Down-Regulation of HLA Class I Antigen is an Independent Prognostic Factor for Clear Cell Renal Cell Carcinoma

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Purpose: We determined the prognostic impact of human leukocyte antigen class I on the survival of patients with clear cell renal cell carcinoma.

Materials and Methods: Immunohistochemical staining for HLA class I was performed on specimens from 45 patients with clear cell renal cell carcinoma. We performed univariate and multivariate analyses of various factors affecting cause specific survival including HLA class I, Fuhrman grade, TNM stage and tumor size. Furthermore, we compared the survival of patients with HLA class I positive renal cell carcinoma to that of those with down-regulated HLA class I using the Kaplan-Meier method and log rank test.

Results: HLA class I was immunohistochemically down-regulated in 17 (37.8%) clear cell renal cell carcinomas. The down-regulation had no correlation with other clinicopathological parameters such as tumor size, perirenal fat invasion, tumor thrombus, TNM stage or nuclear grade. Univariate and multivariate analyses revealed that HLA class I expression, tumor grade and TNM stage were significant factors influencing the disease specific survival of patients with renal cell carcinoma. Patients with HLA class I positive renal cell carcinoma had longer recurrence-free survival than those with down-regulated expression at 5-year followup (95.5% and 61.1%, respectively).

Conclusions: Our data demonstrate that down-regulation of HLA class I on tumor cells is an independent prognostic factor for clear cell renal cell carcinoma. This finding suggests that HLA class I restricted cytotoxic T lymphocytes have an important role in the suppression of renal cell carcinoma.

Key Words: carcinoma, renal cell; histocompatibility antigens class I; prognosis; survival

Metastatic progression will develop after curative surgery in more than 30% of patients with RCC. Therefore, prognostic classification of RCC has been used in an effort to facilitate appropriate counseling of patients and to guide decisions pertaining to surveillance and adjunctive therapy.[1–3] Many prognostic factors have been identified in RCC, and the TNM stage, nuclear grade, sarcomatoid differentiation and tumor size are generally accepted for patients treated surgically.[4,5] However, because of its inherent resistance to chemotherapy and radiotherapy, no satisfactory treatment options exist for patients with advanced RCC at present,[6] and the response rate to immunotherapy using interferon-α and/or interleukin-2 is also unsatisfactory (less than 20%).[7] Therefore, it is important to determine immunological prognostic factors for the survival of patients with RCC.

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Human leukocyte antigen class I has a critical role in recognition and lysis of tumor cells by CTLs, and defects in antigen presentation could allow the tumor to escape from CTLs.[8] Although the abnormalities of HLA class I and antigen processing molecules in RCC have been efficiently investigated,[9] there has been no study evaluating the relationship between down-regulation of HLA class I in RCC tissues and prognosis. In this study we assessed the influence of down-regulation of HLA class I on the survival of patients with RCC.

MATERIALS AND METHODS

Patients

We reviewed the clinical pathology archives of 138 consecutive patients who underwent radical or partial nephrectomy and were diagnosed as having clear cell RCC at the Sapporo Medical University Hospital, Sapporo, Japan from May 1991 to August 1998. Patients whose medical records were incomplete were excluded. We selected 45 of the patients based on the availability of sufficient material for immunohistochemistry. Informed consent was obtained from the patients to use the surgical specimens remaining after pathological diagnosis for the investigational study, which was approved by the Institutional Review Board for Clinical Research at our university. All hematoxylin and eosin stained slides were

