Epithelial Cell Adhesion Molecule (KSA) Expression: Pathobiology and Its Role as an Independent Predictor of Survival in Renal Cell Carcinoma

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ABSTRACT

Purpose: Epithelial cell adhesion molecule (EpCAM) is a widely expressed adhesion molecule in epithelial cancers. The purpose of this study is to determine the protein expression patterns of EpCAM in renal cell carcinoma (RCC) using tissue arrays linked to a clinicopathological database to evaluate both its predictive power in patient stratification and its suitability as a potential target for immunotherapeutic treatment strategies.

Experimental Design: The University of California, Los Angeles kidney cancer tissue microarray contains specimens from 417 patients treated with nephrectomy. EpCAM protein expression in tumors and matched morphologically normal renal tissues was evaluated using anti-EpCAM immunohistochemistry. The resultant expression reactivity was correlated with clinicopathological variables.

Results: EpCAM is consistently expressed in the distal nephron on normal renal epithelium. Clear cell RCCs show minimal and infrequent EpCAM expression, whereas chromophobe and collecting duct RCCs both demonstrate intense and frequent expression. Of 318 clear cell carcinomas used in the analysis, 10% were EpCAM positive in ≥50% of cells, and 8% of patients would be considered candidates for EpCAM-based therapy, based on high expression [≥moderate intensity and frequent (≥50%) expression] and the need for systemic treatment. EpCAM expression was an independent prognostic factor for improved disease-specific survival, with a multivariate hazard ratio of 0.63 (P = 0.017; 95% confidence interval, 0.43–0.92).

Conclusions: EpCAM is a novel prognostic molecular marker in RCC patients, and its positive expression is an independent predictor associated with improved survival. However, high expression in morphologically normal renal tissues and minimal or absent expression in clear cell carcinomas will likely limit the utility of this epithelial marker in targeted treatments of this most common RCC type.

INTRODUCTION

There are approximately 30,000 new cases of renal cell carcinoma (RCC) diagnosed each year, comprising approximately 2% of all cancers and an estimated 12,000 disease-related deaths annually (1). The clear cell type predominates, encompassing approximately 70% of all RCC (2). Metastatic disease accompanies one-third of new RCC diagnoses, and approximately 30% of those treated for localized disease eventually relapse (3). Postoperative prognostication of patients treated with radical nephrectomy remains largely based on tumor stage and nuclear grade (4–8). However, these factors alone are inadequate to predict with accuracy the complex course of this disease. Novel outcome prediction models have incorporated additional clinicopathological variables and promise to improve prediction (9, 10). In addition, molecular tumor markers are likely to become increasingly valuable in prognostication, early tumor detection, stratification of patients for treatment and follow-up regimens, and entry into clinical trials (11).

Epithelial cell adhesion molecule (EpCAM), also known as KSA, KSI1/4, and 17-1 antigen, is a 34–40-kDa glycosylated transmembrane cell surface epithelial protein of 232 amino acids encoded on chromosome 2p21 (12). Previous studies have documented EpCAM expression in a wide spectrum of normal adult epithelial tissues, especially those of glandular and transitional epithelia (13–19). Abundant EpCAM expression is also seen across most epithelial malignancies, including colon, lung, stomach, pancreas, thyroid, breast, ovary, cervix, bladder, and prostate (Refs. 13, 14, and 17–24; reviewed in Ref. 25).

Pancarcinoma expression of EpCAM has been exploited for diagnostic confirmation of epithelial lineage and for detection of disseminated micrometastases (26–30). Given its plasma membrane distribution, significant attention has also been directed to the molecule as a target for immunotherapy using either unconjugated (31–35) or conjugated (36) anti-EpCAM antibodies of varying affinity. Five and 7-year mortality rates were reduced for patients with minimal residual colorectal cancer using the low-affinity anti-EpCAM antibody edrecolomab (Panorex), which has been approved for clinical use in Germany (34, 37).
Recently, humanized low-affinity antibodies have been introduced that provide murine-equivalent complement-dependent and improved antibody-dependant cellular cytotoxicity (complement-dependent cytotoxicity and antibody-dependant cellular cytotoxicity, respectively), with a promise of decreased immunogenicity and toxicity (38). EMD 273066 (huKS-IL2) is a recombinant immunocytokine fusion protein consisting of a monoclonal antibody to EpCAM that is linked to an active interleukin (IL)-2 molecule that has been designed for the targeted delivery of IL-2 to EpCAM-expressing tumor tissues. If effective, this would have tremendous relevance to the treatment of metastatic RCC, as a means of targeted, localized delivery of IL-2 with the potential to reduce systemic toxicity. Clinical studies investigating this protocol are being contemplated, pending empirical pre-clinical justification. To better understand the relative tissue distribution, prognostic impact, and potential for anti-EpCAM-targeted immunotherapy in human renal tissues, we performed an immunohistochemical analysis on malignant and matched morphologically normal tissues in a kidney tissue microarray linked to extensive pathology and clinical datasets.

