Expression of Epithelial Cell Adhesion Molecule in Gallbladder Carcinoma and Its Correlation With Clinicopathologic Variables

Sagit Prince, MD,1 Aliza Zeidman, MD,1 Yoram Dekel, MD,2 Edward Ram, MD,3 and Rumelia Koren, MD4

Key Words: Gallbladder; Carcinoma; Epithelial cell adhesion molecule

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Abstract

Gallbladder carcinoma is rare and fatal, and conventional therapies have been disappointing. Epithelial cell adhesion molecule (EpCAM) serves as a prognostic marker in various carcinomas and is a target of antibody-based therapies. Our purpose was to examine the expression of EpCAM in gallbladder carcinomas in relation to tumor grade, disease stage, and patient survival.

Gallbladder carcinoma tissue specimens from 25 patients attending our center between 1991 and 2004 were immunohistochemically stained for EpCAM. The intensity and extent of staining were analyzed, and the specimens were classified accordingly: (1) weak, weak or no EpCAM expression in less than 10% of the selected area; (2) moderate, moderate expression in 10% to 49% of the selected area; or (3) strong, heavy staining in 50% or more of the selected area. The correlation between EpCAM expression and clinicopathologic variables was analyzed statistically.

EpCAM overexpression predicted decreased survival (P = .005), but EpCAM expression did not correlate with tumor grade (P = .28) or disease stage (P = .10). EpCAM expression in gallbladder tumors may serve as a prognostic factor for poor survival. Its detection may help clinicians select patients likely to benefit from novel molecular therapies.

Gallbladder carcinoma is a rare solid tumor.1,2 It affects mainly older people, with a peak incidence at age 70 to 75 years, and has a female predominance (female/male ratio, 2-6:1).3 It is usually diagnosed at an advanced stage owing to its anatomic position and vague, nonspecific symptoms and signs.2 Median survival is approximately 6 months, and the 5-year survival ranges from 0% to 10% in most reported series.1,3 The exact cause has not been elucidated, although several factors related to genetic predisposition and genetic alterations, including ethnicity and geographic location, apparently have a role.2,3 Other factors that increase the risk of gallbladder carcinoma are gallstones, gallbladder polyps larger than 10 mm in diameter, anomalous pancreaticobiliary junction, and porcelain gallbladder.

Carcinoma of the gallbladder is an aggressive disease that progresses from dysplasia and carcinoma in situ to invasive carcinoma during about 15 years.3 Carcinomas account for 98% of all gallbladder malignancies, and adenocarcinoma accounts for approximately 90% of gallbladder carcinomas.2

The prognosis is based mostly on disease stage3 and is affected also by tumor grade along with the molecular expression of certain proteins and genes.4-7 Approximately half of the patients with gallbladder carcinoma have metastasis at diagnosis.3,8 Therefore, knowledge about spread of the tumor is important for planning the treatment approach.3,9

Surgery offers the only available cure when tumor spread is limited. Unfortunately, gallbladder carcinoma usually manifests initially as a diffuse disease, and only 10% to 30% of affected patients are considered for curative surgery. Besides extensive disease, low functional status is a contraindication for surgery. The outcome of conventional chemotherapy and radiotherapy has been, for the most part,
disappointing. Researchers are, therefore, seeking novel therapies.\textsuperscript{1,3}

Epithelial cell adhesion molecule (EpCAM) was recently identified as a transmembrane glycoprotein involved in cell proliferation, both benign and malignant.\textsuperscript{1,10-12} It has also been found to up-regulate the proto-oncogenes c-myc and cyclin A/E and decrease cell differentiation.\textsuperscript{12-14}

Different carcinomas are characterized by overexpression of EpCAM or de novo EpCAM expression during carcinogenesis. Overexpression of EpCAM in certain carcinomas has led to therapeutic trials, mostly antibody-based trials directed at this molecule.\textsuperscript{11,15-26} However, there are as yet no studies on the effectiveness of antibody-based anti-EpCAM therapy in gallbladder carcinoma.

