Truncation of the β-Catenin Binding Domain of E-cadherin Precedes Epithelial Apoptosis during Prostate and Mammary Involution*

(Received for publication, September 15, 1999, and in revised form, November 4, 1999)

Christopher J. Vallorosi‡§, Kathleen C. Day‡§§, Xin Zhao‡, Michael G. Rashid‡, Mark A. Rubin‡, Keith R. Johnson**, Margaret J. Wheelock**, and Mark L. Day‡‡‡

From the ‡Department of Surgery, Division of Urology, the §Department of Pathology and the ¶University of Michigan Comprehensive Cancer Center, University of Michigan Ann Arbor, Ann Arbor, Michigan 48109, and the **Department of Biology, University of Toledo, Toledo Ohio 43606

A potential target of hormone action during prostate and mammary involution is the intercellular junction of adjacent secretory epithelium. This is supported by the long-standing observation that one of the first visible stages of prostate and mammary involution is the disruption of interepithelial adhesion prior to the onset of apoptosis. In a previous study addressing this aspect of involution, we acquired compelling evidence indicating that the disruption of E-cadherin-dependent adhesion initiates apoptotic programs during prostate and mammary involution. In cultured prostate and mammary epithelial cells, inhibition of E-cadherin-dependent aggregation resulted in cell death following apoptotic stimuli. Loss of cell-cell adhesion in the nonaggregated population appeared to result from the rapid truncation within the cytosolic domain of the mature, 120-kDa species of E-cadherin (E-cad120). Immunoprecipitations from cell culture and involuting mammary gland demonstrated that this truncation removed the β-catenin binding domain from the cytoplasmic tail of E-cadherin, resulting in a non-β-catenin binding, membrane-bound 97-kDa species (E-cad97) and a free cytoplasmic 35-kDa form (E-cad35) that is bound to β-catenin. Examination of E-cadherin expression and cellular distribution during prostate and mammary involution revealed a dramatic reduction in junctional membrane staining that correlated with a similar reduction in E-cad120 and accumulation of E-cad97 and E-cad35. The observation that E-cadherin was truncated during involution suggested that hormone depletion activated the same apoptotic pathway in vivo as observed in vitro. Based on these findings, we hypothesize that truncation of E-cadherin results in the loss of β-catenin binding and cellular dissociation that may signal epithelial apoptosis during prostate and mammary involution. Thus, E-cadherin may be central to homeostatic regulation in these tissues by coordinating adhesion-dependent survival and dissociation-induced apoptosis.

Cadherins are a family of single pass transmembrane glycoproteins that mediate Ca2+-dependent intercellular adhesion (1). In secretory tissues, such as that of the prostate gland and mammary gland, interepithelial membrane adhesion is dependent on the homophilic interaction of E-cadherin (1, 2). Such homophilic cell-cell adhesion results in the formation of desmosomes and adherens junctions that are required for tissue morphogenesis and the maintenance of the differentiated phenotype (1). Cadherins share a common molecular structure; their ectodomain consists of five tandem repeated units of 110 amino acids each that share structural similarities to the immunoglobulin variable-like domain (3). Mutagenesis studies indicate that the homophilic binding between cadherins occurs through interactions at the amino terminus, with binding specificity being confined within the first 113 amino-terminal residues of domain 1 (4). The intracellular domain of E-cadherin is linked to the actin cytoskeleton through its interaction with the cytoplasmic adapter proteins β-catenin, α-catenin, and γ-catenin (plakoglobin) (5–7). The β-catenin binding domain of E-cadherin has been mapped to the residues 815–839 in the cytoplasmic tail, which are required for the adhesive function of the molecule (8). Although central to cell-cell adhesion, E-cadherin has also been implicated in physiologic roles beyond the mechanical interconnection of cells. Recent evidence suggests that E-cadherin may also be associated with regulatory pathways involved in various aspects of cell fate including developmental decisions, cellular differentiation, and cell survival (8–10). Both the prostate and lactating mammary gland are complex tissues with specialized secretory functions that undergo involution upon removal of the appropriate trophic hormones (11). Following depletion of androgenic steroids, the prostate gland will undergo dramatic involution resulting from apoptotic death of the luminal epithelium. Likewise, involution following weaning also results from extensive apoptotic cell death in the mammary gland (12). Thus, homeostatic balance between cell death and cell survival is critically dependent on hormone levels; however, the precise mechanism of hormone-induced cell death in these tissues is still unknown. A potential target of hormone action during involution may be the intercellular junction of the adjacent secretory epithelium. This is supported by the observation that an early visible indication of prostate and mammary involution is the disruption of interepithelial adhesion (11, 13). Thus, loss of lateral adhesion may be involved in an apoptotic response in these tissues destined for involution. Previous studies have demonstrated that anchorage-dependent epithelium will undergo apoptosis following loss of integrin contact with the ECM1 (reviewed in Refs. 14 and 15) or

