Abstract

Purpose: DOC-2/DAB2 (differentially expressed in ovarian carcinoma-2/disabled-2), a potential tumor suppressor gene, is underexpressed in several cancers. Little is known about the expression of this gene in urothelial carcinoma of the bladder (UCB). We profiled DOC-2/DAB2 expression in mouse and human normal and neoplastic urothelium.

Experimental Design: Immunohistochemical staining for DOC-2/DAB2 was carried out on tissue specimens from two transgenic mouse models with urothelium-specific molecular alterations and on a tissue microarray containing cores from 9 normal controls, 44 patients who underwent transurethral resection of the bladder tumor (TURBT), 195 patients who underwent radical cystectomy for UCB, and 39 lymph nodes with metastatic UCB.

Results: Normal mouse urothelium stained uniformly with DOC-2/DAB2. Weaker staining was observed in low-grade, superficial papillary bladder tumors from transgenic mice harboring constitutively active Ha-Ras, whereas carcinoma in situ – like lesions and high-grade bladder tumors from transgenic mice expressing a SV40 T antigen completely lacked DOC-2/DAB2 expression. In human tissues, DOC-2/DAB2 expression was decreased in 11% of normal bladder specimens, 59% of TURBT specimens, 65% of radical cystectomy specimens, and 77% of the metastatic lymph node specimens. Decreased DOC-2/DAB2 expression was associated with advanced pathologic stage (P = 0.023), lymph node metastases (P = 0.050), and lymphovascular invasion (P < 0.001). In univariable, but not in multivariable analysis, decreased DOC-2/DAB2 associated with an increased probability of bladder cancer recurrence (log-rank test, P = 0.020) and bladder cancer – specific mortality (log-rank test, P = 0.023).

Conclusions: Decreased DOC-2/DAB2 expression seems to occur early in bladder tumorigenesis and becomes more prominent in advanced stages of UCB.

The American Cancer Society estimated that bladder cancer will affect ~67,000 persons, resulting in ~13,000 deaths in the United States in 2007 (1). Multiple chromosomal abnormalities have been implicated in bladder tumorigenesis. Chromosome 9 abnormalities have been the most studied entity because these are typically present in the early stages of bladder tumorigenesis (2). Alterations of chromosome 9 are thought to predispose cells to acquire more advanced chromosomal and genetic abnormalities, thus facilitating advanced stages of tumorigenesis (3). Alterations in chromosome 9 have been recognized in bladder cancer specimens (4, 5), and more recent studies narrowed the abnormal area of interest to the 5p13 region (6–8).

DOC-2/DAB2 (differentially expressed in ovarian carcinoma-2/disabled-2) is a candidate tumor suppressor gene originally identified via a search for differentially down-regulated transcripts in human ovarian carcinoma (9, 10). The DOC-2/DAB2 gene has been cloned and localized to chromosome 5p13 (11). Down-regulation of DOC-2/DAB2 has been reported in multiple cancers, including prostate (12), bladder (13), breast (14), esophageal (15), pancreas (16), colon (17), and gestational trophoblastic disease (18).

To our knowledge, DOC-2/DAB2 protein expression has only been reported in bladder cancer cell lines in vitro, but not in animal or human bladder cancer specimens. The aims of the present study are 2-fold: the first is to profile DOC-2/DAB2 expression in several transgenic mouse models of bladder cancer; the second is to determine the association of DOC-2/DAB2 expression with the presence of human urothelial carcinoma of the bladder (UCB), clinical and pathologic characteristics, metastases, and clinical outcomes in UCB patients with different disease stages. Toward the latter goal,
Fig. 1. DOC-2/DAB2 expression in (A) normal urothelium of wild-type mice; (B) hyperplastic urothelium in transgenic mice expressing a constitutively active Ha-ras oncogene; low-grade, superficial papillary bladder tumors of (C) heterozygous and (D) homozygous ras-transgenic mice; (E) carcinoma in situ – like lesions and (F) high-grade bladder tumors derived from transgenic mice expressing a SV40 T antigen. Urothelium-specific transgenic mice were developed using a uroplakin II – controlled promoter.
we assessed the expression of DOC-2/DAB2 in (a) normal bladder urothelial tissue; (b) UCB tissue from patients who underwent transurethral resection of the bladder tumor (TURBT) for Ta, T1, and/or Tis disease; (c) UCB tissue from patients who underwent radical cystectomy and pelvic lymphadenectomy for advanced disease; and (d) UCB metastases in regional lymph nodes from patients treated with radical cystectomy and pelvic lymphadenectomy.

