E-cadherin expression and PSA secretion in human prostate epithelial cells

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Abstract Prostate-specific antigen (PSA) is the most widely used marker for the diagnosis of prostate cancer and is an independent predictor of prostatic capsular invasion. A number of studies have identified E-cadherin, a cell adhesion protein, as a potential invasion suppressor which is decreased in prostate adenocarcinoma. Our goal in the present study was to evaluate E-cadherin expression in primary cultures and determine the relationship between E-cadherin expression and PSA secretion in both primary cultures and the prostate tumor cell line, LNCaP. Immunohistochemical studies and Western blot analysis confirmed greater expression of E-cadherin in normal epithelial cells than tumor-derived prostate cells. This is the first report that the incubation of normal prostate epithelial cells with E-cadherin antibody increases the amount of PSA detected in the media of normal cells as well as in LNCaP. Since E-cadherin may function as an invasion suppressor, an understanding of the decreased expression of this adhesion factor and the impact on PSA secretion may aid in understanding epithelial tumorigenesis.

Keywords PSA · E-cadherin · Prostate · Human · Cell culture

Introduction

Prostate cancer is the result of uncontrolled growth of the epithelial cells, which normally line the ducts of the prostate gland and secrete both prostate-specific antigen (PSA) and prostate-specific acid phosphatase (PSAP). Strict compartmentalization of PSA within the prostate is critical because the identification of its primary structure [20, 26, 35] confirmed that PSA demonstrates a high degree of homology with the proteases belonging to the glandular kallikreins. Acting as a protease, PSA is reported to cleave the insulin-like growth factor I binding protein (IGFIBP), releasing active IGF1 [7, 8, 9]. An increase in serum PSA above 4 ng/ml is utilized in screening for prostate cancer [6]. It is assumed that the increased amount of serum PSA in men with prostate disease is caused by disruption of the epithelial compartmentalization, although the biological mechanism is unknown. Since progression of prostate cancer is associated with a decrease in cell adhesion, the relationship between PSA level and loss of cell adhesion is of potential significance.

A major factor in epithelial cell adhesion is E-cadherin, a transmembrane cell adhesion protein [28, 37]. Umbas et al. [33] reported a decrease in the E-cadherin level in human prostate tumor tissue which was correlated with the pathologic stage. Recently E-cadherin has been shown to function beyond cell adhesion to include cell polarization [21], segregation [32] and the control of invasive behavior [34]. The gene for E-cadherin is on chromosome 16 and this region has been shown to be deleted in 32–56% of prostatic tumors [15, 25]. Changes in cadherin binding properties during both embryonic development and tumor progression [24, 27] result in epithelial cell dissociation which may facilitate the invasive phenotype. Thus E-cadherin has been postulated as an invasion suppressor gene [2, 14, 15].

E-cadherin is a member of the cadherin superfamily and requires the association with catenins for Ca²⁺ dependent cell adhesion and its anchorage to the cytoskeleton [11]. Inactivation of the E-cadherin binding complex has been shown to occur by a variety of mechanisms, including genetic alterations and epigenetic events. Many studies examining the regulation of cadherin expression have focused on the highly
conserved cytoplasmic binding domain which attaches to the catenins and p120 [3, 16,31]. Growth factor receptors have been reported to interfere with binding. For example, the binding of EGF results in the tyrosine receptor phosphorylation of β-catenin and dissolution of the adherens junction [29]. Thus the availability of growth factors may play an important role in the loss of cell adhesion.

The downstream effects of the loss of E-cadherin function have been addressed in a limited number of studies. Day et al. reported that blocking E-cadherin mediated aggregation allowed cells to undergo apoptosis [12]. They demonstrated that E-cadherin-dependent aggregation resulted in retinoblastoma-mediated G1 arrest and survival. Because E-cadherin maintains the integrity of epithelial tissue, we hypothesized that the loss of E-cadherin expression may also be related to a deregulation of PSA secretion. First we characterized age-matched tumor and normal primary prostate cells with regard to E-cadherin expression in the corresponding tissue sections. We then tested the hypothesis by blocking E-cadherin expression with antibody and measuring the effect on intracellular and secreted proteins.