The objective of this study was to examine the expression of EpCAM in gallbladder carcinoma and its correlation with tumor grade, disease stage, and patient survival in order to elucidate its potential as a prognosticator and a target for antibody therapy in this setting.

Materials and Methods

Clinical Characteristics

We studied 25 cases of gallbladder carcinoma diagnosed consecutively at our center between 1991 and 2004.\textsuperscript{1 Table I} There were 21 women and 4 men with a median age of 71 years (range, 50-87 years). Of the 25 patients, 17 (68\%) had 1 or more risk factors for atherosclerotic disease, ie, hypertension (15 [60\%]), diabetes mellitus (9 [36\%]), hyperlipidemia (5 [20\%]), and obesity (6 [24\%]). In addition, 5 patients (20\%) were past or present smokers. Ten patients had an atherosclerotic disease: ischemic heart disease in 7 (28\%) and cerebral ischemic stroke in 3 (12\%).

Drug therapy records were available for 19 patients (76\%), of whom 13 had hypertension and 9 diabetes. All patients with hypertension were receiving drug therapy at the time of diagnosis, mostly calcium channel antagonists (11/19 [58\%]), followed by angiotensin-converting enzyme inhibitors (5/19 [26\%]), diuretics (3/19 [16\%]), and \(\beta\)-blockers (2/19 [11\%]). Of the 19 patients, 6 (32\%) were receiving antihyperglycemic agents, and 5 (26\%) were receiving aspirin.

Of 25 patients, 18 (72\%) had metastasis at diagnosis, including 14 (56\%) with liver metastasis. Of the other 7 patients, 1 (4\%) had stage I disease, 4 had stage II (16\%) disease, and 2 (8\%) had stage III disease.

The median survival was 10.7 months (range, 0.2-83.4 months). The 5-year survival rate was 8.7\%.

Histologic and Immunologic Characteristics

The tumor characteristics are summarized in \textsuperscript{1 Table 2 II}. All tumors were diagnosed following cholecystectomy. Surgical specimens were fixed in formalin and embedded in paraffin for examination with conventional H&E stain. The diagnosis of gallbladder adenocarcinoma was verified in all

\textbf{Table I}

EpCAM Expression in 25 Gallbladder Adenocarcinomas by Clinical Data*  

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients</th>
<th>Strong (n = 8)</th>
<th>Moderate (n = 7)</th>
<th>Weak (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD age (y)</td>
<td>71.3 ± 7.4</td>
<td>73.8 ± 8.5</td>
<td>74.4 ± 5.2</td>
<td>67.2 ± 6.4</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>21 (84)</td>
<td>6 (75)</td>
<td>7 (100)</td>
<td>8 (80)</td>
</tr>
<tr>
<td>Male</td>
<td>4 (16)</td>
<td>2 (25)</td>
<td>0 (0)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Comorbid conditions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>15 (60%)</td>
<td>4 (50)</td>
<td>4 (57)</td>
<td>7 (70)</td>
</tr>
<tr>
<td>Diabetes mellitus type 2</td>
<td>9 (36%)</td>
<td>1 (13)</td>
<td>4 (57)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>5 (20%)</td>
<td>0 (0)</td>
<td>2 (29)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Past stroke</td>
<td>3 (12%)</td>
<td>1 (13)</td>
<td>1 (14)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>7 (28%)</td>
<td>3 (38)</td>
<td>2 (29)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Drug therapy recorded</td>
<td>19 (76%)</td>
<td>5 (63)</td>
<td>6 (87)</td>
<td>8 (80)</td>
</tr>
<tr>
<td>Antihypertensives</td>
<td>13 (68%)</td>
<td>2 (40)</td>
<td>4 (67)</td>
<td>7 (88)</td>
</tr>
<tr>
<td>Diuretics</td>
<td>3 (16%)</td>
<td>0 (0)</td>
<td>1 (17)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Calcium channel antagonists</td>
<td>11 (58%)</td>
<td>2 (40)</td>
<td>3 (50)</td>
<td>6 (75)</td>
</tr>
<tr>
<td>(\beta)-Blockers</td>
<td>2 (11%)</td>
<td>0 (0)</td>
<td>2 (33)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>5 (26%)</td>
<td>1 (20)</td>
<td>2 (33)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Antihyperglycemics</td>
<td>6 (32%)</td>
<td>0 (0)</td>
<td>4 (67)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Sulfonylureas</td>
<td>5 (26%)</td>
<td>0 (0)</td>
<td>4 (67)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Biguanides</td>
<td>1 (5%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>5 (26%)</td>
<td>0 (0)</td>
<td>2 (33)</td>
<td>3 (38)</td>
</tr>
</tbody>
</table>