**Materials and Methods**

**Mouse tissues.** DOC-2/DAB2 expression in normal urothelium and bladder tumors obtained from several transgenic mouse models was initially assessed immunohistochemically as described below. For this purpose, bladder tissues were procured from wild-type as well as transgenic mice specifically expressing a constitutively active Ha-ras oncogene (19) or a SV40 large T antigen (20) in the urothelium under the control of a uroplakin II promoter.

**Patient population.** All human studies were undertaken with the approval and institutional oversight of the Institutional Review Board at the University of Texas Southwestern Medical Center, Dallas, Texas. The study comprised three patient cohorts: 9 patients who underwent cystectomy for causes other than malignancy without evidence of any genitourinary malignancy; 44 patients who underwent TURBT for treatment of Ta, Tis, and/or T1 UCB; and 206 patients who underwent radical cystectomy with bilateral lymphadenectomy.

In the cohort of 44 patients that underwent TURBT, 21 patients (48%) underwent previous TURBTs. Twenty-one patients (48%) were treated with intravesical therapy such as Bacillus Calmette-Guerin and mitomycin C. None of these 44 patients underwent subsequent radical cystectomy at the time of last follow-up.

All 383 consecutive patients admitted at our institution for treatment of UCB with radical cystectomy and bilateral lymphadenectomy during the period from 1987 to 2002 were potential candidates for these analyses. Our analysis was limited to patients who had UCB. Adequately DOC-2/DAB2–stained archival tissue was available for 206 of the 383 cystectomy patients. Histology, grade, stage, and presence of carcinoma in situ were confirmed by blinded review of the original pathology slides. The indications for radical cystectomy were tumor invasion into the muscularis propria or prostatic stroma or Ta, T1, or Tis (carcinoma in situ) refractory to TUR with intravesical chemotherapy and/or immunotherapy. No patient had distant metastatic disease at the time of cystectomy. For each patient, comprehensive clinical and pathologic data elements were collected and entered into an Institutional Review Board–approved database. Multiple data reviews and quality checks were done to assure the accuracy and completeness of data elements. Eleven patients that received neoadjuvant chemotherapy were excluded, leaving 195 patients diagnosed with UCB for final analysis. Median age at radical cystectomy was 66.5 years (range, 38.9-86.0).

**Tissue microarray.** The tissue microarrays comprised (a) noncancer tissue from nine patients who were treated with cystectomy for other causes than malignancy without evidence of any cancer; (b) tumor tissue from the primary, index cancer from 44 patients who were treated with TURBT; (c) tumor tissue from the primary, index cancer from 206 patients who were treated with radical cystectomy; and (d) lymph node tissue involved with cancer from 43 of the 206 patients who were treated with radical cystectomy.

**Table 1.** DOC-2/DAB2 expression in patients with and without UCB

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>DOC-2/DAB2 expression</th>
<th>Normal, Decreased, n (%)</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients without bladder UCB who underwent simple cystectomy</td>
<td>9</td>
<td>8 (88.9)</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Patients treated with TURBT</td>
<td>44</td>
<td>18 (40.9)</td>
<td>26 (59.1)</td>
</tr>
<tr>
<td>Patients treated with radical cystectomy</td>
<td>195</td>
<td>68 (34.9)</td>
<td>127 (65.1)</td>
</tr>
<tr>
<td>Primary, index UCB*</td>
<td>9</td>
<td>9 (23.1)</td>
<td>30 (76.9)</td>
</tr>
<tr>
<td>UCB metastases to lymph nodes†</td>
<td>39</td>
<td>6 (15.4)</td>
<td>33 (84.6)</td>
</tr>
</tbody>
</table>

*After exclusion of 11 patients that received neoadjuvant chemotherapy.
†After exclusion of six patients that received neoadjuvant chemotherapy.

