Claudin-1 Immunohistochemistry for Distinguishing Malignant From Benign Epithelial Lesions of Prostate

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BACKGROUND. Claudins are a family of approximately 23 integral membrane tight junction (TJ) proteins that maintain cell polarity and paracellular barrier functions in epithelial and endothelial cells. Although Claudin-1 was demonstrated to be typically downregulated in various cancers, the precise expression patterns of this protein in normal and neoplastic tissues remain poorly characterized.

METHODS. Using immunohistochemistry, the expression of Claudin-1 was investigated in prostate tissue samples arranged in a tissue microarray (TMA) format and comprising elements of normal prostatic epithelium (n = 6), benign prostatic hyperplasia (BPH; n = 38), prostatic intraepithelial neoplasia (PIN; n = 11), and prostate adenocarcinoma (n = 48). The Claudin-1 expression pattern was compared with that of the basal cell-specific markers, p63, and HMW cytokeratin (34βE12), by employing double-labeling techniques in conjunction with image analysis methods utilizing color deconvolution algorithms.

RESULTS. In benign prostatic epithelium, pronounced Claudin-1 expression was observed in the basal cell layer with no staining in luminal cells. Prostate adenocarcinoma specimens from 98% (47/48) patients lacked Claudin-1 immunostaining, and no cases contained >5% immunopositive tumor cells.


KEY WORDS: normal prostate; basal layer marker; immunohistochemistry

INTRODUCTION

Prostate-specific antigen (PSA) testing facilitates early diagnosis of prostate cancer, but up to 60% of PSA tests are false-positive, making histological confirmation of diagnosis critical. Skinny needle biopsy is prone to sampling errors and inconsistent interpretations, suggesting a need for complementary diagnostic methods that provide more accurate diagnosis.

Claudins are a family of >20 integral membrane tight junction (TJ) proteins that maintain cell polarity and paracellular barrier functions in epithelial and endothelial cells (reviewed in [1]). Claudin-1 localizes to tight junctions [2], and its expression is reduced in proliferating compared to quiescent epithelial cells [3]. Moreover, reduced Claudin-1 expression has been reported for various cancers (reviewed in [4]), though not previously explored in prostate cancer. In this report, we determined the pattern of Claudin-1 protein expression in normal prostate, preneoplastic prostatic tissue, and prostate adenocarcinomas, using immunohistochemistry.

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MATERIALS AND METHODS

Patient Specimens

Prostate cancer tissue microarrays (TMAs) were constructed, as described [5]. An IRB-approved protocol was used to retrieve archival paraffin blocks from the Sharp HealthCare hospitals of San Diego. The TMAs included trans-urethral retrograde prostatectomy (TURP) and radical prostatectomy specimens from 66 patients with locally confined (stage T2) and locally advanced disease (stage T3).

Immunohistochemistry

Dewaxed tissue sections were immunostained using an automated immunostainer (DAKO Universal Staining System) and employing the Envision-Plus-horseradish peroxidase system (DakoCytomation, Inc., Carpinteria, CA) [6]. Rabbit polyclonal anti-Claudin-1 antibody (Zymed Laboratories, Inc., San Francisco, CA) was used at 1.25 μg/mL. Also employed for immunostaining were mouse monoclonal antibodies to Bcl-2, p63, and keratin 34βE12 (high molecular weight keratin) and rabbit monoclonal to AMACR (DakoCytomation).

For double-labeling procedures, tissue sections were stained as above using Claudin-1 rabbit polyclonal antiseraum (SG chromagen, Vector Lab, Inc., Burlingame, CA; black) followed by mouse monoclonal antibodies to Bcl-2, p63, or keratin 34βE12 (DAB chromagen, DakoCytomation; brown), using Nuclear Red (DakoCytomation) for counterstaining.

An automated image analysis system (Aperio Technology Inc., Vista, CA) was employed to visualize Claudin-1 and Bcl-2, p63, keratin 34βE12, or AMACR stainings separately, applying a color deconvolution algorithm [7]. Quantification of immunohistochemical staining was performed using color translation and an automated thresholding algorithm (Aperio Technology).