ACE, angiotensin-converting enzyme; EpCAM, epithelial cell adhesion molecule.

* Data are given as number (percentage) unless otherwise indicated. Percentages in subcategories are based on the numbers for the categories, eg, 13/19 for patients receiving antihypertensives and 1/6 for patients receiving diuretics and with moderate EpCAM expression.
EpCAM in Gallbladder Carcinomas

Table 2
EpCAM Expression in 25 Gallbladder Adenocarcinomas by Pathologic Data*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients</th>
<th>Strong (n = 8)</th>
<th>Moderate (n = 7)</th>
<th>Weak (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor grade†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well-differentiated</td>
<td>5 (20)</td>
<td>1 (13)</td>
<td>0 (0)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>16 (64)</td>
<td>5 (63)</td>
<td>6 (86)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>4 (16)</td>
<td>2 (25)</td>
<td>1 (14)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Other tissue characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysplasia</td>
<td>11 (44)</td>
<td>2 (25)</td>
<td>4 (57)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Ulceration or necrosis</td>
<td>9 (36)</td>
<td>3 (38)</td>
<td>4 (57)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Inflammation</td>
<td>12 (48)</td>
<td>2 (25)</td>
<td>5 (71)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Existence of gallstones</td>
<td>19 (76)</td>
<td>4 (50)</td>
<td>7 (100)</td>
<td>8 (80)</td>
</tr>
<tr>
<td>Tumor stage‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1 (4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>II</td>
<td>4 (16)</td>
<td>0 (0)</td>
<td>2 (29)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>III</td>
<td>2 (8)</td>
<td>0 (0)</td>
<td>1 (14)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>IV</td>
<td>18 (72)</td>
<td>8 (100)</td>
<td>4 (57)</td>
<td>6 (60)</td>
</tr>
</tbody>
</table>

EpCAM, epithelial cell adhesion molecule.

* Data are given as number (percentage).

† The stronger the EpCAM stain, the greater tendency for better differentiation (P = .28, not significant).

‡ The stronger the EpCAM stain, the greater the tendency for a more advanced stage (P = .10, borderline significance).

cases. Six tumors (24%) were of the papillary type, and the remainder were of the nodular and tubular types. Gallstones were found in 19 (86%) of 22 patients tested. Most of the tumors were moderately differentiated, followed by well differentiated and poorly differentiated, and the tumor was histologically associated with dysplasia, ulceration or necrosis, or inflammation in similar numbers of cases (Table 2).

Immunohistochemical Staining

For the present study, 4-µm-thick sections of the paraffin blocks containing the most representative areas of the tumor were stained immunohistochemically for EpCAM. The specimens were oven heated for 4 hours and deparaffinized by soaking in xylene for 2 cycles of 15 minutes each, followed by washing with 100% ethanol for 2 cycles of 5 minutes each and then with 95% ethanol for 2 cycles. The slides were rinsed with purified water for 3 minutes and placed in 3% oxygen water for 10 minutes to neutralize endogenous peroxidase activity. This step was followed by rinsing with purified water for 3 cycles, incubating with trypsin for 10 minutes at 37°C, and repeated washing with purified water and then with phosphate-buffered saline (PBS). The staining procedure was performed as follows: the slides were covered with a solution containing a monoclonal antibody against EpCAM (Biocare Medical, Concord, CA) in a concentration of 1:100 for 1 hour, washed with PBS, incubated in a solution containing horseradish-peroxidase polymer conjugate for 15 minutes, washed with PBS, incubated with diaminobenzidine tetrahydrochloride chromogen for 5 minutes, washed in running water for 3 minutes, stained with Mayer hematoxylin for 5 minutes, washed with running water, soaked in 96% ethanol for 1 minute and 100% ethanol for 1 minute, soaked in xylene, and covered with Permount (Biomeda, Burlingame, CA).