Fig. 2. DOC-2/DAB2 expression in (a) normal bladder tissue; (B) UCB; (C) cancerous lymphoid tissue.
Tissue microarrays were built using a manual tissue arrayer (Beecher Instruments). The index tumor, defined as the largest and/or highest tumor stage and grade, and areas of normal transitional cell not adjacent to cancer cells were identified and circled on the H&E slides. Triplicate 0.6-mm cores were obtained from the circled areas of tumor and/or normal transitional cell and transferred to a recipient paraffin block. Multiple sausage internal controls were also placed with the standard controls.

**Immunohistochemistry.** Paraffin sections containing mouse as well as arrayed human bladder tissues were routinely deparaffinized, hydrated, and treated with 3% hydrogen peroxide in methanol for 15 min to quench the endogenous peroxidase activity. For antigen retrieval, the sections were microwave treated at maximum power for 15 min in 0.01 mol/L citrate buffer (pH 6.0). After an overnight incubation at 4°C with the anti-DOC-2/DAB2 antibody (1:100 dilution of monoclonal DOC-2/p96 antibody from BD Transduction Laboratories), a biotinylated goat anti-rabbit secondary antibody (Vector Laboratories), a biotinylated goat anti-rabbit secondary antibody was applied. The slides were then incubated with avidin conjugated with peroxidase and developed in a solution containing 3,3'-diaminobenzidine (DAB) and H2O2. All sections were then counterstained with hematoxylin, dehydrated, mounted, and observed by light microscopy.

The expression of DOC-2/DAB2 was scored by assigning a proportion score and an intensity score according to Allred's scoring protocol (21). The proportion score was assigned, which represented the estimated proportion of positive-staining cells (0: none; 1: <1/100; 2: 1/100 to 1/10; 3: 1/10 to 1/3; 4: 1/3 to 2/3; and 5: >2/3). The intensity score was assigned, which represented the average intensity of positive cells (0: none; 1: weak; 2: intermediate; and 3: strong). The proportion and intensity scores were combined to obtain a total score, which ranged from 0 to 8. All slides were scored independently by two investigators who were blinded to patient clinical information. In a preliminary study, we assessed the discriminative value of DOC-2/DAB2 as a continuous variable and as a categorical variable with serial increments of cutoffs for DOC-2/DAB2 positivity with regard to bladder cancer characteristics and prognosis (data not shown). Patients with a total score of 3 or less were at higher risk of bladder cancer recurrence and mortality compared with patients with a total score of more than 3. DOC-2/DAB2 immunoreactivity was therefore assigned to one of two categories of staining in the tumor cells: decreased expression (total score ≤3) or normal expression (total score >3).

**Pathology.** Staff pathologists with expertise in genitourinary pathology examined all specimens according to previously published protocols. The 2002 tumor-node-metastasis classification was used for pathologic staging, and the 1973 WHO classification was used for pathologic grading. To ensure the validity of the data extraction, two clinicians read pathology reports of 162 consecutive cystectomy patients, while blinded to patient clinical parameters and the findings of the other reviewer. Inter-reader reliability measured using the intraclass correlation coefficient was >0.95 for all pathologic parameters.

**Follow-up.** Patients generally were seen postoperatively at least every 3 to 4 months for the first year, semiannually for the second year, and annually thereafter. When patients died, the cause of death was determined by the treating physicians, by chart review corroborated by death certificates, or by death certificates alone. Most patients who were identified as having died of bladder cancer had progressive, widely disseminated, and often symptomatic metastases at the time of death. Perioperative mortality (death within 30 days of surgery) was censored at time of death for bladder cancer–specific survival analyses.

