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Bladder Cancer

CEACAM1: A Novel Urinary Marker for Bladder Cancer Detection

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Abstract

Background: Carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein; CEACAM1) is expressed in normal bladder urothelium and in angiogenically activated endothelial cells, where it exhibits proangiogenic properties.
Objective: The aim of this study was to evaluate the value of urinary CEACAM1 for detection of urothelial carcinoma of the bladder (UCB).
Design, setting, and participants: This prospective study included 175 patients.
Measurements: Immunohistochemistry for CEACAM1 was performed on UCB sections of 10 patients. Enzyme-linked immunosorbent assay (ELISA) for CEACAM1 was performed on urine specimens of healthy volunteers ($n = 30$), patients with benign prostatic hyperplasia (BPH; $n = 5$), severe cystitis ($n = 5$), non–muscle-invasive UCB ($n = 72$), muscle-invasive UCB ($n = 21$), or past history of UCB without evidence of disease ($n = 42$). Western blot analysis was performed on a subgroup of these subjects ($n = 53$).
Results and limitations: CEACAM1 immunostaining in normal urothelium disappears in non-invasive UCB but appears in endothelial cells of adjacent vessels. Western blotting revealed presence of CEACAM1 in the urine of no healthy volunteers, of 76% of noninvasive UCB patients, and of 100% of invasive UCB patients. ELISA analysis confirmed that urinary CEACAM1 levels were significantly higher in UCB patients compared with control subjects (median: 207 ng/ml vs 0 ng/ml; $p < 0.001$). The area under the curve for UCB detection was 0.870 (95% confidence interval [CI]: 0.810–0.931). In multivariable logistic regression analyses that adjusted for the effects of age and gender, higher CEACAM1 levels were associated with cancer presence (hazard ratio [HR]: 2.89; 95% CI: 2.01–4.15; $p < 0.001$) and muscle-invasive cancer (HR: 5.53; 95% CI: 1.68–18.24; $p = 0.005$). The cut-off level of 110 ng/ml yielded sensitivity of 74% and specificity of 95% for detecting UCB. Sensitivity was 88% for detecting high-grade UCB and 100% for detecting invasive-stage UCB. Larger studies are necessary to establish the diagnostic and prognostic roles of this highly promising novel marker in UCB.
Conclusions: Urinary CEACAM1 levels discriminate UCB patients from non-UCB subjects. Moreover, urinary levels of CEACAM1 increased with advancing stage and grade.

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1. Introduction

Urothelial carcinoma of the bladder (UCB), the fourth most common cancer in men and the ninth most common cancer in women, results in significant morbidity and mortality [1]. At initial diagnosis, about 70% of patients have cancers confined to the epithelium or subepithelial connective tissue. These cancers are usually managed with endoscopic resection and selective use of intravesical therapy. The recurrence rate for these tumors ranges from 50% to 70%, and 10–15% progress to muscle invasion over a 5-yr period [2,3]. This high rate of disease recurrence requires lifelong surveillance, consisting of serial cystoscopy and cytology. These tests are invasive and expensive, with considerable interuser and interinstitutional variability [4–6]. Additionally, although urine cytology has a reasonable sensitivity for the detection of high-grade UCB, it lacks sensitivity to detect low-grade tumors [7]. Therefore, there is a need for new urine markers that may help in UCB detection and surveillance [8,9].

Tumor growth and progression depend on angiogenesis, which is regulated by angiogenic activators and inhibitors [10,11]. In the intricate angiogenesis system, cell adhesion molecules play an important role in vascular morphogenesis and endothelial signaling [12]. Human carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein; CEACAM1) is such a molecule with proangiogenic activity [13]. We have previously observed that CEACAM1, which is ubiquitously expressed in the luminal surface of normal bladder urothelium, is downregulated in bladder cancer cells while it is concurrently upregulated in endothelial cells of adjacent blood vessels [14]. This differential switch in CEACAM1 expression is accompanied by an upregulation of proangiogenic and prolymphangiogenic factors [15–17]. Interestingly, the upregulation of CEACAM1 during this angiogenic activation of endothelial cells is detectable in both the endothelial cells in a membrane-bound form and in the supernatant of these cells [13]. This presence suggests that soluble CEACAM1 forms might be released into body fluids during angiogenic activation. Based on these findings, the aims of this study were to assess whether CEACAM1 is detectable in urine and whether its level could help differentiate bladder cancer patients from healthy subjects.