* This study was supported by the SPORE in Prostate Cancer P50 CA69568 (to M. L. D.) from the National Institutes of Health and by Grant TPRN-98-111-01 CSM from the American Cancer Society (to M. L. D.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡‡ To whom correspondence should be addressed: Box 0944, Rm. 6219 CGC, 1500 E. Medical Center Dr., Ann Arbor, MI 48109. Tel.: (734) 647-8121; Fax: (734) 647-9271; E-mail: mday@umich.edu.

1 The abbreviations used are: PKC, protein kinase C; TPA, 12-O-tetradecanoylphorbol-13-acetate; PAGE, polyacrylamide gel electro-
inhibition of integrin-mediated organoid formation (16). Likewise loss of integrin contact with the ECM will induce apoptosis of prostate and mammary epithelial cells (17). It has been suggested that just as integrins function to mediate cell-ECM interactions in anchorage-dependent survival, cadherins might also act in such a capacity, residing a direct functional role in the regulation of adhesion-dependent survival. This has now been demonstrated in several studies in which intercellular adhesion-dependent survival is uniquely regulated through the E-cadherin cell-adhesion system (10, 18, 19). These studies showed that homophilic binding of cadherin molecules on adjacent cells may transduce apoptotic suppressive signals and that the specific disruption of E-cadherin-mediated adhesion was required for apoptosis to occur.

The PKCα family of serine/threonine protein kinases represents a prominent signal transduction mechanism activated by extracellular contact (20–23), steroid hormones (24–28), and peptide growth factors (29–30). Historically, PKC activity has been associated with the regulation of cell growth and differentiation; however, recent studies have demonstrated that the PKC family may regulate apoptotic programs as well (31, 32). A potential target of PKC action, with important ramifications in intercellular contact and tissue homeostasis, is E-cadherin (33–37). We have recently demonstrated that inducible overexpression of the PKCα isozyme (PKCα) or treatment with the PKC activator, TPA, initiated an E-cadherin-dependent program of cellular aggregation that was required for the survival of mammary and prostate epithelial cells (10, 38).

In the present study, we have examined a mechanism by which E-cadherin is rapidly truncated in the cytosolic domain. Co-immunoprecipitations from cell culture and involving mammary gland demonstrated that this truncation removed the β-catenin binding domain from the cytoplasmic tail of E-cadherin, resulting in a non-β-catenin binding, membrane-bound 97-kDa species (E-cad97) and a free cytoplasmic 35-kDa form (E-cad35) that complexes with β-catenin. Examination of E-cadherin expression and cellular distribution during prostatic and mammary involution revealed a dramatic reduction in junctional membrane staining that correlated with a similar reduction in E-cad120 and accumulation of E-cad97 and E-cad35. Coupled with the observation that E-cad97 and E-cad35 accumulated during involution suggested that hormone depletion signaled the same apoptotic pathway in vivo as observed in vitro. Based on these findings, we hypothesize that truncation of E-cadherin results in the loss of β-catenin binding and cellular dissociation that may signal epithelial apoptosis during prostate and mammary involution. E-cadherin may then play a predominant role in the regulation of prostate and mammary homeostasis by coordinating adhesion-dependent survival and dissociation-induced apoptosis.