**Statistical analysis.** The \( \chi^2 \) test was used to evaluate the association between DOC-2/DAB2 and clinicopathologic parameters. Differences in variables with a continuous distribution across dichotomous categories were assessed using the Mann-Whitney \( U \) test. The Kaplan-Meier method was used to calculate survival functions, and differences were assessed with the log-rank statistic. Multivariable survival analyses were done using the Cox proportional hazards regression model. The lowest quartile was used as the reference category when calculating the

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**Table 2.** Association of DOC-2/DAB2 expression with clinicopathologic characteristics of 195 consecutive patients treated with radical cystectomy and bilateral lymphadenectomy for UCB

<table>
<thead>
<tr>
<th>Number of patients (%)</th>
<th>DOC-2/DAB2 expression</th>
<th>Normal</th>
<th>Decreased</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (%)</td>
<td></td>
<td>68 (34.9)</td>
<td>127 (65.1)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Age (y), median (range)</td>
<td></td>
<td>66.5 (35.2-86.0)</td>
<td>63.3 (38.8-80.5)</td>
<td>68.9 (35.2-86.0)</td>
</tr>
<tr>
<td>Gender (%)</td>
<td>Female</td>
<td>38 (19.5)</td>
<td>9 (23.7)</td>
<td>29 (76.3)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>157 (80.5)</td>
<td>59 (37.6)</td>
<td>98 (62.4)</td>
</tr>
<tr>
<td>Final pathologic tumor stage (%)</td>
<td>Ta, Tis, T1</td>
<td>38 (19.5)</td>
<td>15 (39.5)</td>
<td>23 (60.5)</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>58 (29.7)</td>
<td>28 (48.3)</td>
<td>30 (51.7)</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>69 (35.4)</td>
<td>19 (27.5)</td>
<td>50 (72.5)</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>30 (15.4)</td>
<td>6 (20.0)</td>
<td>24 (80.0)</td>
</tr>
<tr>
<td>Final pathologic grade (%)</td>
<td>Grade 1 or 2</td>
<td>15 (7.7)</td>
<td>6 (40.0)</td>
<td>9 (60.0)</td>
</tr>
<tr>
<td></td>
<td>Grade 3</td>
<td>180 (92.3)</td>
<td>62 (34.4)</td>
<td>118 (65.6)</td>
</tr>
<tr>
<td>Carcinoma in situ (%)</td>
<td>Negative</td>
<td>110 (56.7)</td>
<td>39 (35.5)</td>
<td>71 (64.5)</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>84 (43.3)</td>
<td>28 (33.3)</td>
<td>56 (66.7)</td>
</tr>
<tr>
<td>Lymph node status (%)</td>
<td>N0</td>
<td>132 (68.0)</td>
<td>52 (39.4)</td>
<td>80 (60.6)</td>
</tr>
<tr>
<td></td>
<td>N1, N2</td>
<td>62 (32.0)</td>
<td>15 (24.2)</td>
<td>47 (75.8)</td>
</tr>
<tr>
<td>Lymphovascular invasion (%)</td>
<td>Negative</td>
<td>101 (52.1)</td>
<td>46 (45.5)</td>
<td>55 (54.5)</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>93 (47.9)</td>
<td>21 (22.6)</td>
<td>72 (77.4)</td>
</tr>
</tbody>
</table>

*Mann Whitney \( U \) test.  
\( \chi^2 \) test.  
Lymph node status, lymphovascular status, and carcinoma in situ status were not available for one patient.
hazards ratios. Statistical significance in this study was set as \( P < 0.050 \). All reported \( P \) values are two sided. All analyses were done with SPSS version 13.0 (SPSS Inc.).

### Results

Expression of DOC-2/DAB2 in normal and neoplastic urothelium of transgenic mice. Preliminary evaluation of DOC-2/DAB2 expression in normal urothelium and bladder tumors was carried out using transgenic mouse models specifically expressing various transgenes in the urothelium. Normal urothelium was labeled uniformly throughout basal, intermediate, and superficial layers, with mostly cytoplasmic staining (Fig. 1A). Significantly weaker DOC-2/DAB2 staining was found in hyperplastic urothelium in transgenic mice expressing a constitutively active Ha-Ras oncogene (ras transgenic; Fig. 1B). Weaker membranous and cytoplasmic staining was observed in low-grade, superficial papillary bladder tumors of heterozygous (Fig. 1C) and homozygous ras-transgenic hosts (Fig. 1D). In contrast, carcinoma in situ-like lesions and high-grade bladder tumors derived from transgenic mice expressing a SV40 T antigen completely lacked DOC-2/DAB2 expression (Fig. 1E and F). These data reveal a heterogeneous pattern of DOC-2/DAB2 expression in different phenotypic variants of bladder tumors and set a stage for human studies.