MATERIALS AND METHODS

Patients. Following study protocol 99-233 approval by the University of California, Los Angeles Institutional Review Board, a retrospective study was performed with outcome assessment based on chart review of clinical and pathological data. A total of 395 formalin-fixed paraffin-embedded primary kidney cancer specimens and 9 oncocytomas were obtained from the Department of Pathology at the University of California, Los Angeles Medical Center from nephrectomy cases occurring between 1985 and 2000. Malignant tumors were graded according to Fuhrman et al. (4). Pathological tumor subtyping and tumor staging were performed according to the 1997 Union Internationale Contre le Cancer and American Joint Committee on Cancer tumor-node-metastasis (TNM) classification of malignant tumors (2, 39). T stage was determined from surgical pathology. N and M stages were determined by clinical, radiographic, and/or postoperative pathological data. The Eastern Cooperative Oncology Group (ECOG) performance status and metastatic status were determined at initial presentation (40). Patients with localized disease are those with M and N stage = 0. Sixty-four percent of patients with metastases at nephrectomy were treated with IL-2-based immunotherapy. Disease recurrence was monitored by a protocol of physical examination, liver function tests, and radiographic exams of the chest, abdomen, and pelvis every 6–12 months.

Tissue Microarrays and Whole Tissue Sections. At least three core tissue biopsies 0.6 mm in diameter were taken from selected morphologically representative regions of each paraffin-embedded renal tumor and precisely arrayed using a custom-built instrument (Beecher Instruments) as described previously (41). Additional core tissue biopsies were taken from morphologically benign-appearing surrounding renal tissue for each tumor. To compare the tissue array results with whole tissue sections an additional 20 matched tumor/normal whole tissue specimens were obtained from the University of California, Los Angeles Human Tissue Resource Center.

Immunohistochemistry. Four-μm-thick sections of the tissue microarray block were transferred to glass slides using a paraffin sectioning aid system (Instrumedics Inc., Hackensack, NJ) to support the integrity and maintain the array grid of the 0.6-mm array elements. Four-μm-thick whole tissue sections were mounted on conventional glass slides. All sections were heated at 56°C for 25 min, deparaffinized in three changes of xylene, and rehydrated through a descending series of ethanol. A biotin-free immunohistochemical staining technique was used (EnVision + System, HRP Mouse Kit; DAKO). After endogenous peroxidase blocking with a 0.03% hydrogen peroxide solution for 5 min, EpCAM antigens were retrieved through a 10-min pepsin digestion (Digest-All 3; Zymed) at 37°C. Blocking of nonspecific protein binding was accomplished by incubation for 30 min with 3% normal goat serum. The sections were then incubated for 30 min at room temperature with an anti-EpCAM murine monoclonal antibody recognizing an extracellular epidermal growth factor-like domain of EpCAM (IgG2a clone KS 1/4; 20 μg/ml final concentration; BD Pharmingen, San Diego, CA). Subsequently, the sections were incubated with a goat antimouse-conjugated peroxidase-labeled polymer secondary antibody (DAKO EnVision + ) for 30 min at room temperature. The sections were visualized with application of diaminobenzidine substrate chromagen solution and hematoxylin counterstain. Sections from a single moderate to poorly differentiated colon carcinoma sample with associated matched morphologically normal colon tissue were used for both EpCAM antibody optimization experiments and as positive tissue controls for all experiments. A murine antibody isotype-matched to the EpCAM antibody clone (IgG2a; Sigma-Aldrich, St. Louis, MO) was used as a negative control for all experiments. Mouse pankeratin (4/5/6/8/10/13) was used as a positive assay control.

EpCAM Protein Expression Scoring. All sections were examined using an Olympus BX-40 bright-field microscope (Olympus). Quantitative assessment of EpCAM expression was performed by a single pathologist (D. B. S.) blinded to clinicopathological variables. Where multiple normal histology components were present (proximal tubules, distal tubules, collecting ducts, and glomeruli), each was scored separately. Staining was well localized to the plasma membrane of renal epithelium. Target cells within each tissue spot were scored, noting the proportion of cells staining at each intensity level, using a 0–3 scale (0 = negative, 1 = weak, 2 = moderate, 3 = strong).

To survey a wide range of EpCAM staining variables, the staining of each tissue spot was first represented by maximal staining intensity and overall positivity. These spot-level representations were then pooled using maximum, median, mean, and minimum strategies to survey for the best tumor expression representation. We used a survival tree analysis with outcome disease-specific survival to find the most significant staining score as well as its cutoff point. The overall score used for subsequent statistical outcomes analysis was the pooled median EpCAM expression frequency of all evaluable tumor spots of each case. EpCAM was considered positive if the pooled median staining positivity was ≥5% of the tumor cells in each case.

Statistical Analysis. The primary outcome of interest was disease-specific survival. The length of follow-up was the time from nephrectomy to death or last contact. A Kaplan-Meier
EpCAM protein expression in morphologically normal renal tissues by immunohistochemistry. Characteristic staining pattern in morphologically normal renal tissues on tissue arrays following the direction of flow. In the proximal nephron, the glomerular tufts and Bowman’s capsule (A, ●), the convoluted (A, ●) and straight proximal tubules (B, ●), and the loops of Henle (C, arrowheads) typically display overall negative to weak staining. More prominent staining begins in the thick ascending limbs and straight distal tubules of the medullary rays (A, ●) and increases to a maximal expression in distal convoluted tubules of the cortical labyrinth (A, arrowheads). Positivity, although more heterogeneous in pattern, continues in the collecting ducts extending into the cortical medullary rays (A, arrow) and outer and inner medulla (B and C, arrows, respectively). EpCAM expression ceases in the transitional epithelium of the pelvis and proximal ureter (D). Original objective: ×10, A–D; ×40, insets.