**EXPERIMENTAL PROCEDURES**

**Tissue Culture Reagents and Chemicals**—The cell line LNCaP (ATCC) was propagated in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Inc.). The cells were kept at 37 °C in a humidified atmosphere of 5% CO2 and subcultured weekly. The SUM185 mammary epithelial cell line (gift of Dr. Stephen P. Ethier) was grown as described (10). Cell viability was followed by trypan blue exclusion (Life Technologies, Inc.) or, by the colorimetric MTS assay (Promega). Chemicals and PKC activator, TPA, were used at 50 ng/ml concentration as a pretreatment 90 min prior to PKC activation. Treatment with tunicamycin (Roche Molecular Biochemicals), 1.3 × 105 LNCaP cells were plated in 100-mm^2 dishes for 48 h. Tunicamycin (1.5 μg/ml) was added to the cells 8 h prior to TPA treatment.

**Protein Analysis and Western Blot**—To prepare protein lysates from tissue culture cells and frozen prostate and mammary tissues, the following buffer was added at the time of lysis: 50 mM Tris, pH 7.5, 120 mM NaCl, 0.5% Nonidet P-40, 0.5 mM phenylmethylsulfonyl fluoride, 50 μg/ml leupeptin, 50 μg/ml aprotinin, 200 μM orthovanadate. The cells were allowed to lyse for 1 h (on ice), lysates were centrifuged, and the supernatants were collected and quantitated. All proteins were quantified using a Bradford assay, separated by 6, 10, or 12% Tris-glycine precast NOVEX gels and analyzed using the NOVEX and enhanced chemiluminescence (Amersham Pharmacia Biotech) detection systems as described previously (38). For treatment with 10 and 12% gels, the transfer or blotting time was increased to 60 min.

**Membrane Extractions**—For experiments involving Western analysis of detergent-extracted membranes, cells were lysed as described above. Following incubation on ice, the cell lysates were centrifuged and the supernatants decanted. The insoluble, precipitated pellet was then washed with Tris-buffered saline and centrifuged again. The insoluble, detergent-insoluble membrane pellet was then analyzed by Western blot.

**Antibodies**—Antibodies utilized for the detection of 120- and 97-kDa E-cadherin were HECDD-1 (Zymed Laboratories Inc. Laboratories) and SC7870 (Santa Cruz) and E-9 (39). For the detection of 120- and 35-kDa E-cadherin, the following antibodies were employed: SC1499 (Santa Cruz), 4A2 (40) and E2 (gift of Dr. W. James Nelson). The anti-E-cadherin C20820 (Transduction Laboratories) was employed for the rat tissue staining. For β-catenin immunoprecipitations and Western blots, the CAT-5H10 antibody (Zymed Laboratories Inc.) was used. The appropriate horseradish peroxidase-conjugated anti-rabbit, anti-goat, anti-mouse, or anti-rat secondary antibodies were obtained from Amresco and Jackson Immuno Research Labs, Inc.

