Epithelial to Mesenchymal Transition in Clear Cell Renal Cell Carcinoma with Rhabdoid Features

Masaaki Sugimoto^a Kenichi Kohashi^a Momoe Itsumi^b Masaki Shiota^b
Tatsuro Abe^a Yuichi Yamada^a Kentaro Kuroiwa^b Seiji Naito^c Yoshinao Oda^a

^aDepartment of Anatomic Pathology, Pathological Sciences, and ^bDepartment of Urology, Graduate School of Medical Sciences, Kyushu University, and ^cDepartment of Urology, Harasanshin General Hospital, Fukuoka, Japan

Key Words

Epithelial to mesenchymal transition · Rhabdoid features · Clear cell renal cell carcinoma · E-cadherin · Vimentin

Abstract

Aims: The aims of this study were to investigate the association of renal cell carcinoma (RCC) displaying rhabdoid features and morphologically mesenchymal characteristics with epithelial to mesenchymal transition (EMT), and to clarify the expression of EMT markers. **Methods:** We investigated the expression of EMT markers (E-cadherin, vimentin, Snail, Slug, ZEB1, ZEB2 and Twist1) using immunohistochemistry, Western blotting and real-time polymerase chain reaction in 18 cases of clear cell RCC (ccRCC) with rhabdoid features and 74 ccRCC cases with Fuhrman grade 1–3 (G1 to G3). **Results:** In ccRCCs with rhabdoid features, low E-cadherin and high vimentin expression were found. In G1 to G3 ccRCCs, low E-cadherin expression and high expression of vimentin, ZEB1 and ZEB2 were found. There was no significant difference in the immunoexpression of E-cadherin and vimentin between the two ccRCC groups. **Conclusions:** The rhabdoid features may histologically and biologically be associated with EMT in ccRCC. There is a possibility that in G1

to G3 ccRCCs showing epithelial structures, other cell-cell adhesion mechanisms apart from E-cadherin adhesion may continue to work, and that ccRCC with rhabdoid features may be caused by an inactivation or loss of these mechanisms.

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Introduction

Renal cell carcinoma (RCC) accounts for approximately 3% of all adult malignancies and 90–95% of all renal neoplasms [1, 2]. Gökden et al. [3] initially described rhabdoid features in RCCs. The incidence of rhabdoid features was reported in approximately 1.4–7.4% of all RCCs [3–9]. We reported previously that the biological behavior of RCCs with rhabdoid features is highly aggressive and presents a very poor prognosis [5], which has been confirmed by other investigators [7–10]. Rhabdoid features were identified in several RCC histological subtypes, most commonly observed in clear cell RCC (ccRCC) [11], the most conventional histopathological subtype [12]. RCC is defined as a malignant neoplasm arising from the epithelium of the renal tubules [13]. Interest-

ingly enough, in their histological structures, ccRCCs without rhabdoid features are similar to epithelial tumors, whereas ccRCCs with rhabdoid features are similar to mesenchymal tumors.

Epithelial to mesenchymal transition (EMT) is an essential morphologic conversion in which epithelial cells lose their epithelial characteristics and acquire a mesenchymal phenotype [14]. There is increasing evidence that EMT occurs during cancer progression, by which tumor cells acquire the capacity to migrate, invade and metastasize [14]. Loss of cell-cell adhesion is a prerequisite of EMT and involves a functional loss of E-cadherin. Several EMT regulators, such as Snail [15], Slug [16], ZEB1 [17], ZEB2 [18] and Twist1 [19], have been identified as E-cadherin repressors. One of several mesenchymal markers related to EMT is vimentin expression [20], which is widely used as a marker of EMT [21].

To our knowledge, no molecular or biological studies of RCC with rhabdoid features and morphologically mesenchymal characteristics have been published to date. We hypothesized that the presence of EMT in RCC with rhabdoid features may cause morphological change to mesenchymal characteristics. The aims of this study were to investigate the association between RCC with rhabdoid features and EMT, and to clarify the expression of EMT markers such as E-cadherin, vimentin, Snail, Slug, ZEB1, ZEB2 and Twist1 in RCC using immunohistochemistry, Western blotting and real-time polymerase chain reaction (PCR).