Fig. 1

EPCAM protein expression patterns were evaluated in both tissue arrays and a survey of 20 matched tumor/normal whole tissue sections that provided confirmatory geographic localization of renal structures and displayed staining patterns in good agreement with that seen on the arrays (data not shown). After exclusion of non-RCC tumor histologies, a total of 404 cases from 400 patients, encompassing 2068 total tissue array spots, were represented, and 1909 spots (92%) were evaluable for EpCAM expression (1450 of 1583 tumor and 459 of 485 matched normal samples). Included were 395 cases of RCC by immunohistochemistry is seen in Fig. 3. Whereas clear cell (Fig. 3A), papillary (Fig. 3B) and sarcomatoid (Fig. 3E) RCC show little EpCAM expression, chromophobe (Fig. 3F) and collecting duct (Fig. 3G) RCC both demonstrate strong positivity, with expression in papillary RCC falling intermediate to the above-mentioned groups, with the basophilic (Fig. 3C) subtype typically expressing EpCAM less abundantly than the eosinophilic (Fig. 3D).

The maximal membrane staining intensity and the positive cellular staining frequency distributions for these normal renal epithelial components are depicted in Fig. 2, A and B, respectively. Maximal staining to at least a moderate level was seen in 0% of 268 array tissue spots containing glomeruli (78% are negative), 18% of 402 spots containing proximal tubules (9% are negative), 100% of 417 spots containing distal convoluted tubules (0% are negative), and 85% of 68 spots containing collecting ducts (0% are negative; Fig. 2A). The proportion of cells staining (frequency) followed a similar pattern (Fig. 2B). Three percent of spots with glomeruli, 3% of spots with proximal tubules, 99% of spots with distal convoluted tubules, and 78% of spots with collecting ducts had positive staining in ≥50% of the cells of that component, showing that the staining in distal tubules is not only stronger but also more frequent.

EpCAM expression visible by immunohistochemistry ceases to be expressed in the transitional epithelium of the renal pelvis and proximal ureter. With exceedingly rare exception, amounting to only three vessel profiles seen in over 2000 array spots, EpCAM expression is negative in the smooth muscle of vasculature. It is also universally negative in nerve, inflammatory white cells, stromal fibroblasts, and endothelium.

Representative EpCAM protein expression in renal tumors by immunohistochemistry is seen in Fig. 3. Whereas clear cell (Fig. 3B) and sarcomatoid (Fig. 3E) RCC show little EpCAM expression, chromophobe (Fig. 3F) and collecting duct (Fig. 3G) RCC both demonstrate strong positivity, with expression in papillary RCC falling intermediate to the above-mentioned groups, with the basophilic (Fig. 3C) subtype typically expressing EpCAM less abundantly than the eosinophilic (Fig. 3D).

estimator was used to visualize disease-specific survival distributions. The log-rank statistic was used to test whether different groups had different survival distributions. Univariate and multivariate Cox proportional hazards models were used to relate a variety of potential prognostic factors to survival. Schoenfeld residuals were used to verify the proportional hazards assumption. To identify the optimal staining score of EpCAM expression and its cutoff value, we used a survival tree model that is implemented in the RPART function of the freely available software R. The robustness of the cutoff was confirmed by surveying a wide range of cutoffs. The Pearson χ² statistic was used to correlate dichotomized marker expression with pathology covariates. All Ps are two sided, and P < 0.05 is considered significant. Statistical analyses were performed using the R software.

RESULTS

EpCAM protein expression patterns were evaluated in both tissue arrays and a survey of 20 matched tumor/normal whole tissue sections that provided confirmatory geographic localization of renal structures and displayed staining patterns in good agreement with that seen on the arrays (data not shown). After exclusion of non-RCC tumor histologies, a total of 404 cases from 400 patients, encompassing 2068 total tissue array spots, were represented, and 1909 spots (92%) were evaluable for EpCAM expression (1450 of 1583 tumor and 459 of 485 matched normal samples). Included were 395 cases of RCC.

EpCAM protein is consistently expressed in matched morphologically normal renal epithelium by immunohistochemistry and is most prominent in the distal nephron, where it is typically abundantly expressed (Fig. 1). Expression predominates in basolateral cellular membranes, and, rarely, a weak cytoplasmic staining is also seen. Following the direction of flow, glomerular tufts are universally negative for EpCAM; however, scattered and weak expression is occasionally seen in the simple squamous epithelium of the parietal layer of Bowman’s capsule. The convoluted and straight proximal tubules and the loops of Henle typically display overall negative to weak staining. More prominent staining begins in the thick ascending limbs and straight distal tubules of the medullary rays, and staining increases to a maximal expression in distal convoluted tubules of the cortical labyrinth. Positivity, although more heterogeneous in pattern, continues in the collecting ducts extending into the cortical medullary rays to the outer and inner medulla.