Immunohistochemical Evaluation

The tumors were stained heterogeneously by EpCAM. The most strongly stained tumor area in each case was selected, and EpCAM expression was evaluated and graded according to the intensity and extent of staining as follows: weak staining, no staining or very weak staining in less than 10% of the selected area; moderate, moderate staining in 10% to 49% of the selected area; or strong, heavy staining in 50% or more of the selected area.

Statistical Analysis

The association between EpCAM expression and clinicopathologic variables was analyzed by the 2-tailed t test and the Pearson χ² test. Kaplan-Meier survival curves were plotted. A P value of less than .05 was considered statistically significant. BMTP software (1993) was used for data management and analysis.

Results

EpCAM Expression and Its Correlation With Clinical and Pathologic Variables

EpCAM expression was cytoplasmic in all cases. Our assessment of the intensity and extent of staining yielded strong expression of EpCAM in 8 cases (32%) Image 1, moderate expression in 7 (28%), and weak expression in 10 (40%) Image 2.
The correlations of the degree of EpCAM staining with selected clinical variables (age, sex, comorbid conditions, and medication use) and pathologic variables (tumor grade, disease stage, and presence of inflammation, dysplasia, and necrosis or ulceration) are shown in Tables 1 and 2. None of these variables was associated with EpCAM expression.

Survival Analysis

Kaplan-Meier analysis was used to calculate the impact of classic clinicopathologic features and EpCAM expression on survival. As expected, disease stage ($P = .02$) and tumor grade ($P = .03$) were of prognostic value, whereas age and sex were not significant. Stronger expression of EpCAM was associated with decreased survival ($P = .005$) with cases with strong staining having a survival time of 5.2 months compared with 10.8 months for cases with moderate staining and 21.6 months for cases with weak staining. Cox regression analysis revealed a statistically significant correlation among disease stage, patient survival, and EpCAM expression ($P = .019$). Interestingly, subgroup analysis confirmed a statistically significant impact of EpCAM expression on survival, but the impact of disease stage was less significant.

In the clinical analysis, the presence of gallstones correlated significantly with improved prognosis, with a median survival of 16.4 months for patients with gallstones vs 2.4 months for patients without ($P = .0001$). The effect of treatment with calcium channel antagonists on improved survival was of borderline significance. Median survival was 17.7 months in patients who were treated with calcium channel antagonists vs 8.2 months in patients who were not ($P = .066$).

Discussion

The main finding of the present study was the correlation of strong EpCAM expression in gallbladder carcinomas with prognosis. Of the 25 samples (from 25 patients) examined histologically, 60% showed strong to moderate EpCAM expression. This rate is close to the 63.6% severe-moderate EpCAM expression reported by Varga et al in the only other such study in gallbladder cancer and agrees with the report of EpCAM expression in other cancers of the colon, stomach, prostate, and lung. Our high rate of EpCAM expression combined with lack of a significant correlation of EpCAM
expression with tumor grade and disease stage may indicate that anti-EpCAM antibody–based therapy may be effective in gallbladder carcinoma. This assumption is further supported by our finding of a significant correlation between EpCAM overexpression and decreased patient survival (P = .005), establishing EpCAM as a prognostic factor with potential usefulness for the selection of patients most likely to benefit from antibody drug trials. This study corroborates the results of Varga et al.,1 which was the first to show that EpCAM expression is an independent marker of survival in gallbladder carcinoma.