Materials and Methods

Materials

We used the 860 formalin-fixed, paraffin-embedded tumor samples from patients who underwent either partial or radical nephrectomy for suspected RCC at the Department of Urology, Kyushu University Hospital, Fukuoka, from 1989 to 2014. This was a single-institution retrospective study. Of the 860 patients, we excluded those who had deficient clinical data or had the non-ccRCC subtype. The remaining 695 patients presenting with ccRCC comprised the study cohort. None of these patients had received neoadjuvant or adjuvant therapy. The diagnosis of rhabdoid features in ccRCC was based on light microscopic examination with hematoxylin and eosin staining according to the ISUP grading system [11]. As a control group, we randomly selected ccRCCs with nonrhabdoid features. Of these 695 patients, we immunohistochemically examined 18 ccRCCs with rhabdoid features and 74 ccRCCs with nonrhabdoid features: 55 ccRCCs with Fuhrman grade 1 (G1) or 2 (G2), i.e. Fuhrman grade 1 + 2 (G1 + 2), and 19 ccRCCs with Fuhrman grade 3 (G3). We also examined the adjacent nonneoplastic renal tissues of the ccRCCs with rhabdoid features, G1, G2 and G3, respectively. Frozen samples of each ccRCC with rhabdoid features, G1, G3 and their adjacent non-

neoplastic renal tissues were obtained at surgery and were available for the Western blotting analysis and real-time PCR analysis. A part of the obtained frozen tissue was formalin fixed, embedded into paraffin, and stained with hematoxylin and eosin. These sections were reviewed and confirmed for histological subtype, presence of rhabdoid cells and Fuhrman nuclear grade. This study was approved by the Institutional Review Board of Kyushu University (IRB No. 27-230).

Immunohistochemistry

Immunohistochemical staining was performed using the primary antibodies of epithelial markers against cytokeratin AE1/AE3 (mouse monoclonal, clone AE1/AE3, 1/1,000 dilution; DAKO Cytomation, Glostrup, Denmark), cytokeratin CAM5.2 (mouse monoclonal, clone CAM5.2, 1/20 dilution; BD Biosciences, Franklin Lakes, N.J., USA) and epithelial membrane antigen (EMA; mouse monoclonal, clone E29, 1/400 dilution, DAKO Cytomation) and the primary antibodies of EMT markers listed in table 1. Four-micrometer-thick tissue sections from 10% formalin-fixed, paraffin-embedded material were deparaffinized in xylene and dehydrated in ethanol. Endogenous peroxidase activity was blocked by incubation in methanol containing 0.3% H₂O₂ for 30 min. Antigen retrieval was achieved by the method shown in table 1. The slides were then incubated with primary antibodies at 4°C overnight and subsequently incubated with biotin-free horseradish peroxidase enzyme-labeled polymer (Envision plus System; DAKO Cytomation) for 40 min at room temperature. The labeled antigens were visualized using 3,3'-diaminobenzidine tetrahydrochloride as a chromogen. Counterstaining was performed with hematoxylin. Appropriate positive and negative controls were prepared for all antibodies. Nonspecific staining was not observed in any negative control sections.

Evaluation of Immunohistochemical Staining

Three pathologists (M.S., K.K., Y.O.) who were blinded to the clinicopathological variables independently reviewed the immunohistochemical staining for each tumor and its adjacent nonneoplastic renal tissue. They evaluated the membranous immunorexpression of E-cadherin, the cytoplasmic immunorexpression of vimentin, and the nuclear immunorexpression of Snail, Slug, ZEB1, ZEB2 and Twist1. We performed a semiquantitative estimation based on the intensity and extent of stained cells for each case, as previously described [22, 23]. The staining intensity was graded from 0 to 3 (0 = no staining at all, 1 = weak, 2 = moderate, 3 = strong). The extent was also graded from 0 to 3 (0 = <5%, 1 = 5–25%, 2 = 26–75%, 3 = >75%). The intensity and extent scores were summed to obtain the composite score (0–6). The immunohistochemical stains for AE1/AE3, CAM5.2 and EMA were evaluated as positive or negative. In the comparison between two types of ccRCCs, i.e. ccRCCs with rhabdoid features and ccRCCs with nonrhabdoid features, the immunohistochemical scores based on their adjacent nonneoplastic renal tissues were regraded.