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6 http://www.r-project.org/.
subtype. Interestingly, oncocytomas (Fig. 3H) display only scattered and focal positivity in single or sparsely clustered cells that are otherwise histomorphologically indistinct. Low-grade papillary and collecting duct carcinoma cells generally retain basal or basolateral staining. In high-grade papillary tumors, staining sometimes becomes more uniformly circumferential, as is likewise seen in tumors of the clear cell and chromophobe types, highlighting the frequent loss of cellular polarity in these tumors. Occasionally, focally strong EpCAM concentrations occur at cellular interfaces, suggesting either a remnant or aberrant recapitulation of basolateral polarity.

The maximal membrane staining intensity and the positive cellular staining frequency distributions for renal tumor epithelium, by tumor type, are depicted in Fig. 4, A and B, respectively. Maximal staining to at least a moderate intensity level was seen in 20% of 1200 clear cell spots, 10% of 49 sarcomatoid spots, 49% of 130 papillary spots, 100% of 22 chromophobe spots, and 71% of 21 collecting duct RCC spots. The proportion

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**Fig. 2** EpCAM protein expression distribution in normal renal tissue components by immunohistochemistry. The staining distribution of matched morphologically normal renal tissues, by histological category, is shown. The proportion of spots staining with the stated maximal intensity (A), negative, weak, moderate, strong; or staining frequency category (B), 0–24%, 25–49%, 50–74%, 75–100%, is depicted for each histological category. NL, normal; PT, proximal tubule; DCT, distal convoluted tubule; CD, collecting duct.

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**Fig. 3** EpCAM protein expression in renal cell carcinoma (RCC) and oncocytoma by immunohistochemistry. Characteristic staining pattern of renal tumors on tissue arrays is shown; morphologically normal renal cortex is included for reference. A, matched morphological normal with strong staining corresponding to distal convoluted tubules, and relative negativity of other components. B, RCC clear cell, negative staining. C, RCC papillary basophilic, moderate basolateral staining. D, RCC papillary eosinophilic, strong basolateral staining. E, RCC sarcomatoid, negative staining. F, RCC chromophobe, strong circumferential staining. G, RCC collecting duct, strong basolateral staining. H, oncocytoma, moderate but only focal staining. Original objective: ×10, A–H; ×40, insets.)
of cells staining at ≥50% within these groups is similarly
distributed (13%, 4%, 45%, 100%, and 71%, respectively; Fig.
4B). However, whereas 59% of 27 oncocytoma spots stained to
at least a moderate intensity level, only 4% of the spots had
≥50% tumor cell positivity. Therefore, minimal and infrequent
staining is seen in clear cell and sarcomatoid tumors, an inter-
mediate intensity and frequency are seen in papillary tumors as
a group, strong and frequent staining is present in both chromo-
phobe and collecting duct tumors, and moderate but rare stain-
ing is seen in oncocytomas.

The maximal membrane staining intensity and the positive
staining frequency distributions for clear cell and papillary
tumors stratified by grade are shown in Fig. 4, C and D,
respectively. There is only a subtle increasing trend in EpCAM
expression intensity (Fig. 4C) with increasing grade in the clear
cell cases. Thirteen percent of grade 1, 20% of grade 2, 24% of
grade 3, and 22% of grade 4 tumor spots had a ≥moderate
maximal intensity. In papillary tumors, a ≥moderate maximal
intensity is seen in 42% of grade 1, 51% of grade 2, and 50% of
grade 3 tumor spots. However, a more distinctive rise in expres-
sion is seen when only the strongest staining group is examined,
with 10%, 30%, and 45% of tumor spots staining strongly in
grades 1, 2, and 3, respectively (note, no grade 4 papillary
tumors are present).

Outcomes analyses were limited to RCCs of the clear cell
type. The study end point was disease-specific death. A total of
318 of 341 (93%) clear cell RCCs were evaluable for outcome
studies. The clinical characteristics of 318 patients at the time of
nephrectomy for clear cell RCC are summarized in Table 1. The
median age of this group was 61 years (range, 27–88 years), and
the male:female ratio was 2:1 (Table 1). The median tumor size
was 7 cm, and 49% of patients had metastatic disease at the time

Fig. 4 EpCAM protein expression distribution of renal cell carcinoma and oncocytoma by immunohistochemistry. The staining distribution of renal
tumors, by category, is shown [matched normal distal convoluted tubule (NL DCT) is included for reference, as the strongest staining normal
components]. The proportion of spots staining with the stated maximal intensity (A), □, negative, □, weak, □, moderate, □, strong; or staining
frequency category (B), □, 0–24%, □, 25–49%, □, 50–74%, □, 75–100%, is depicted for each histological category. The proportion of clear cell or
papillary renal cell carcinoma spots staining with the stated maximal intensity (C), □, negative, □, weak, □, moderate, □, strong; and staining
frequency category (D), □, 0–24%, □, 25–49%, □, 50–74%, □, 75–100%, is depicted stratified by tumor grade. GD, grade; Pap, papillary.
of nephrectomy. The median follow-up was 28 months overall (range, 0.03–142 months), 55 months for surviving patients (range, 0.3–142 months), and 14 months for patients dead of disease at last follow-up (range, 0.3–100 months).