**Immunoprecipitation Experiments**—LNCaP, MCF-7 cells, and rat prostate and breast tissue lysates were prepared and quantitated as described above. For the immunoprecipitation reactions, the following lysis buffer was used: 50 mM Tris, pH 7.5, 0.1 mM calcium chloride, 120 mM NaCl, 0.5% Nonidet P-40, 40 μM phenylmethylsulfonyl fluoride, 50 μg/ml leupeptin, 50 μg/ml aprotinin, and 200 μM orthovanadate. The lysates were precleared with the appropriate Sepharose beads (Zymed Laboratories Inc.) and then incubated with 5 μg of the requisite antibody, followed by the appropriate horseradish peroxidase-conjugated protein A (for rabbit/goat antibodies) or protein G (for mouse/rat antibodies) prior to the immunoprecipitation reactions. Note that all beads were diluted with an equal volume of Tris-buffered saline (50 mM Tris, pH 7.6, 120 mM NaCl) with 2.5% milk prior to use (termed blocked beads). Following the preclearing step, lysates were aliquoted into microcentrifuge tubes, where 1 mg of protein was used per immunoprecipitation reaction. Normal rabbit or mouse, or anti-rat secondary antibodies were obtained from Amersham's or Zymed Laboratories and used at 1:100 dilution. The immunoprecipitation reaction with prostate and breast tissues. For each immunoprecipitation reaction, the volumes were equalized to 500 μl with lysis buffer, 5 μg of primary antibody was added, and the reaction was carried out at 4 °C overnight, rotating end over end. The next day, the corresponding protein A- or protein G-conjugated Sepharose beads were added (120 μl of blocked beads/immunoprecipitation tissue), and this secondary reaction was allowed to continue mixing end over end for 90 min at 4 °C. The beads were then pelleted (microcentrifuge, 4 °C at 9000 rpm for 5 min) and washed four times with 1 ml of lysis buffer (+ CaCl2 and protease inhibitors). Finally, 40 μl of 2× reducing sample buffer was added to each pellet (washed beads), and samples were then heated at 100 °C for 5 min. Supernatants were then loaded onto gels for Western analysis.

**Animal Studies**—Male, 250-g Harlan Sprague-Dawley rats were castrated at the indicated times, the ventral prostate were excised and fixed (or frozen), and histology was performed. Frozen tissue sections (3 μm) from the castrated rats were fixed in 100% methanol and stained with the rat anti-E-cadherin antibody (Transduction Laboratories C20820) at 1:40,000 dilution. Nursing Harlan Sprague-Dawley rats were obtained with litters. The mothers were allowed to nurse their pups for 3–4 weeks. The pups were taken from the mothers on day 0 (lactating) and the involving breast tissue was harvested each day following weaning for 8 days. The excised tissue was fixed and embedded in paraffin or frozen, and the resulting sections (3 μm) were stained with the 4A2 antibody at 1:800 dilution. For both prostate and mammary sections, the secondary antibody was a biotinylated horse anti-mouse (Vector Labs BA-2001) diluted 1:200 and conjugated with peroxidase (brown). The sections were counter-stained with hematoxylin, dehydrated, and mounted.

Downloaded from http://www.jbc.org/ by guest on July 21, 2018
Truncation of E-cadherin during Involution

FIG. 1. E-cadherin truncation precedes apoptosis. Protein extracts were obtained from LNCaP and SUM185 cells at the times indicated following TPA treatment. An identical 24-h time point was also harvested from a culture pretreated with staurosporine (STS). Immunoblot analysis employing HECD-1 antibody depicts the ratios of E-cad120 and E-cad97 over this time course. Viability was measured by trypan blue exclusion and represents the average of triplicate cell counts.

RESULTS

Truncation of E-cadherin Precedes Apoptosis of Prostate and Mammary Epithelium—In a previous study we demonstrated that, in subconfluent cultures of prostate and mammary epithelial cells, overexpression and activation of PKCα or treatment with the phorbol ester, TPA, resulted in two populations: 1) surviving aggregated cells and 2) dissociated apoptotic cells (10). Survival of the aggregated population required E-cadherin-mediated adhesion. Because only aggregated cells survived, we postulated that some perturbation to the cell-cell adhesion mechanism might signal apoptosis in the nonaggregated population. To investigate this further, we examined the expression of E-cadherin in both prostate and mammary epithelial cells following PKC activation. Immunoblot analysis revealed strong expression of full-length E-cad120 following PKC activation in both the LNCaP (prostate) and SUM185 (mammary) cell lines (Fig. 1). The maximum accumulation of E-cad120 at 6 h coincided precisely with the onset of cellular aggregation (10). Concurrent with induction of E-cad120 was the rapid accumulation of a novel 97-kDa form of E-cadherin (E-cad97). E-cad97 was detectable by 6 h following PKC activation and increased steadily over the next 42 h, preceding significant cell death by 18 h. Staurosporine, a potent, nonspecific catalytic inhibitor of protein kinase C (41), suppressed apoptosis and the generation of E-cad97 (Fig. 1). To determine whether E-cad97 was uniquely associated with the nonaggregated apoptotic population, we examined the expression of E-cad120 and E-cad97 in both the aggregated and nonaggregated cells. Because of decreased anchorage, the nonaggregated cells were separated from aggregated cells by gentle agitation as early as 12 h following TPA treatment. We found that E-cad97 was strongly expressed in this nonaggregated population, which was approximately 50% apoptotic by 24 h, whereas very little expression of E-cad97 could be detected in the aggregated, surviving population (Fig. 2). Comparison of viability and E-cadherin expression at the 12- and 24-h time points demonstrated that the accumulation of E-cad97 preceded the onset of cell death.