Western Blotting

We performed a Western blotting analysis of each pair of frozen samples of three ccRCCs with rhabdoid features and their adjacent nonneoplastic renal tissues, using the primary antibodies listed in table 1. The protein concentrations were quantified using a Protein Assay (Bio-Rad, Hercules, Calif., USA) based on the Bradford method. Aliquots (30 µg of protein) were separated by

Table 1. Primary antibodies used for immunohistochemical staining and Western blotting

Antibody	Source	Species	Clonality	Dilution	Antigen retrieval
<i>Immunohistochemical staining</i>					
E-cadherin	BD	Mouse	M, 36/E-cadherin	1:1,000	pH 6.0, microwave 20 min
Vimentin	DAKO	Mouse	M, V9	1:25	Not done
Snail	Abcam	Mouse	P, SNAIL	1:100	TRS, pressure cooker 20 min
Slug	Abcam	Rabbit	P, SLUG	1:100	pH 6.0, microwave 20 min
ZEB1	Abcam	Rabbit	P, AREB6	1:50	TRS, microwave 20 min
ZEB2	Abcam	Rabbit	P, SIP1	1:100	TRS, microwave 20 min
Twist1	Sigma	Rabbit	P, Twist1	1:100	pH 6.0, microwave 20 min
<i>Western blotting</i>					
E-cadherin	BD	Mouse	M, 36/E-cadherin	1:40,000	
Vimentin	BD	Mouse	M, RV202	1:500	
β -Actin	Sigma	Mouse	M, AC-15	1:50,000	

Abcam = Abcam Inc. (Cambridge, Mass., USA); BD = BD Biosciences; DAKO = DAKO Cytomation; M = monoclonal; P = polyclonal; Sigma = Sigma-Aldrich (St. Louis, Mo., USA); SIP1 = Smad interacting protein 1; TRS = target retrieval solution.

4–20% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride microporous membranes (GE Healthcare Bio-Sciences, Piscataway, N.J., USA) using a semi-dry blotter. The membranes were then incubated with the primary antibodies for 1 h at room temperature, followed by incubation with peroxidase-conjugated secondary antibodies for 40 min at room temperature. The bound antibodies were visualized using an ECL kit (GE Healthcare Bio-Sciences) and images were obtained using an image analyzer (Ez-Capture MG; Atto, Tokyo, Japan). We quantitated the E-cadherin and vimentin bands on imaging film by measuring their densities (ImageJ software, US National Institutes of Health, <http://imagej.nih.gov/ij/>). The density values were expressed as a ratio of the corresponding β -actin densities.

RNA Isolation, Reverse Transcription and Quantitative Real-Time PCR

A real-time PCR analysis was performed in each pair of frozen samples of three ccRCCs with rhabdoid features, seven ccRCCs with G1, nine ccRCCs with G3, and the adjacent nonneoplastic renal tissues of each ccRCC. Total RNA was extracted using the NucleoSpin RNA II kit (Clontech, Mountain View, Calif., USA) and then reverse-transcribed using iScript (Bio-Rad). The resulting complementary deoxyribonucleic acids were diluted to 1:5–10 and served as templates for real-time PCR using TaqMan Gene Expression Assays for E-cadherin (CDH1, Hs01023894_m1), vimentin (VIM, Hs00185584_m1), Snail (SNAIL, Hs00195591_m1), Slug (SNAIL2, Hs00950344_m1), ZEB1 (Hs00232783_m1), ZEB2 (Hs00207691_m1), TWIST1 (Hs00361186_m1) and 18S rRNA (Hs99999901_s1; all from Life Technologies, Carlsbad, Calif., USA), and TaqMan Gene Expression Master Mix (Life Technologies) on a CFX Connect Real-Time System (Bio-Rad). The transcript levels of the target genes were normalized to the corresponding 18S rRNA transcript levels. All values represent the results of at least three independent experiments.

Statistical Analysis

A paired t test was used to compare the immunohistochemical proteins and mRNA expressions in the tumor tissues with their paired adjacent nonneoplastic renal tissue samples. We compared two groups – ccRCCs with rhabdoid features and G1 to G3 ccRCCs – using a t test for continuous variables. Probability values of $p < 0.05$ were considered significant. All statistical analyses were performed using JMP 11.0.0 software (SAS Institute, Cary, N.C., USA).

Results

Analyses for ccRCCs with Rhabdoid Features

Immunohistochemical Analyses for ccRCCs with Rhabdoid Features

Intracytoplasmic inclusion bodies in the rhabdoid areas of ccRCCs were immunohistochemically positive for one or more of the epithelial markers: AE1/AE3 (17/18), CAM 5.2 (8/18) and EMA (8/18). Figure 1 shows immunohistochemical findings of EMT markers and figure 2 summarizes the results of the immunohistochemical analyses for EMT markers in ccRCCs with rhabdoid features and their adjacent nonneoplastic renal tissues. In ccRCCs with rhabdoid features, the immunohistochemical score of vimentin was significantly higher ($p = 0.0005$) and those of E-cadherin and Twist1 were significantly lower ($p < 0.0001$, $p = 0.0110$, respectively) than those of their adjacent nonneoplastic renal tissues (fig. 2). The immunohistochemical protein expressions of Snail, Slug, ZEB1 and ZEB2 showed no significant differences in