The staining distribution of the 318 cases of clear cell RCC grouped as described are seen in Fig. 5; 41% and 59% of patients were EpCAM positive and negative, respectively, by the dichotomized criteria. These EpCAM expression groups

<table>
<thead>
<tr>
<th>Table 1 Clinicopathologic characteristics and EpCAM expression in 318 patients with renal cell carcinoma, clear cell type</th>
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<tbody>
<tr>
<td>Characteristic</td>
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<td>----------------</td>
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<tr>
<td>All patients</td>
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<td>Gender (n = 318)</td>
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<td>Age (yrs) (n = 316)</td>
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<td>Tumor size (cm) (n = 316)</td>
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<td>Grade (n = 318)</td>
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<td>pT stage (tumor stage) (n = 317)</td>
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<tr>
<td>pN stage (regional nodes) (n = 318)</td>
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<td>pM stage (distant metastasis) (n = 318)</td>
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<td>Localized (n = 318)</td>
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<td>ECOG PS (n = 318)</td>
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<td>Recurrence (n = 163)</td>
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<td>Survival (n = 318)</td>
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<sup>a</sup> EpCAM, epithelial cell adhesion molecule; NS, not significant; ECOG, Eastern Cooperative Oncology Group; PS, performance status.

<sup>b</sup> The positive and negative groups have pooled median positive frequencies of staining of 5%, and 5%, respectively.

<sup>c</sup> P<sub>s</sub> are Pearson χ² test unless otherwise noted.

<sup>d</sup> Kruskal-Wallis test.

<sup>e</sup> Recurrence in patients with localized disease at nephrectomy.

<sup>f</sup> Univariate Cox proportional hazards model.
were significantly associated, with pT stage (P = 0.047), overall stage (P = 0.037), and localized disease (P = 0.024) using the Pearson χ², with EpCAM negativity contributing to higher stages and decreased localization. Negative groups also have overall larger tumors, less organ confinement, and higher ECOG performance status, but these associations do not reach statistical significance (Table 1). In lower-risk patients (localized disease at nephrectomy), a slightly higher proportion of EpCAM-negative patients recur compared with EpCAM-positive patients (20% and 14%, respectively; P = 0.37), and they recur more quickly as well (median follow-up, 7 and 13 months, respectively; P = 0.33, univariate Cox proportional hazards analysis).

Univariate Cox proportional hazards analysis of established prognostic factors ECOG performance status, pN, pT, pM, tumor localization and grade, and EpCAM expression and their relationship to disease-specific survival for all patients are shown in Tables 2–4, respectively. EpCAM expression is a significant univariate predictor of increased survival for all patients (Table 2: P = 0.0068; hazard ratio = 0.62; 95% confidence interval, 0.44–0.88) and for patients negative for distant metastases (Table 4; P = 0.033; hazard ratio = 0.46; 95% confidence interval, 0.23–0.94). However, EpCAM is not a significant univariate survival predictor in the patient group with distant metastases at nephrectomy (P = 0.74).

Multivariate Cox proportional hazards analysis of established prognostic factors, including ECOG performance status, pN, pT, pM, and grade, with EpCAM expression and their relationship to disease-specific survival for all patients are shown in Table 5. Notably, EpCAM expression remains a significant and independent predictor of survival (Table 3; P = 0.017; hazard ratio, 0.63; 95% confidence interval, 0.43–0.92). Whereas ECOG performance status, pT, pM, and grade are associated with an increase in disease-specific death, higher EpCAM expression is associated with a decrease in disease-specific death. EpCAM expression did not remain a significant predictor in multivariate analyses with the patient groups stratified for either distant metastases or overall tumor localization (data not shown). Kaplan-Meier estimates of disease-specific survival curves according to dichotomized EpCAM expression were examined for all patients and for patient groups stratified by grade [1 and 2 (low) versus 3 and 4 (high)], pT stage [1 and 2 (low) versus 3 and 4 (high)], group stage [I and II (low) versus III and IV (high)], regional nodal status (positive

![EpCAM Pooled Median Primary Tumor Expression Positivity](image)

**Table 2** Univariate Cox proportional hazards analysis for disease-specific survival for renal clear cell carcinoma (all patients)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>P</th>
<th>Hazard ratio</th>
<th>95% CI</th>
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<td>Distant metastases (pM)</td>
<td>318</td>
<td>&lt;0.0001</td>
<td>3.71</td>
<td>2.64–5.33</td>
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<tr>
<td>ECOG PS</td>
<td>315</td>
<td>&lt;0.0001</td>
<td>2.64</td>
<td>2.05–3.39</td>
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<tr>
<td>Stage (pT)</td>
<td>317</td>
<td>&lt;0.0001</td>
<td>2.17</td>
<td>1.79–2.64</td>
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<tr>
<td>Grade</td>
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<td>&lt;0.0001</td>
<td>2.16</td>
<td>1.72–2.72</td>
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<tr>
<td>Nodal status (pN)</td>
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<td>&lt;0.0001</td>
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<td>1.53–2.44</td>
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<tr>
<td>EpCAM expression b</td>
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<td>0.0068</td>
<td>0.62</td>
<td>0.44–0.88</td>
</tr>
<tr>
<td>Localized</td>
<td>313</td>
<td>&lt;0.0001</td>
<td>0.14</td>
<td>0.09–0.21</td>
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</tbody>
</table>

* CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; PS, performance status; EpCAM, epithelial cell adhesion molecule.

b Dichotomized value of pooled median staining frequency scorings for all tumor samples within each case, cutoff at 5% positive expression in tumor cells.

were shown in Tables 2–4, respectively. EpCAM expression is a significant univariate predictor of increased survival for all patients (Table 2: P = 0.0068; hazard ratio = 0.62; 95% confidence interval, 0.44–0.88) and for patients negative for distant metastases (Table 4; P = 0.033; hazard ratio = 0.46; 95% confidence interval, 0.23–0.94). However, EpCAM is not a significant univariate survival predictor in the patient group with distant metastases at nephrectomy (P = 0.74).