RESULTS

To characterize the expression of Claudin-1 in human prostate, we employed TMAs containing radical prostatectomy specimens from 66 patients. Tissue samples comprised elements of benign prostatic hyperplasia (BPH; n = 38), prostatic intraepithelial neoplasia (PIN; n = 11), and prostate adenocarcinoma (n = 41). Gleason score data were available for all tumors, while clinical stage information (T2–T3) (according to International Union against Cancer criteria) was known for 48% of patients. In addition, non-neoplastic prostate epithelium from 9% of cases

Fig. 1. Immunohistochemical detection of Claudin-1 expression in normal prostate. TMA slides containing prostate specimens were double stained with the Claudin-1 (SG, black) and HMW cytokeratin antibodies (DAB, brown) (panels A, H) or with the Claudin-1 (SG, black) and p63 antibodies (DAB, brown) and counterstained with Nuclear Red. The black (B, I) and brown (C, J) for HMW cytokeratin; D, K for p63) colors were separated in the annotated regions using a color deconvolution algorithm (Aperio Technology). Quantification of immunohistochemical staining for Claudin-1 (E, L), HMW cytokeratin (F, M), and p63 (G, N) was performed using color translation and an automated thresholding algorithm (Aperio Technology). Yellow/orange/red pixels visualize positive immunostaining, whereas blue pixels depict immunonegative areas. Note colocalization of Claudin-1 and HMW cytokeratin or p63 in basal cells. 10× and 15× digital zooms were applied for the images presented (A, H, and B–D, E–G, I–N, respectively).
was available for comparison of protein expression in non-transformed versus neoplastic epithelium.

In normal prostatic epithelium, Claudin-1 immunostaining was detected exclusively in the basal cell layer, showing cytosolic and membranous intracellular localization. No staining was observed in the secretory epithelial cells or in stroma. A comparison of Claudin-1 immunostaining with 34βE12, p63, and Bcl-2 immunolabeling of serial sections (not shown), as well as double-labeling with monoclonal antibodies to HMW cytokeratin, p63 (Fig. 1), or Bcl-2 (not shown), confirmed that the Claudin-1 immunopositive cells are basal cells. In addition, Claudin-1 protein was detected in the perineurium of peripheral nerves, consistent with prior reports [8].

BPH specimens retained a normal Claudin-1 staining pattern, whereas increasing grades of PIN demonstrated intermittent Claudin-1 labeling, reflecting progressive disruption of the basal cell layer. Similarly, discontinuous Claudin-1 staining was observed in some normal-appearing glands surrounded by inflammatory cells.

**Fig. 2.** Claudin-1 immunostaining in normal and malignant prostate tissue. Using single- (A — Claudin-1), or double-labeling (B, G — Claudin-1 and HMW cytokeratin), Claudin-1 protein was detected by immunohistochemistry in benign glands but not in adenocarcinomas. A color deconvolution algorithm allowed for color separation in the annotated regions (SG — black, C, H; DAB — brown, D, I, J). Quantification of immunohistochemical staining for Claudin-1 (E, L), HMW cytokeratin (M), and p63 (F, N) was performed using color translation and an automated thresholding algorithm. Claudin-1 immunostaining was detected in the perineurium of peripheral nerves (K).
In 98% (47/48) of prostate cancer cases, malignant cells were uniformly Claudin-1 immunonegative (Fig. 2). Among these 47 Claudin-1 negative tumors, 8 (17%) showed positive staining with Bcl-2 antibody, excluding poor tissue processing as an explanation for the immunonegativity. The one adenocarcinoma displaying any immunopositivity for Claudin-1 exhibited only weak intensity staining in occasional cells (≈2%). Double-labeling with alpha-methylacyl coenzyme A racemase (AMACR), an enzyme selectively expressed in neoplastic glandular epithelium, demonstrated lack of Claudin-1 and AMACR co-expression. Thus, the immunostainings for these proteins were mutually exclusive. We conclude therefore that Claudin-1 expression is uniformly lost in prostate cancers.

**DISCUSSION**

In this study, we demonstrate that Claudin-1 expression in human prostate is confined to the basal cell layer, and that essentially all primary prostate adenocarcinomas lack expression of this TJ protein. Our protein data complement results obtained by others at the RNA level, suggesting reduced Claudin-1 expression in tumors compared to normal prostate [9], but for the first time localize Claudin-1 expression at the single-cell level. Loss of Claudin-1 protein expression in prostate cancer is consistent with the notion that disruption of tight junctions is associated with loss of cell-cell contact, de-differentiation, and invasiveness during tumorigenesis (reviewed in [4]).

Epithelial-mesenchymal transition (EMT) is a recently recognized phenomenon associated with epithelial carcinogenesis (reviewed in [10]). The EMT-inducing transcription factors Snail and Slug were recently implicated as potential repressors of Claudin-1 expression [11]. In addition, Claudin-1 was recently identified as a possible target of β-Catenin/Tcf signaling in colorectal carcinogenesis [12,13]. Cooperation between Snail and LEF-1 transcription factors has been shown to be essential for TGF-β-induced EMT associated with the loss of Claudin-1 and E-Cadherin [14]. Thus, the loss of Claudin-1 expression in prostate cancer may be an indication of EMT. The absence of Claudin-1 protein expression may provide a marker for aiding in pathological diagnosis of prostate cancers.

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