Fig. 1. Representative immunohistochemical findings of E-cadherin, vimentin, Snail, Slug, ZEB1, ZEB2 and Twist1 in ccRCCs with rhabdoid features, ccRCCs with non-rhabdoid features (Fuhrman grade 1–3), and adjacent nonneoplastic renal tissues. Original magnification $\times 400$. The E-cadherin expression was negative and vimentin expression was positive in ccRCCs with rhabdoid features and ccRCCs with non-rhabdoid features. ZEB1 and ZEB2 expression was positive in ccRCCs with nonrhabdoid features.

ccRCCs with rhabdoid features compared to their adjacent nonneoplastic renal tissues (fig. 2). Our analysis of the immunohistochemical scores showed that the immunoeexpression of E-cadherin protein was decreased ap-

proximately 0.23-fold ($\sim 1.1/4.7$) and the immunoeexpression of vimentin protein was increased approximately 2.45-fold ($\sim 2.7/1.1$) in ccRCCs with rhabdoid features compared to their adjacent nonneoplastic renal tissues.

Fig. 2. Immunohistochemical scores and mRNA expression levels of EMT markers (E-cadherin, vimentin, Snail, Slug, ZEB1, ZEB2 and Twist1) in ccRCCs with rhabdoid features. Each tumor (T) was compared with its adjacent nonneoplastic renal tissue (N). Only E-cadherin showed matching results of expression obtained by immunohistochemistry and real-time PCR. The protein and

mRNA expression levels of E-cadherin were lower in ccRCCs with rhabdoid features than their adjacent nonneoplastic renal tissues. The statistical analysis was performed with a paired t test. The mean immunohistochemical score is presented above each column. The immunohistochemical scores are means $\pm$ SD. * $p < 0.05$, *** $p < 0.001$.

Western Blot Analysis of ccRCCs with Rhabdoid Features

We performed Western blotting to investigate the protein expression of E-cadherin and vimentin in ccRCCs with rhabdoid features. We observed that the expression of E-cadherin was significantly lower ($p = 0.0233$) and that of vimentin was significantly higher ($p = 0.0264$) in ccRCCs with rhabdoid features than in their paired adjacent nonneoplastic renal tissues (fig. 3a). Our analysis of the optical density of bands by Western blotting showed that the protein expression of E-cadherin was decreased 0.27-fold and that of vimentin was increased 2.46-fold in the ccRCCs with rhabdoid features compared to their adjacent nonneoplastic renal tissues (fig. 3b). The expression of E-cadherin and

vimentin shown by the Western blotting reflected almost the same levels as their immunohistochemical scores.

Quantitative Real-Time PCR Analysis of ccRCCs with Rhabdoid Features

In ccRCCs with rhabdoid features, the mRNA expression level of E-cadherin was significantly downregulated ($p = 0.0221$) and the expression level of vimentin was not significantly different between the tumors and their adjacent nonneoplastic renal tissues ($p = 0.7850$; fig. 2). The mRNA expression levels of Snail, Slug, ZEB1, ZEB2 and Twist1 showed no significant differences between ccRCCs with rhabdoid features and their adjacent nonneoplastic renal tissues (fig. 2).

Fig. 3. Expression of E-cadherin and vimentin at the protein level in ccRCCs with rhabdoid features (T) and their paired adjacent nonneoplastic renal tissues (N) investigated by Western blot analysis. Significantly lower expression of E-cadherin and significantly higher expression of vimentin were observed in ccRCCs with rhabdoid features compared to the nonneoplastic renal tissues. **a** Immunoblots show the expression of E-cadherin and vimentin in ccRCC with rhabdoid features. β -Actin served as a loading control. **b** The data on the relative protein expression ratio normalized to β -actin are expressed as the mean $\pm$ SD of densitometric analysis results from three separate experiments. * $p < 0.05$ compared to nonneoplastic renal tissue, by t test.