Multivariate Cox proportional hazards analysis of established prognostic factors, including ECOG performance status, pN, pT, pM, and grade, with EpCAM expression and their relationship to disease-specific survival for all patients are shown in Table 5. Notably, EpCAM expression remains a significant and independent predictor of survival (Table 3; P = 0.017; hazard ratio, 0.63; 95% confidence interval, 0.43–0.92). Whereas ECOG performance status, pT, pM, and grade are associated with an increase in disease-specific death, higher EpCAM expression is associated with a decrease in disease-specific death. EpCAM expression did not remain a significant predictor in multivariate analyses with the patient groups stratified for either distant metastases or overall tumor localization (data not shown). Kaplan-Meier estimates of disease-specific survival curves according to dichotomized EpCAM expression were examined for all patients and for patient groups stratified by grade [1 and 2 (low) versus 3 and 4 (high)], pT stage [1 and 2 (low) versus 3 and 4 (high)], group stage [I and II (low) versus III and IV (high)], regional nodal status (positive

**Table 3** Univariate Cox proportional hazards analysis for disease-specific survival for renal clear cell carcinoma (patients with distant metastases)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>P</th>
<th>Hazard ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECOG PS</td>
<td>146</td>
<td>0.0037</td>
<td>1.60</td>
<td>1.16–2.19</td>
</tr>
<tr>
<td>Grade</td>
<td>146</td>
<td>0.018</td>
<td>1.44</td>
<td>1.06–1.94</td>
</tr>
<tr>
<td>Stage (pT)</td>
<td>146</td>
<td>0.012</td>
<td>1.39</td>
<td>1.08–1.79</td>
</tr>
<tr>
<td>Nodal status (pN)</td>
<td>142</td>
<td>0.16</td>
<td>1.21</td>
<td>0.93–1.58</td>
</tr>
<tr>
<td>EpCAM expression b</td>
<td>146</td>
<td>0.74</td>
<td>0.94</td>
<td>0.63–1.39</td>
</tr>
</tbody>
</table>

* CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; PS, performance status; EpCAM, epithelial cell adhesion molecule.

b Dichotomized value of pooled median staining frequency scorings for all tumor samples within each case, cutoff at 5% positive expression in tumor cells.

were shown in Tables 2–4, respectively. EpCAM expression is a significant univariate predictor of increased survival for all patients (Table 2: P = 0.0068; hazard ratio = 0.62; 95% confidence interval, 0.44–0.88) and for patients negative for distant metastases (Table 4; P = 0.033; hazard ratio = 0.46; 95% confidence interval, 0.23–0.94). However, EpCAM is not a significant univariate survival predictor in the patient group with distant metastases at nephrectomy (P = 0.74).

Multivariate Cox proportional hazards analysis of established prognostic factors, including ECOG performance status, pN, pT, pM, and grade, with EpCAM expression and their relationship to disease-specific survival for all patients are shown in Table 5. Notably, EpCAM expression remains a significant and independent predictor of survival (Table 3; P = 0.017; hazard ratio, 0.63; 95% confidence interval, 0.43–0.92). Whereas ECOG performance status, pT, pM, and grade are associated with an increase in disease-specific death, higher EpCAM expression is associated with a decrease in disease-specific death. EpCAM expression did not remain a significant predictor in multivariate analyses with the patient groups stratified for either distant metastases or overall tumor localization (data not shown). Kaplan-Meier estimates of disease-specific survival curves according to dichotomized EpCAM expression were examined for all patients and for patient groups stratified by grade [1 and 2 (low) versus 3 and 4 (high)], pT stage [1 and 2 (low) versus 3 and 4 (high)], group stage [I and II (low) versus III and IV (high)], regional nodal status (positive

**Table 4** Univariate Cox proportional hazards analysis for disease-specific survival for renal clear cell carcinoma (patients without distant metastases)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>P</th>
<th>Hazard ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECOG PS</td>
<td>169</td>
<td>&lt;0.0001</td>
<td>3.81</td>
<td>1.99–7.27</td>
</tr>
<tr>
<td>Nodal status (pN)</td>
<td>171</td>
<td>&lt;0.0001</td>
<td>3.63</td>
<td>2.23–5.92</td>
</tr>
<tr>
<td>Stage (pT)</td>
<td>171</td>
<td>&lt;0.0001</td>
<td>2.50</td>
<td>1.75–3.57</td>
</tr>
<tr>
<td>Grade</td>
<td>166</td>
<td>0.00026</td>
<td>2.33</td>
<td>1.48–3.66</td>
</tr>
<tr>
<td>EpCAM expression b</td>
<td>172</td>
<td>0.033</td>
<td>0.46</td>
<td>0.23–0.94</td>
</tr>
</tbody>
</table>

* CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; PS, performance status; EpCAM, epithelial cell adhesion molecule.