Truncation of E-cadherin during Prostate and Mammary Involution—We analyzed expression of E-cadherin proteins in involuting rat prostate and mammary glands to determine whether E-cadherin was truncated in response to reduced hormone levels in vivo. Prostate and mammary involution involves extensive regression of the ductal architecture to an atrophic state following castration or weaning, respectively (11–13). The predominance of apoptotic cells in prostatic ducts observed 3 to 4 days following castration indicates that regression results from programmed cell death of the luminal epithelium. To determine whether E-cadherin is truncated prior to apoptosis in vivo, we examined expression of E-cadherin in the rat ventral prostate following castration and the rat mammary gland following weaning. The glandular epithelium constitutes approximately 70–90% of the total cell mass. Apoptotic cells begin to appear by day 3 following castration or weaning, where more than 80% of the cells had died by day 7 (42, 43). Immunoblot analysis of E-cadherin protein in the rat ventral prostate following castration revealed a dramatic reduction of E-cad120 expression by 24 h, which is the time E-cad97 begins to appear (Fig. 3A). Although slightly delayed, the conversion of E-cad120 to E-cad97 was observed during mammary involution as well (Fig. 3A). E-cad97 from both involuting prostate gland and mammary gland migrated precisely with E-cad97 observed in the LNCaP and SUM185 cell lines. Immunohistochemical analysis demonstrated that E-cadherin was localized exclusively in the junctional membranes of the luminal epithelium in the ventral prostate gland and mammary gland of untreated rats (Fig. 3B, a and c). In comparison, glands from the 48-h castrates and the 48-h weaned females exhibited dramatic reductions in E-cadherin membrane staining (Fig. 3B, b and d), which coincided precisely with the reduction of E-cad120 observed by immunoblot analysis. In a previous study, we demonstrated by TUNEL analysis (which detects fragmented DNA) that apoptotic nuclei were prominent throughout the luminal epithelium in the 72-h castrates and only in cells that had lost E-cadherin staining in the junctional membrane (10).

Mapping the E-cadherin Truncation Site—To specifically map the truncation of E-cad120, we employed a variety of region- and peptide-specific antibodies in immunoblot analysis of TPA-treated LNCaP lysates. The epitopes of these antibodies are schematically depicted in Fig. 4A. As shown in the diagram, the monoclonal antibodies HECD-1 (44), E9 (39), and SC-7870 (46), raised against various regions of the extracellular domain, recognize both E-cad120 and E-cad97 (Fig. 4B). However, antibodies 4A2, E2 (47), and SC-1499 (48), which are specific for various cytosolic epitopes, recognize only E-cad120. We predicted that a single cleavage event in this region would result in the accumulation of two peptides: the 97-kDa species and a smaller peptide with a molecular mass of 25–35 kDa. When the cytosolic-specific antibodies (Fig. 4B, c-f) were used in immunoblots of LNCaP lysates resolved on 12% acrylamide gels, we detected a PKC-inducible 35-kDa fragment of E-cadherin, which we have designated E-cad95 (Fig. 4C). Considering the molecular masses of the two fragments and the antibody mapping suggests that E-cad120 was truncated proximal to the transmembrane domain in the cytosolic domain. We predicted
that a clip in this region would result in membrane-bound 97-kDa fragment and free cytosolic 35-kDa fragment. To confirm the cellular location of these peptides, we addressed whether E-cad97 and E-cad35 were associated with the cell membrane. E-cad97 could only be detected in the pelleted fraction of PKC activated cells and not in the supernatant, suggesting membrane localization of E-cad97. However, when we specifically examined the membrane using a second Triton X-114 phase separation of cellular proteins, we found that E-cad97 and E-cad120 were detected in the Triton X-114 fraction and not in the aqueous fraction (Fig. 5). Examination of the cytosolic fraction revealed only E-cad35.