Analyses of ccRCCs with Nonrhabdoid Features (G1 to G3)

Immunohistochemical Analyses of ccRCCs with Nonrhabdoid Features

Figure 1 shows the immunohistochemical findings and figure 4 summarizes the results of the immunohistochemical analyses of G1 to G3 ccRCCs and their adjacent nonneoplastic renal tissues. The immunohistochemical scores for vimentin, ZEB1 and ZEB2 were significantly higher ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$, respectively) and those for E-cadherin, Snail and Slug were significantly lower ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$, respectively) in these ccRCCs compared to their adjacent nonneoplastic renal tissues (fig. 4). The immunohistochemical score of Twist1 showed no significant difference between G1 to G3 ccRCCs compared to their adjacent nonneoplastic renal tissues (fig. 4).

Quantitative Real-Time PCR Analysis of ccRCCs with Nonrhabdoid Features

In G1 to G3 ccRCCs, the mRNA expression levels of E-cadherin were significantly downregulated ($p = 0.0174$) and those of vimentin, ZEB1 and ZEB2 were significantly upregulated compared to their adjacent nonneoplastic renal tissues ($p = 0.0193$, $p = 0.0259$, $p = 0.0119$, respectively; fig. 4). The mRNA expression levels of Snail, Slug and Twist1 showed no significant differences between G1 to G3 ccRCCs and their adjacent nonneoplastic renal tissues.

Comparison of Immunohistochemical Scores or mRNA Expression between ccRCCs with Rhabdoid Features and ccRCCs with Nonrhabdoid Features (G1 to G3)

Regarding the combination of the immunorepression of EMT markers between the 18 ccRCCs with rhabdoid features and the 74 G1 to G3 ccRCCs, the immunohistochemical scores of ZEB1, ZEB2 and Twist1 were significantly lower ($p = 0.0475$, $p < 0.0001$, $p < 0.0001$, respectively) in ccRCCs with rhabdoid features than in G1 to G3 ccRCCs (fig. 5). In ccRCCs with rhabdoid features, the immunorepression of E-cadherin protein was lower and the immunorepression of vimentin protein was higher compared to G1 to G3 ccRCCs, although these differences were not significant (fig. 5). With regard to the mRNA expression level, E-cadherin, vimentin, Snail, Slug, ZEB1, ZEB2 and Twist1 were significantly downregulated in ccRCCs with rhabdoid features compared to G1 to G3 ccRCCs ($p = 0.0027$, $p = 0.0047$, $p = 0.0445$, $p = 0.0044$, $p = 0.0035$, $p = 0.0035$, $p = 0.0035$, respectively; fig. 5).

Discussion

To the best of our knowledge, this is the first report of an immunohistochemical and molecular biological study of EMT markers in ccRCCs with rhabdoid features. Although ccRCCs with rhabdoid features also show the immunoreactivity of epithelial markers, the histological findings of the rhabdoid features in ccRCCs are the epithelioid and mesenchymal structures but not epithelial structures, and there seems to be a loss of intercellular adhesion and cell polarization. In contrast, the histological findings of G1 to G3 ccRCCs, namely nonrhabdoid features, are epithelial structures. We thus hypothesized that EMT may biologically lead to the histologically mesenchymal rhabdoid features in ccRCCs. Our study was performed using patients with ccRCCs with rhabdoid

Fig. 4. Immunohistochemical scores and mRNA expression levels of EMT markers (E-cadherin, vimentin, Snail, Slug, ZEB1, ZEB2 and Twist1) in ccRCCs with nonrhabdoid features: G1 to G3. Each tumor (T) was compared with its adjacent nonneoplastic renal tissue (N). The immunohistochemistry and real-time PCR results are matched for E-cadherin, vimentin, ZEB1 and ZEB2 expression. In

G1 to G3 ccRCCs, the protein and mRNA expression levels of E-cadherin were significantly lower and those of vimentin, ZEB1 and ZEB2 were significantly higher than in the adjacent nonneoplastic renal tissues. The mean immunohistochemical score is presented above each column. The immunohistochemical scores are means $\pm$ SD. * $p < 0.05$, *** $p < 0.001$ by t test.

features and a morphologically mesenchymal structure, and the control group consisted of those with ccRCCs with nonrhabdoid features and a morphologically epithelial structure.

E-cadherin and vimentin are generally recognized as hallmarks of EMT. It is widely known that several transcription factors (i.e. Snail, Slug, ZEB1, ZEB2 and Twist1) repress E-cadherin promoter activity, and that these factors are EMT-regulated markers. In previous studies, the immunoexpression of E-cadherin, vimentin and ZEB2 protein in RCC was significantly associated with a poor prognosis [24–26]. Our present findings revealed lower expression of protein and mRNA of E-cadherin in both ccRCCs with rhabdoid features and ccRCCs with non-rhabdoid features compared to their normal renal tissues.