Expression of DOC-2/DAB2 in human normal bladder urothelial cells, UCB, and cancerous lymph node tissues. DOC-2/DAB2 was predominantly expressed in the superficially located umbrella cells of normal urothelium. The staining was primarily cytoplasmic, with occasional weak nuclear labeling (Fig. 2). Table 1 shows DOC-2/DAB2 expression in subjects with and without bladder urothelial carcinoma. DOC-2/DAB2 expression was decreased in 1:9 (11.1%) of noncancerous bladder specimens, 26:44 (59.1%) of the specimens from patients who underwent exclusively TURBT, 127:195 (65.1%) of the specimens from patients who underwent radical cystectomy, and 30:39 (76.9%) of the metastatic lymph node specimens.

Association of DOC-2/DAB2 expression with clinicopathologic characteristics of patients who underwent TURBT for UCB. Median age at TURBT was 64.5 years (range, 41.1 to 89.3). Seven patients were of female gender (15.9%). Fifteen (34.1%) had Ta UCB, nine (20.5%) had Tis UCB, and 20 (45.5%) had T1 UCB. Five (11.4%) had grade 1 disease, 21 (47.7%) had grade 2 disease, and 18 (40.9%) had grade 3 disease. None of the patients underwent radical cystectomy within a median follow-up of 40.4 months (range, 0.3 to 124.6).

DOC-2/DAB2 expression was decreased in the primary index cancer in 26 of 44 (59.1%) of the patients who underwent TURBT for bladder Ta, T1, and/or Tis. Expression of DOC-2/DAB2 was not associated with gender, tumor grade, stage, previous TURBT, or previous intravesical therapy (\( P \) values ≥0.164).

Association of DOC-2/DAB2 expression with clinicopathologic characteristics and clinical outcomes of patients who underwent radical cystectomy for UCB. DOC-2/DAB2 expression was decreased in the primary index cancer in 127 of 195 patients (65.1%) who were treated with radical cystectomy and bilateral lymphadenectomy. Pathologic characteristics of the 195 cystectomy patients and association with DOC-2/DAB2 expression are shown in Table 2. A total of 56 of the 195 patients (28.7%) underwent adjuvant chemotherapy, and 14:195 (7.1%) underwent adjuvant radiation therapy for positive lymph node status and/or extravesical extension. Exactly 23 of 38 patients (60.5%) with low-stage (Ta, Tis, and T1) UCB and 9 of 15 patients (60.0%) with low-grade (grades 1 and 2) UCB had decreased expression of DOC-2/DAB2. Decreased expression of DOC-2/DAB2 was associated with advanced pathologic stage (\( P = 0.023 \)), metastases to lymph nodes (\( P = 0.050 \)), and lymphovascular invasion (\( P = 0.001 \)).

Disease recurred in 87:195 patients (44.6%), and 89:195 (45.6%) patients were dead at the time of analysis. Of these 89 patients, 71 patients died of metastatic bladder cancer, and 18 patients died of other causes without evidence of disease progression. The mean follow-up was 49.3 months (median, 33.4; range, 1.3 to 183.4) for patients alive at the time of analysis. Kaplan-Meier plots showed that a decreased expression of DOC-2/DAB2 was associated with an increased probability of tumor recurrence (log-rank test, \( P = 0.020 \); Fig. 3A) and bladder cancer-specific mortality (log-rank test, \( P = 0.023 \); Fig. 3B). In multivariable Cox proportional hazards regression analyses (Table 3), DOC-2/DAB2 expression was not independently associated with disease recurrence (\( P = 0.383 \)) or disease-specific survival (\( P = 0.607 \)).
**Table 3. Multivariable Cox regression analyses of pathologic features, adjuvant chemotherapy, and DOC-2/DAB2 expression for the prediction of disease recurrence and disease-specific survival of 195 patients treated with radical cystectomy and bilateral lymphadenectomy for UCB**