Materials and methods

Cell cultures

For cell culture experiments, we utilized our in vitro culture system for normal and neoplastic prostate epithelial cells. This has been described previously [18]. We obtained prostatectomy specimens from the patients at the University of Pittsburgh Medical Center in order to develop cell cultures of prostate epithelial cells. A piece of tissue (approximately 1 cm³) was removed by a pathologist from an area that was confirmed by adjacent sections as consisting entirely of normal or tumor tissue. The selection of areas of interest was performed by a pathologist based on standard gross morphologic features. The tumor nodules were firm, gray to yellow in color, more homogenous in consistency, and without areas of cystic change. The normal areas were foci of unremarkable prostate tissue. The selected piece of tissue was bisected with part of it submitted for primary culture and part for histologic evaluation. The histologic evaluation was performed at the time of microscopic diagnostic evaluation. The selected tissues were used in the study only if the microscopic morphology corroborated the gross impression.

These specimens for culture were cut into smaller pieces and dissociated with collagenase to create primary cell cultures of prostate cells. The initial cultures were passaged to create multiple replicates for experimental purposes and cells were maintained in a chemically-defined media (CDM).

Growth media

The chemically-defined media adapted for use in prostate cultures consisted of a 1:1 mixture of Ham’s F12 and William’s Media E with the following supplements: sodium bicarbonate (2.5 g/l); albumin (0.8 g/l); α-glucose (1.26 g/l); HEPES (3.57 g/l); sodium pyruvate (4.4 mg/l); ascorbic acid (17.6 mg/l); ethanolamine (4.2 µl/l of 98% solution, Sigma) and linoleic acid (2.2 ml/l) of 1 mg/ml stock solution in 0.08% fatty-acid free BSA. The media was then adjusted to a pH of 7.2, sterile-filtered and stored at 4°C. Dexamethasone (10⁻⁷ M) and gentamycin (50 mg/l) were added immediately before use. A premixed additive, ITS, supplied insulin (5 µg/ml), transferrin (5 µg/ml) and selenium (5 ng/ml)(Sigma).

Fungizone was added to some of the cultures to prevent fungal contaminations (Cell Zone).

Immunohistochemistry

Prostate epithelial cells were grown in primary culture from samples of both benign and neoplastic tissue. After approximately one week in culture, the cells were subcultured and plated in equal numbers on coverslips. Immunohistochemistry was performed as previously described [17]. Briefly incubation with anti-uvomorulin (Decma clone 20.1:1500) (Sigma, St. Louis, MO) took place in a humidified chamber for 1 h, then biotinylated secondary antibody was added for thirty minutes. Slides were developed using the diaminobenzene reaction and counterstained with hematoxylin. The negative controls were incubated with PBS in place of the primary antibody.

Western blot

Benign and tumor tissues obtained from prostatectomy were cultured separately in CDM until 80% confluent, and then passaged. The cells were lysed and analyzed by Western blot according to the procedure of Laemmli [19].

An equal amount of protein, as measured by the BCA method, was loaded in each lane [30]. For cadherin analysis, 50 µg of protein of each normal/tumor pair was separated on a 7.5% polyacrylamide gel, transferred to nitrocellulose and immunoblotted with anti-uvomorulin (1:1000).

To test the role of E-cadherin on PSA secretion, normal cells were incubated with vehicle control (0.1% sodium azide), E-cadherin antibody (1:100), or no treatment (control) for 24 h. The media from these cultures was collected in the presence of protease inhibitors and frozen until analyzed for total protein by the BCA method [30]. Protein samples (25 µg) were loaded in each lane, with separate lanes for PSA standard and molecular weight markers. The proteins were separated by 12% polyacrylamide gel electrophoresis, then transferred to nitrocellulose membranes and immunoblotted with anti-PSA (1:2000) or anti-PSAP (1:2500). A similar analysis was performed on LNCaP cells in the presence and absence of two different E-cadherin antibodies (Zymed and Transduction sources). Fifteen micrograms of LNCaP protein obtained from media was applied to each lane and separated on a 12% polyacrylamide gel. Twenty micrograms of LNCaP cellular protein from each experimental treatment was loaded in each lane of a separate gel. The proteins were then transferred to nitrocellulose membranes and immunoblotted with anti-PSA for comparison of secreted and cellular products.

Results

Immunohistochemistry

Differences in E-cadherin expression between the normal and tumor cell populations were observed immunohis-