Post-translational Modifications—A common post-translational modification of E-cadherin is the addition of N-linked sugar moieties to the mature protein. Although we did not believe that differential glycosylation would explain the various forms of E-cadherin, we did want to rule out this possibility. Tunicamycin is an efficient inhibitor of N-linked oligosaccharide synthesis and is commonly used to ascertain the glycosylation state of eukaryotic proteins. LNCaP cells were cultured in the presence of tunicamycin for 8 h prior to treatment with TPA, and protein extracts were prepared. Immunoblot analysis revealed that the molecular mass of the 120- and 97-kDa species changed substantially (Fig. 6). In cells pretreated with tunicamycin, the HECD1 antibody recognized four species of E-cadherin. Minor bands were detected at 120 and 97 kDa, representing the glycosylated forms, and major bands migrating at 115 and 92 kDa, representing the nonglycosylated forms. To rule out that the truncation is generated through transcriptional mechanisms, we examined the levels of E-cadherin mRNA to determine whether the truncated fragments resulted from alternatively spliced transcripts. E-cadherin mRNA isolated from TPA-treated LNCaP cells revealed the accumulation of a single, 4.3-kb transcript by Northern analysis (data not shown). Performing multiple reverse tran-
We have previously demonstrated that E-cadherin functions in a novel adhesion-dependent survival pathway that suppressed apoptosis of prostate and mammary epithelial cells (10). Although full-length E-cadherin was necessary for survival in the aggregated cells, its expression was transient as the 120-kDa form was depleted in the dissociated cells destined to undergo apoptosis. We believe that loss of E-cadherin resulted from the rapid, post-translational truncation of the molecule and not from transcriptional events. However, to rule out transcriptional mechanisms, we examined E-cadherin mRNA by Northern blot and reverse transcriptase-polymerase chain reaction analysis and found only the accumulation of a single transcript in both experiments. We have also rejected two common post-translational modifications to explain the appearance of E-cad35 and E-cad35. Both N-linked glycosylation and dephosphorylation, were dismissed in studies using tunicamycin (Fig. 6) and orthovanadate (data not shown), respectively.

Truncation of a diverse set of physiologically important cell surface proteins such as membrane-anchored growth factors, their receptors, ectoenzymes, and cell adhesion molecules occurs through proteolysis (39, 49–52, 54). PKC activation has been shown to elicit the proteolytic truncation of several transmembrane proteins, including the NGF receptor and the LAR and PTP tyrosine phosphatases (49, 50). Cleavage of cell surface proteins has dramatic effects on their biological function. For example, cleavage of membrane-bound growth factors abolishes membrane-anchored function and liberates the factor for free diffusion to neighboring cells (51). Ectodomain cleavage of several tyrosine phosphatases results in cellular redistribution of the intracellular domain with profound functional effect (54). Various adhesion molecules can be processed from their mature forms, producing membrane-bound fragments and soluble fragments, the functional consequences of which are unknown. During involution, degradation of the lateral and basal cell junctions has been associated with the activation of a number of proteolytic pathways, such as cathepsin D, matrix metalloproteases, Ca2+-dependent calpains, and plasminogen activators (55–60). Although many of these proteases likely function in the degradation of extracellular matrix proteins during prostate and mammary involution, their ability to target membrane adhesion proteins has not been ruled out. Taken together these results suggest that the truncation of E-cadherin results from a single post-translational event that is likely proteolytic in nature. However, we have examined a variety of protease inhibitors, including inhibitors of serine proteases, Ca2+-dependent proteases, and caspases, and have not demonstrated a reduction in E-cadherin truncation.