<table>
<thead>
<tr>
<th>Pathologic tumor grade*</th>
<th>Recurrence</th>
<th>Disease-specific survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazards ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Pathologic tumor stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ta, Tis, T1</td>
<td>1.00</td>
<td>Reference</td>
</tr>
<tr>
<td>T2</td>
<td>0.95</td>
<td>0.35-2.58</td>
</tr>
<tr>
<td>T3</td>
<td>2.05</td>
<td>0.78-5.41</td>
</tr>
<tr>
<td>T4</td>
<td>3.80</td>
<td>1.38-10.47</td>
</tr>
<tr>
<td>Trend</td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td>1.80</td>
<td>1.04-3.11</td>
</tr>
<tr>
<td>Lymph node metastases</td>
<td>1.89</td>
<td>1.14-3.14</td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td>1.31</td>
<td>0.81-2.13</td>
</tr>
<tr>
<td>Decreased DOC-2/DAB2 expression †</td>
<td>0.80</td>
<td>0.49-1.30</td>
</tr>
</tbody>
</table>

Abbreviation: 95% CI, 95% confidence interval.
*Pathologic tumor grade was analyzed as dichotomous variable (grade 3 versus grade 1 and 2).
† DOC-2/DAB2 expression was analyzed as a dichotomous variable (score >3 versus ≤3).

**Discussion**

UCB is a heterogeneous disease that present in two major phenotypes (22). About 80% of UCB present as low-grade papillary tumors, with limited potential to metastasize, whereas 20% present with high-grade, invasive tumors, with up to 50% of patients dying of metastases within 2 years. Alterations in chromosome 5 have been recognized in bladder cancer specimens (4, 5), and more recent studies narrowed the abnormal area of interest to the 5p13 region (6–8). As DOC-2/DAB2 gene is located on chromosome 5p13 (11), we decided to evaluate its association with bladder tumorigenesis in transgenic mice and human UCB specimens.

DOC-2/DAB2 expression was strong in normal urothelium of wild-type mice, but decreased in urothelial hyperplasia and low-grade papillary urothelial carcinoma in urothelium-specific Ras-transgenic mice. Moreover, DOC-2/DAB2 expression was absent in high-grade and invasive urothelial carcinoma in urothelium-specific SV40 T antigen-transgenic mice. This suggests that the loss of DOC-2/DAB2 expression is an early event in bladder cancer tumorigenesis, as well as an important factor in bladder cancer progression in a murine model. In humans, we also found that only 11.1% of patients without UCB had decreased DOC-2/DAB2 expression. Conversely, 59.1% of TURBT bladder specimens, 65.1% of radical cystectomy bladder specimens, and 76.9% of lymph node specimens from patients with UCB had decreased DOC-2/DAB2 expression. Interestingly, similar to the results we found in transgenic mice, DOC-2/DAB2 expression seems to be decreased early in human UCB: 60.5% of patients with low-stage UCB and 60.0% with low-grade UCB had decreased expression of DOC-2/DAB2. In one study of ovarian carcinoma, DOC-2/DAB2 was decreased in 74% of tumors studied (23). The loss of DOC-2/DAB2 expression in ovarian epithelial tumors did not correlate with tumor grade; subsequently, it was postulated that the loss of DOC-2/DAB2 is an early event in ovarian tumorigenesis (24). Similarly, no relation was found between the level of DOC-2/DAB2 expression and stage of colon cancer (17). Moreover, in esophageal squamous cell carcinoma, loss of DOC-2/DAB2 occurs as early as in hyperplasia (15). Taken together, these findings suggest a potential role for DOC-2/DAB2 in early tumorigenesis.