Our data also showed a higher expression of vimentin protein by immunohistochemistry and Western blotting in both types of ccRCCs compared to their normal renal tissues. We found that in ccRCCs with rhabdoid features an upregulated expression of vimentin mRNA and increased expression of other EMT markers were not present. However, the tumor cells of ccRCCs with rhabdoid features transform from clear cell histology to the histological findings of mesenchymal tumors. Therefore, we suggest that the rhabdoid features in ccRCCs may be histologically and biologically associated with EMT in terms of the histological characteristics of rhabdoid features and the protein expression of E-cadherin and vimentin. Using more cases than the previous report [5], we indicated and confirmed that ccRCCs with rhabdoid features were re-

Fig. 5. Immunoexpression scores of EMT markers (E-cadherin, vimentin, Snail, Slug, ZEB1, ZEB2 and Twist1) were compared between G1 to G3 ccRCCs (C) and ccRCCs with rhabdoid features (R). The immunohistochemical scores of ZEB1, ZEB2 and Twist1 were significantly lower ($p = 0.0475$, $p < 0.0001$, $p < 0.0001$, respectively) in ccRCCs with rhabdoid features than G1 to G3 ccRCCs. In ccRCCs with rhabdoid features, the immunoexpression of E-cadherin, Snail and Slug protein was lower and the immunoex-

pression of vimentin protein was higher compared to the corresponding values of G1 to G3 ccRCCs, although this difference was not significant. The mRNA expression levels of E-cadherin, vimentin, Snail, Slug, ZEB1, ZEB2 and Twist1 were significantly lower in ccRCCs with rhabdoid features than G1 to G3 ccRCCs. The mean immunohistochemical score is presented above each column. The immunohistochemical scores are means $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by t test.

lated to the high aggressiveness and poor prognosis which we showed in our report [27].

On the other hand, although the mRNA expression of E-cadherin or vimentin was significantly lower in ccRCCs with nonrhabdoid features than ccRCCs with rhabdoid features, there was no significant difference in the protein expression of E-cadherin and vimentin between the two types of ccRCC. Unlike ccRCCs with rhabdoid features, ccRCCs with nonrhabdoid features showed the histological findings of epithelial tumors and we observed an up-regulated expression of vimentin mRNA and increased expression of both protein and mRNA of ZEB1 and ZEB2 in ccRCCs with nonrhabdoid features. These results led

to our presumption that ccRCCs with rhabdoid features are in a state of inactivation or loss of other cell-cell adhesion mechanisms – except for E-cadherin adhesion – and that these mechanisms continue to work in ccRCCs with nonrhabdoid features.

A positive correlation between ‘high’ Snail or ‘high’ Slug expression and the poor survival of patients in various cancers has been generally reported, including gastric cancer [28] and metastatic non-small cell lung cancer [29]. We found that the expression of Snail or Slug was not higher in ccRCCs with rhabdoid features and with nonrhabdoid features compared to their adjacent nonneoplastic renal tissues. However, for bladder cancer, two reports have con-

tradictory results concerning prognosis and Snail protein expression detected by immunohistochemistry [30, 31]. There are also contradictory reports concerning prognosis and Snail expression by immunohistochemistry in RCC [32, 33]. To our knowledge, no contradictory studies about Slug expression similar to those of Snail expression have been reported. Mikami et al. [34] reported that Slug protein expression was lower in RCC at higher stages. However, the Snail and Slug protein expression in the present series showed no significant association with prognosis. Further studies are needed to clarify the low protein expression of Snail and Slug in RCC.

In summary, this is the first report of an immunohistochemical and molecular biological study of EMT markers in ccRCCs with rhabdoid features. In terms of the histological characteristics of rhabdoid features and the protein expression of E-cadherin and vimentin, we conclude that the rhabdoid features may histologically and biologically be associated with EMT in ccRCC. In contrast, the histological findings between the ccRCC with G1 to G3 – namely nonrhabdoid features and ccRCCs with

rhabdoid features – differ, and ccRCC with nonrhabdoid features shows the histological findings of epithelial tumors. However, there were no significant differences in the immunoexpression of E-cadherin and vimentin protein between these two types of ccRCC. There is thus a possibility that, in ccRCC with nonrhabdoid features, other cell-cell adhesion mechanisms than E-cadherin adhesion may continue to work, and that ccRCC with rhabdoid features may be caused by an inactivation or loss of these mechanisms.

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Disclosure Statement

The authors declare that there are no potential conflicts of interest.

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