The characteristics of our study group were in line with those in previous reports on gallbladder carcinomas: median age, 71 years; female/male ratio, 5.1:1; and increased propensity for gallstones (86% vs 65%-90% in the literature).2,3 The 10.7-month median survival for our group was slightly higher than the approximately 6 months noted by others.3 Also in agreement with earlier studies, we found a significant correlation between survival and tumor grade (P = .03) and stage (P = .02).1,3

Surprisingly, the presence of gallstones was significantly associated with better prognosis (P = .0001). Accordingly, Wakai et al.27 reported a borderline statistical correlation of gallstones and improved survival in patients with gallbladder carcinoma (P = .072). Gallstones are also an established risk factor for the disease, although apparently not the only one, because not all patients with gallbladder cancer have gallstones and gallbladder cancer develops in only 1% to 3% of all patients with gallstones.3 This correlation might be attributable in part to the known correlation of the expression of certain genes with gallstones.3 The presence of gallstones could be associated with better survival because their surgical treatment leads to earlier detection of the malignancy.

A correlation of borderline significance was observed between treatment with calcium channel antagonists and improved survival (P = .066). This finding has not been reported previously. It may have been due, however, to a selection bias: patients receiving more extensive therapy for comorbidities may have better functional status, and better control of comorbidities may contribute to improved survival.

Therapeutic trials of monoclonal antibodies directed against EpCAM have shown that they may induce antibody-based cellular cytotoxicity22,24-26 by adhering to cytokines such as interleukin 2 or complement-based cytotoxicity23,28 by activating T-cytotoxic cells. MT201, an IgG1 monoclonal antibody, has moderate affinity to EpCAM, and others, such as ING-1, have high affinity.22,26,29 Many studies use a bispecific antibody that can bridge CD3+ T cells to tumor cells positive for EpCAM. Some investigators have attempted intracellular gene insertion using, for example, a thymidine kinase gene, to make the cells responsive to specific drugs, such as ganciclovir.14,21 So far, research has been limited to in vitro and ex vivo trials. In the few in vivo experiments in animals and preliminary in vivo studies in humans,22,27 the monoclonal antibodies were found to have a good safety profile and could be used with low effector-to-target concentrations.17,22,27

The findings of the present study have important therapeutic implications. The results of conventional radiation and chemotherapy in patients with gallbladder cancer have been disappointing.2,3 Although surgery remains a curative treatment option, most patients are not candidates for this approach because of low functional status owing to older age and the presence of comorbidities.2,3 In our study, more than 60% of patients had hypertension, diabetes, and/or hyperlipidemia, and 40% had atherosclerotic disease. Furthermore, most patients, as in our group as well, have a diffuse malignancy at diagnosis.2,3 Several preliminary studies have shown that EpCAM-directed immunotherapy is successful in primary and secondary malignancies and holds promise as a complementary therapy against colonic, breast, and ovarian carcinoma.23,26,27 Our study indicates that it may also benefit patients with gallbladder carcinoma who are unable to withstand aggressive surgery.

We found that moderate to strong EpCAM staining in most gallbladder carcinomas, together with the correlation between EpCAM expression and survival and lack of a significant correlation between EpCAM expression and tumor grade or stage, suggests that anti-EpCAM molecular therapy may be effective in gallbladder carcinoma. In addition,
EpCAM may serve as a feasible tool for selecting the patients most likely to benefit from such therapy.

The sample size in our study was small because of the malignancy’s relative rarity. Therefore, more research on EpCAM expression in gallbladder carcinoma and trials with anti-EpCAM molecular therapy are needed to corroborate these findings.

From the Departments of 1Internal Medicine B, 2Institute of Urology, 3General Surgery A, and 4Pathology, Rabin Medical Center, Petach Tikva, affiliated with Sackler Faculty of Medicine, Tel-Aviv University, Israel.

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Address reprint requests to Dr. Zeidman: Dept of Internal Medicine B, Hasharon Hospital, Petach Tikva, Israel.

References