DOC-2/DAB2 expression was more frequently decreased in patients with higher pathologic stage, lymph node metastasis, and lymphovascular invasion. DOC-2/DAB2 expression was not decreased significantly in patients with higher grades, possibly because only 7.7% of the patients had grades 1 and 2 disease, which may have limited the power of this comparison. On multivariable analysis that adjusted for standard clinicopathologic parameters, only pathologic stage and lymph node metastases, but not decreased DOC-2/DAB2, were independent predictors of disease recurrence and disease-specific survival. Because decreased DOC-2/DAB2 expression occurs early in bladder tumorigenesis, it is possible that this marker is not useful to independently predict outcomes such as recurrence, bladder cancer—specific survival, and overall survival.

DOC-2/DAB2 is a phosphoprotein involved in signal transduction, containing multiple SH3 binding motifs at the COOH terminus (10). These motifs are conserved in the mouse homologue of DOC-2/DAB2, p96/Dab2, suggesting important biological functions associated with these motifs (25, 26). The SH3 binding motifs of the mouse p96/Dab2 protein interact with the SH3 domains of growth factor receptor binding protein 2 (Grb2) to reduce the binding between Grb2 and Sos (25). DOC-2/DAB2 inhibits Ras-mediated mitogenic stimulation by binding to Grb2 (27, 28) and negatively regulates Src in the prostate (29). DOC-2/DAB2 is also a negative regulator of Wnt signaling via stabilization of β-catenin degradation complex (30). We have recently shown, in urothelial carcinoma cell lines, that DOC-2/DAB2 transcription regulation is mediated by the epigenetic modulation through histone acetylation and by specific transcription factors such as GATA6 (13), but not by DNA methylation (see Supplementary Data). Similarly, in esophageal cancer, DNA methylation was infrequently associated with DOC-2/DAB2 gene promoter region (15). Taken together, these findings suggest that DOC-2/DAB2 acts as a homeostatic factor that prevents mitogen-activated signaling pathways from overactivation and seems to be a strong
suppressor of cancer cell growth. In bladder tumors with normal expression of DOC-2/DAB2 (~35%), it is possible that another member of the DOC-2/DAB2 signaling pathway is altered, but not DOC-2/DAB2 per se, therefore contributing to early bladder cancer pathogenesis.

In addition to its role as a signaling molecule, DOC-2/DAB2 plays a role in cell-cell adhesion. The presence of a basement membrane in breast and ovarian carcinomas correlates with the expression of DOC-2/DAB2, and plating DOC-2/DAB2–transfected tumor cells on a basement membrane rescued the cells from death (31). Moreover, expression of DOC-2/DAB2 in DOC-2/DAB2–negative ovarian and breast cancer cells results in cell death (31). These results indicate that DOC-2/DAB2 acts as a tumor suppressor gene by controlling the position of epithelial cells to the basement membrane. Loss of DOC-2/DAB2 may contribute to the development of gestational trophoblastic diseases. DOC-2/DAB2–transfected tumor cells on a basement membrane rescued the cells from death (31). Moreover, re-expression of DOC-2/DAB2 in DOC-2/DAB2–negative ovarian and breast cancer cells results in cell death (31). However, little is known whether DOC2/DAB2, in this functional capacity, has any impact on the urothelium and its predilection to develop malignancy.

Potential limitations to our study should be noted. The use of immunohistochemistry has its inherent limitations with regards to choice of antibody, specimen handling, staining techniques, and scoring protocols. To minimize variability due to these factors, we used a tissue microarray and a standardized scoring protocol. In addition, the staining patterns were read separately by two pathologists who were blinded to clinical outcomes, resulting in good reproducibility. The small number of patients and relatively short duration of follow-up might have limited the ability to detect significant associations with clinical outcomes.

Conclusions

DOC-2/DAB2, a phosphoprotein involved in modulating signal transduction, is frequently decreased in patients with UCB and seems to be an early event in bladder tumorigenesis. Further studies are needed to elucidate the molecular consequences of DOC-2/DAB2–decreased expression in the pathogenesis of UCB and the potential of DOC-2/DAB2–targeted therapy.

References

Clinical Cancer Research

Decreased DOC-2/DAB2 Expression in Urothelial Carcinoma of the Bladder

Jose A. Karam, Shahrokh F. Shariat, Hong-Ying Huang, et al.


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