b Dichotomized value of pooled median staining frequency scorings for all tumor samples within each case, cutoff at 5% positive expression in tumor cells.
Table 5  Multivariate Cox proportional hazards analysis for disease-specific survival for renal clear cell carcinoma (all patients); (n = 304)

<table>
<thead>
<tr>
<th>Variable</th>
<th>P</th>
<th>Hazard ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metastases (pM)</td>
<td>&lt;0.0001</td>
<td>2.77</td>
<td>1.80–4.27</td>
</tr>
<tr>
<td>ECOG PS</td>
<td>&lt;0.0001</td>
<td>1.76</td>
<td>1.33–2.34</td>
</tr>
<tr>
<td>Stage (pT)</td>
<td>&lt;0.0001</td>
<td>1.65</td>
<td>1.31–2.09</td>
</tr>
<tr>
<td>Grade</td>
<td>0.018</td>
<td>1.38</td>
<td>1.06–1.79</td>
</tr>
<tr>
<td>Nodal status (pN)</td>
<td>0.052</td>
<td>1.30</td>
<td>0.99–1.69</td>
</tr>
<tr>
<td>EpCAM expression$^b$</td>
<td>0.017</td>
<td>0.63</td>
<td>0.43–0.92</td>
</tr>
</tbody>
</table>

$^a$ CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; PS, performance status; EpCAM, epithelial cell adhesion molecule.

$^b$ Dichotomized value of pooled median staining frequency scores for all tumor samples within each case, cutoff at 5% positive expression in tumor cells.

or negative), distant metastasis status (positive or negative), tumor localization (yes or no), and ECOG performance status status (0 or >0). A comparison of the differences between the survival curves yielded significant log-rank P-s for several subgroups (Fig. 6, A–E). The median estimated disease-free survival times for EpCAM-positive and -negative patient groups for all clear cell patients were >11.09 and 3.8 years, respectively (Fig. 6A).

**DISCUSSION**

EpCAM mediates Ca$^{2+}$-independent homotypic cell-cell adhesions involved in the maintenance of epithelial integrity (42), and it plays a morphoregulatory role required for normal embryonic development and homeostasis of mature tissues (15, 16, 43–46). Reactivation of embryonic patterns of expression is known to accompany regeneration and repair of adult epithelial tissues (15). Increasing EpCAM expression is frequently directly related to cellular proliferation and inversely related to cellular differentiation, in particular, terminal differentiation (16, 18). For example, in normal colonic crypts, the germinal region of the colonic mucosal lining displays high EpCAM expression that steadily decreases as cells differentiate and migrate toward the villi, where programmed cell death occurs (47). Likewise, stem and basal cells, the active and partially differentiated progenitors of skin epithelium, express EpCAM, whereas the differentiated keratinocytes do not (48).

Metaplastic, dysplastic, and malignant proliferations of epithelium similarly often show *de novo* or overexpression of EpCAM (14, 18, 49, 50). Tumor progression has in some cases been clearly linked to increasing EpCAM expression, and abundant expression in early lesions may serve as an early detection marker of impending invasive malignancies (14, 18, 49, 51). Therefore, normal EpCAM regulation provides a flexible regulatory plasticity required for normal epithelial development and maintenance, whereas EpCAM dysregulation upsets these delicate regulatory balances, facilitating aberrant cellular proliferation and dedifferentiation.

The expectation that EpCAM, like other adhesion molecules, provides invasion-suppressor properties to epithelia through cell-cell aggregation has been demonstrated in *in vitro* and clinical models. Normally nonadhesive cell lines have been induced to aggregate through transfection of EpCAM and, in addition, have been shown to have reduced mobility and invasive behaviors (52). EpCAM-transfected tumor cells have shown reduced metastases in *in vivo* mouse models (53). Negative or limited EpCAM expression in primary laryngeal carcinoma has been linked to the presence of nodal metastases and in colorectal carcinoma to a poor prognosis (53, 54). However, this finding is not consistent across all studies and tumor types. Elevated EpCAM expression has been linked to increasing lymph node metastases, recurrence, and mortality in breast cancer (49). This dualistic role highlights the complex multifunctionality of EpCAM and its interactions with other prominent molecules. Importantly, elevated EpCAM has a negative effect on E-cadherin-mediated adhesion by decreasing the association of the cadherin-catenin-cytoskeleton complex (55, 56). Some studies report that loss of E-cadherin expression leads to a decrease in differentiation and an increase in both invasive tumor behavior and lymph node metastases, resulting in overall poorer prognosis (57–62). E-cadherin also has a growth suppressor function by inducing cell cycle arrest via up-regulation of the cyclin-dependent kinase p27 (63, 64). It is likely that only a full profile of these and other key regulatory components would provide a sufficient picture of metastatic mechanisms in RCC.

The survival tree-derived cutoff of 5% of tumor cells positive is a reasonable assay cutoff that may be used to generate EpCAM-positive and -negative patient groupings with clinical relevance. Eighty-eight percent of patients in the negative group had ≤5% cellular positivity in all spots of their cases, whereas 90% of the positive group had >5% cellular positivity in all spots of their case. Thus, even though a median pooling was found to be statistically optimal to distinguish each group, the two groups closely approach fundamentally negative and positive expression.