2. Patients and methods

2.1. Patient population

We obtained approval of the institutional review board of the University Hospital Grosshadern of Ludwig-Maximilians-University Munich. All participants gave written informed consent. Voided urine samples from 175 subjects were collected prospectively in the morning and frozen at −20 °C within 30 min until their analysis. The urine samples were obtained from 30 healthy volunteers, 5 subjects with biopsy-proven benign prostatic hyperplasia (BPH), 5 subjects with severe cystitis, 42 subjects with a history of non–muscle-invasive UCB but without current disease, and 93 patients with actual UCB (17 with pTis, 43 with pTa, 12 with pT1, and 21 patients with pT2–pT4). To exclude renal insufficiency as a reason for CEACAM1 in the urine, no patients with diabetes mellitus or renal insufficiency were included in this study.

2.2. Immunohistochemistry

Paraffin-embedded tissue blocks of human urinary bladder ($n = 10$) were obtained from the Department of Pathology of Ludwig-Maximilians-University Munich. Normal urothelium and primary cancer areas from each patient with non–muscle-invasive ($n = 8$) and muscle-invasive tumors ($n = 2$) were stained according to a previously published protocol [15,18]. Briefly, we used mouse monoclonal antibody (mAb) 4D1/C2 against CEACAM1 (50 ng/ml). Immunostaining was visualized by a modified nickel-enhanced glucose oxidase and peroxidase technique, and sections were counterstained with Calcium Red.

2.3. Western blot analysis

Voided urine samples of a subgroup of the patients (10 healthy volunteers, 3 subjects with biopsy-proven BPH, 5 with severe cystitis, 10 with a history of non–muscle-invasive UCB but without current disease, and 25 with UCB) were analyzed using 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under nonreduced conditions. Volume of loaded sample was 20 μl of the urine sample with 5 μl of 5x Laemmli's loading buffer. CEACAM1 isolated from human granulocytes was used as the positive control [13]. Proteins were transferred to a nitrocellulose membrane (Schleicher & Schell, Germany) and incubated with the antibodies 4D1/C2 and T84.1. Subsequently, the membrane was exposed to the secondary antibody (goat antimouse IgG; Sigma, Germany) conjugated with horseradish peroxidase. The antigen–antibody complex was detected by enhanced chemiluminescence using ECL reagents (Amersham-Pharmacia, Freiburg, Germany) and visualized by autoradiography. The x-ray films were digitized for further use.

2.4. Sandwich enzyme-linked immunosorbent assay for CEACAM1

We measured CEACAM1 levels in all 175 voided urine specimens. Maxisorp enzyme-linked immunosorbent assay (ELISA) immunoplates (Nunc, Wiesbaden, Germany) were coated with 50 μl of 5 μg/ml polyclonal rabbit antihuman carcinoembryonic antigen (CEA; Dako, Glostrup, Denmark; antibody specifically binds to CEACAM1, CEACAM3, CEACAM4, CEACAM5, CEACAM6, CEACAM7, and CEACAM8). Remaining binding sites were blocked with 5% bovine serum albumin (BSA)–phosphate buffered saline (PBS) solution. Plates were then incubated with 50 μl urine diluted with 50 μl PBS containing 3% BSA. The standards were prepared using purified native CEACAM1-Fc protein in different concentrations [19]. Bound CEACAM1 was labeled by incubating overnight with 100 μl mouse mAb anti-CEACAM1 (clone 4D1C2). Plates were washed and incubated with peroxidase-coupled AffiniPure F(ab′)2 fragment goat antimouse antibody (Jackson ImmunoResearch, West Grove, PA, USA). The enzyme reaction was visualized using 150 μl tetramethylbenzidine (Sigma) as substrate. Absorbance was detected at 450 nm in a Sunrise ELISA reader (Tecan, Männedorf, Switzerland). Samples were measured in triplicate. The reproducibility of our ELISA was affirmed by measuring linearity, intrarun precision, interrun precision, analytical sensitivity, dilution verification, and reference range.

2.5. Statistical analysis

The associations between categorical data were tested using the Fisher exact test or the χ^2 test. Spearman rank correlation coefficients were used to examine the correlations between continuous variables. Differences in continuous variables across categorical variables were