Mapping the E-cadherin fragments with multiple antibodies indicated that the truncation occurred in the cytosolic region in close proximity to the transmembrane domain. This hypothesis was strengthened by cell fractionation studies localizing the E-cad35 to the membrane and E-cad35 to the cytosol. And co-immunoprecipitation experiments demonstrated the loss of both E-cad35 and E-cad35, only E-cad35 co-precipitated with β-catenin (see Fig. 7). Even long exposures of the immunoblot failed to demonstrate the presence of E-cad35 in the β-catenin precipitates (data not shown). To demonstrate that truncation has the same functional effect in vivo, we performed co-immunoprecipitations using extracts from involuting mammary gland. β-Catenin co-immunoprecipitations of 3-day post-weaning mammary extracts followed by E9 E-cadherin immunoblots demonstrated abundant E-cad35 binding but, as in the cell culture experiments, complete absence of E-cad35 binding. This experiment suggested that truncation of E-cad35 removed the β-catenin binding domain resulting in a non-β-catenin binding E-cad35. We next addressed whether cytosolic E-cad35 retained the ability to bind β-catenin. When the same cell culture and tissue extracts were immunoprecipitated with a β-catenin monoclonal antibody and the immunoprecipitated products immunoblotted with carboxymethyl-terminal-specific SC-1499, which recognizes both E-cad35 and E-cad35, and run on 12% acrylamide gels, both fragments were found to precipitate with β-catenin.

**DISCUSSION**

We have previously demonstrated that E-cadherin functions in a novel adhesion-dependent survival pathway that suppressed apoptosis of prostate and mammary epithelial cells (10). Although full-length E-cadherin was necessary for survival in the aggregated cells, its expression was transient as the 120-kDa form was depleted in the dissociated cells destined to undergo apoptosis. We believe that loss of E-cadherin resulted from the rapid, post-translational truncation of the molecule and not from transcriptional events. However, to rule out transcriptional mechanisms, we examined E-cadherin mRNA by Northern blot and reverse transcriptase-polymerase chain reaction analysis and found only the accumulation of a single
The results of this study may provide an explanation for both a protective role in cells by suppressing apoptosis through a common pathway; however, such a divergent mechanism has not been previously described. There are conflicting thoughts as to the functional role of E-cadherin in the regulation of cell survival: one view suggests that E-cadherin played an important role in cells by suppressing apoptosis through aggregation (10, 17). Another view suggests that the disruption of E-cadherin-dependent adhesion initiates cell death (10, 18).

Considering the coordinated regulation between cell survival and cell death that must be maintained for normal growth, it is not surprising that apoptosis has emerged as a fundamental component in the regulation of tissue homeostasis. Therefore, it is feasible that cell survival and apoptosis are regulated through a common pathway; however, such a divergent mechanism has not been previously described. There are conflicting thoughts as to the functional role of E-cadherin in the regulation of cell survival: one view suggests that E-cadherin played a protective role in cells by suppressing apoptosis through aggregation (10, 17). Another view suggests that the disruption of E-cadherin-dependent adhesion initiates cell death (10, 18).
Truncation of E-cadherin during Involution
Truncation of the β-Catenin Binding Domain of E-cadherin Precedes Epithelial Apoptosis during Prostate and Mammary Involution

Christopher J. Vallorosi, Kathleen C. Day, Xin Zhao, Michael G. Rashid, Mark A. Rubin, Keith R. Johnson, Margaret J. Wheelock and Mark L. Day

J. Biol. Chem. 2000, 275:3328-3334.
doi: 10.1074/jbc.275.5.3328

Access the most updated version of this article at http://www.jbc.org/content/275/5/3328

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 59 references, 22 of which can be accessed free at http://www.jbc.org/content/275/5/3328.full.html#ref-list-1