown).

Results from Western blot analysis of the 18 matched normal tumor pairs are shown in Fig. 1. In all of the tumor samples there is significant accumulation of E-cad	extsuperscript{97}. In the normal specimens, 6 samples were devoid of the truncated band whereas the remaining 12 samples exhibited, in most cases, moderate accumulation of E-cad	extsuperscript{97}. To further characterize this apparent increase in E-cad	extsuperscript{97} in the tumor relative to its case matched normal, densitometry was performed to quantitate band intensity. Then, the fraction of truncated E-cadherin

Fig. 1. Western blot analysis distinguishes truncated and full-length E-cadherin in clinical prostate cancer specimens. Of 18 total specimens only 4 demonstrated a higher E-cad	extsuperscript{97}/E-cad	extsuperscript{120} ratio in the normal aspect of the prostate gland compared with the tumor (A). The remaining 14 of 18 samples demonstrated a higher E-cad	extsuperscript{97}/E-cad	extsuperscript{120} ratio in the tumor aspect of the prostate gland compared with normal tissue (B).

was determined for both the tumor and normal samples for each patient. The net difference between the ratio of E-cad	extsuperscript{97}/E-cad	extsuperscript{120} in the tumor relative to its case-controlled normal emphasizes a relative increase of E-cad	extsuperscript{97} in the cancerous phenotype as graphically illustrated in Fig. 2. The actual densitometric data from individual specimens and the differences in ratios between tumor tissue and matched normal tissue controls are given in Table 1. In only four patients was the ratio of E-cad	extsuperscript{97}/E-cad	extsuperscript{120} greater in the normal tissue as compared with its case-matched tumor. In the remaining 14 patients, there is an increase in the E-cad	extsuperscript{97}/E-cad	extsuperscript{120} ratio in tumor aspect of the prostate gland compared with case-matched normal. The mean ratio of E-cad	extsuperscript{97}/E-cad	extsuperscript{120} for the normal group was 0.50 and for the tumor was 0.76. For the 18 patients, distribution of the E-cad	extsuperscript{97}/E-cad	extsuperscript{120} ratio was not normal in nature, and, therefore, a nonparametric analysis was performed. The Wilcoxon matched pairs test established a significant difference between the E-cad	extsuperscript{97}/E-cad	extsuperscript{120} ratio in the tumor relative to its matched normal, P = 0.035 (Fig. 2).
The β-catenin binding domain of E-cadherin, which is essential for the adhesive function of E-cad, has been mapped to the residues 815–839 in the cytoplasmic tail (10) and in in vitro and in vivo studies has been shown to be removed following truncation of E-cad120 (18). To determine whether this occurs in human prostate cancer, we used immunoprecipitation to examine the loss of β-catenin binding in the truncated E-cad97. When lysates from localized prostate cancer specimens were immunoprecipitated with a β-catenin monoclonal antibody and the immunoprecipitated products immunoblotted with the HECD-1 antibody, which recognizes both E-cad120 and E-cad97, only E-cad120 coprecipitated with β-catenin (see Fig. 3). Even long exposures of the immunoblot failed to demonstrate the presence of E-cad97 in the β-catenin precipitates (data not shown). This experiment suggested that truncation of E-cad120 removed the β-catenin binding domain, resulting in a nonfunctional, truncated E-cad97 with a resultant decrease in the E-cad/β-catenin functional complex available for intercellular adhesion.

Discussion

There have been many studies linking a decrease in E-cadherin expression to an increased aggressiveness of prostate cancer. Demarzo et al. (1) evaluated 76 localized prostate cancers for E-cad expression using immunohistochemical staining with HECD-1 antibody. In addition to finding a correlation with tumor grade, they found that decreased E-cad expression along with preoperative prostate-specific antigen and Gleason score were predictive of pathological stage in a multivariate analysis. Others have correlated decreased expression of E-cadherin along with preoperative prostate-specific antigen and Gleason score with the malignant potential in prostate tumor cells. This is supported by one study that has previously demonstrated the role of E-cadherin-dependent aggregation in prostate and mammary epithelial survival and the correlation of E-cadherin truncation and inactivation in cells destined to undergo apoptosis (13, 18). Thus, the question arises, does the truncation and inactivation of E-cadherin correlate with apoptosis and the malignant phenotype of prostate carcinoma? There are two possible scenarios: because of the correlation between E-cadherin truncation and apoptosis, the accumulation of E-cad97 may reflect an increase in apoptotic index in prostate tumor cells. This is supported by one study that has demonstrated a positive correlation between apoptosis and an increasing malignant potential in prostate cancer (22). In the second scenario, we postulate that the accumulation of E-cad97 truncation may reflect early apoptotic signaling events resulting from the breakdown of adhesive interactions. However, although these cells may be in a detached state they are unable to execute apoptosis because of downstream mutations or inactivating events. Therefore, the truncated species may be accumulating in tumor cells that are not undergoing apoptosis. Undoubtedly, further analysis that will delineate the apoptotic and proliferative indices will help answer these questions.

Our study has used frozen tissue that was histologically confirmed as either normal prostatic tissue or tumor to analyze E-cad processing on the protein level. We have described a functional aberration in E-cad as a consequence of cleavage with subsequent loss of the β-catenin binding motif. As can be appreciated from the Western analysis of the 18 matched tumor normal pairs, there is a statistically significant increase in the ratio of the truncated E-cad97 to the native E-cad120 in the tumor relative to its case-matched normal control. The relative loss of β-catenin binding domain in the tumor, as demonstrated by increased accumulation of E-cad97 that is not present in the functional complex with β-catenin, provides an alternative mechanism for the loss of E-cadherin function.

It is prudent to restate that inactivation of E-cadherin in prostate cancer does result from prodigious allelic loss (7). Disruption of E-cadherin transcription does represent one mechanism by which E-cadherin is inactivated in this disease (7–9). aberrant translational regulation and possibly posttranslational events have also been proposed as alternative mechanisms of E-cadherin dysfunction, but these possibilities have not previously been evaluated in clinical prostate cancer. Our findings indicate that the posttranslational truncation of E-cadherin is a novel and readily attainable end point for evaluating
loss of E-cadherin function that is significantly associated with the malignant transformation of the human prostate.

Acknowledgments

We thank Kathleen Day and Xin Zhao for expert technical assistance.

References


Posttranslational Truncation and Inactivation of Human E-Cadherin Distinguishes Prostate Cancer from Matched Normal Prostate

Michael G. Rashid, Martin G. Sanda, Christopher J. Vallorosi, et al.

Cancer Res 2001;61:489-492.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/2/489

Cited articles
This article cites 22 articles, 12 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/2/489.full.html#ref-list-1

Citing articles
This article has been cited by 11 HighWire-hosted articles. Access the articles at:
/content/61/2/489.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.