In the present study, we found that loss of EpCAM was associated with tumor invasion and mobility, seen as increased pT stage (*P* = 0.047), overall stage (*P* = 0.037), and metastases (*P* = 0.024). There was predominantly an increase in intermediate pT stages (pT2–3), where the tumors are larger and transitioning into increasingly aggressive, invasive behaviors. The benefits of positive EpCAM expression in clear cell renal tumors are demonstrated by its univariate association with improved survival (logistic regression *P* = 0.00431; Cox model *P* = 0.0068). Despite the relationship of EpCAM to key prognostic variables, it remains an independent predictor of improved survival in multivariate Cox proportional hazards analysis (*P* = 0.017). EpCAM expression successfully stratifies clear cell RCC patients (*P* = 0.00632) and can stratify patients who have tumors of high grade (*P* = 0.00291), have no regional node (*P* = 0.00381) or distant metastasis (*P* = 0.0285), or have a clinically favorable performance status (*P* = 0.044; Fig. 6, A–E). In each case, positive EpCAM expression was associated with improved survival. Negative expressers in all of these groups had consistently lower proportions of localized tumor and had minimally larger tumors (data not shown).

EMD 273066 (huKSI-IL2) is a recombinant immunocyto- kine fusion protein consisting of a monoclonal antibody to EpCAM that is linked to an active IL-2 molecule that has been designed for the targeted delivery of IL-2 to EpCAM-expressing
tumor tissues and is being considered as a targeted therapy for patients with metastatic RCC. Candidates for EpCAM-based therapy ideally should have a high-intensity EpCAM tumor expression, low or absent normal tissue expression, and regional or distant metastatic disease. Fifty-nine percent of 1200 clear cell RCC samples were completely negative for EpCAM expression. Eighty-one percent of 318 RCC clear cell cases were negative for EpCAM in at least one of the representative case samples. Only 33% of clear cell cases contain any regions of positivity that will react moderately or strongly with the EpCAM antibody. Only 10% were positive in ≥50% of cells (pooled median positivity), and only 8% of patients would be considered candidates for EpCAM-based therapy based on high expression (≥50% expression) and the need for systemic treatment (metastases at nephrectomy). Furthermore, we found that EpCAM is universally expressed in morphologically normal renal epithelium, particularly that of the distal nephron. One hundred percent of samples containing distal tubules and/or collecting ducts stained positively (most stained moderately or strongly). This abundant EpCAM expression in normal renal tissues greatly increases the risk of toxic side effects.

EpCAM expression in malignant renal tumor types suspected of deriving from distal nephron components, especially chromophobe and collecting duct carcinomas, have higher overall maximal EpCAM protein expression than those that potentially derive from proximal segments (clear cell carcinomas). Therefore, EpCAM expression in renal tumors may at least partially reflect the native expression in the normal nephron components from which they are likely derived. The more heterogeneous expression of papillary carcinomas may possibly represent their derivation from a region of distal tubules with transitioning native expression. Sarcomatoid tumors in this sampling expressed the lowest EpCAM staining of all tumor types. Fourteen of 15 cases with sarcomatoid histology derived from clear cell tumors, and only 6% of these sarcomatoid regions reached a level of moderate staining (80% were totally negative). Of note, the only sarcomatoid tumor samples staining strongly were those derived from a papillary tumor that was also a strong expresser. Therefore, EpCAM expression in sarcomatoid tumors appears to correlate in this small sampling with the RCC subtypes from which they derive, supporting the hypothesis that sarcomatoid differentiation may derive from a variety of RCC types (2). In addition, a shift to mesenchymal-like differentiation may be accompanied by a natural reduction in EpCAM, which generally remains specific for epithelium. Interestingly, oncocytomas, like chromophobe carcinomas, are both derived from intercalated cells, and yet oncocytomas have more varied and much more focal staining than their malignant counterpart. Elevated EpCAM in scattered cells of oncocytomas possibly represents early signals of rapid growth and/or malignant transformation, and further investigation with regard to the meaning of this finding is warranted.

In conclusion, our tissue array-based investigation of EpCAM expression demonstrates that EpCAM is a novel prognostic and diagnostic molecular marker in RCC patients.
EpCAM Is a Predictor of Survival in RCC

tive EpCAM expression in clear cell RCC is an independent predictor of improved survival and is associated with a higher rate of organ-confined disease, decreased incidence of metastatic disease at diagnosis, and decreased systemic disease recurrence rates after nephrectomy for patients staged with localized disease at the time of surgery. EpCAM is uniformly seen in morphologically benign renal tissue specimens, predominantly within the distal nephron. The majority of RCCs ostensibly developing from the proximal renal tubules (clear cell carcinomas comprising >90% of all renal tumors) are completely negative or display only weak positivity for EpCAM expression in 67% of cases. High expression in normal renal tissues, low or absent expression in the most common renal tumors, and the fact that tumors with higher expression are associated with an improved survival and decreased need for systemic therapy severely decrease enthusiasm for the development of EpCAM-targeted treatment for patients with clear cell RCC. Future functional studies of EpCAM in RCC are needed to clarify the associations observed in this immunohistochemical study.

REFERENCES


Epithelial Cell Adhesion Molecule (KSA) Expression: Pathobiology and Its Role as an Independent Predictor of Survival in Renal Cell Carcinoma

David B. Seligson, Allan J. Pantuck, Xueli Liu, et al.


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