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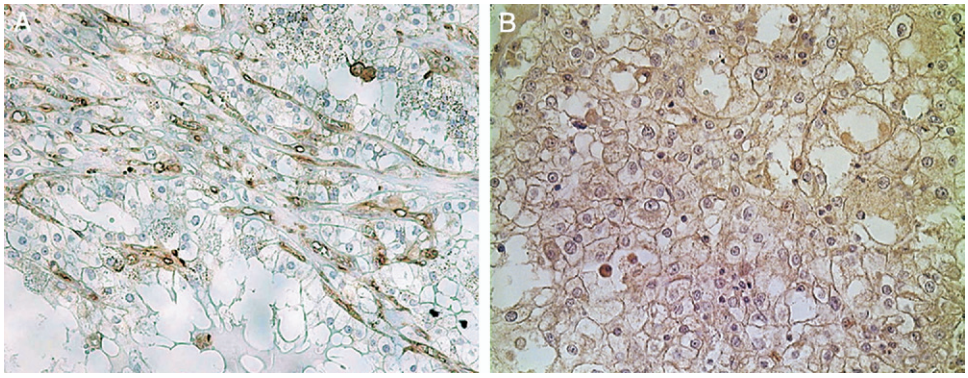

FIG. 1. Representative pictures of immunohistochemical staining with mAb reacting to HLA class I (EMR8-5) in RCC. *A,* in tumor cells staining is seen in cytoplasm but not in cell membrane, which demonstrates down-regulation of HLA class I (score 1). *B*, cell membranes of tumor cells are completely stained (score 2).

reviewed, and all of these specimens showed clear cell RCC. Median patient age at operation of the 27 male patients and 18 female patients was 61 years (range 24 to 80). Median followup was 63 months (range 3 to 117). All hematoxylin and eosin stained slides were reviewed, and clinical stage was assigned using the 2002 TNM classification of malignant tumors. There were 22 cases of stage I, 9 cases of stage II, 7 cases of stage III and 7 cases of stage IV disease. Fuhrman grade distribution was 15 cases with G1-2, 22 cases with G3 and 8 cases with G4. Median tumor diameter was 5.5 cm (range 1.2 to 18). No patients with stage I, II or III RCC underwent immunotherapy before recurrence.

Immunohistochemistry and Scoring

Immunohistochemical staining with the monoclonal anti-pan HLA class I antibody EMR8-5, established at our laboratory, was performed as previously described.[10] Human tonsil sections were used as positive controls for HLA class I. Negative controls had the primary antibody replaced by buffer. All specimens were reviewed independently using light microscopy in at least 5 areas at ×400 magnification by investigators who were blinded to clinicopathological data (IH and TT). The membrane immunoreactivity level for HLA class I was categorized from undetectable to +2. A score of zero was defined as undetectable staining. A score of +1 was defined as faint, incomplete membrane staining in more than 20% of the tumor cells, or as moderate to complete staining in cytoplasm but negative membrane staining in the tumor cells (fig. 1, *A*). Finally, a score of +2 was defined as complete membrane staining in more than 80% of the tumor cells (fig. 1, *B*). HLA class I expression was then classified as down-regulated (scores 0 and 1) or positive (score 2).

Statistical Analysis

We tested the relationships between HLA class I expression and the other clinicopathological parameters, ie the TNM stage, tumor diameter, tumor thrombus, perinephric fat invasion and Fuhrman grade, by logistic regression tests. Disease specific survival was assessed by the Kaplan-Meier method, and differences between 2 groups were compared using the log rank test. Univariate and multivariate regression analyses according to the Cox proportional hazards regression model, with disease specific survival as the dependent variable, were used to evaluate the down-regulation of HLA class I for potential independent prognostic factors. A value of $p < 0.05$ was considered to indicate statistical significance. The calculations were performed using JMP™ software.

RESULTS

Immunohistochemical study of HLA class I in cancer cells revealed that 1, 16 and 28 of the 45 cases had scores 0, 1 and 2, respectively. In other words, HLA class I was down-regulated in 17 (37.8%) of the clear cell RCCs. All normal cells of proximal convoluted tubules, the origin of clear cell RCC, and tumor infiltrating lymphocytes showed positive staining by EMR8-5. Logistic regression analysis revealed no relationship between HLA class I expression and tumor size ($p = 0.6286$), Fuhrman grade ($p = 0.6806$), perinephric fat invasion ($p = 0.8696$), tumor thrombus ($p = 0.7633$) or TNM stage ($p = 0.6869$).

The 5-year disease specific survival was 81.6% for all patients. Univariate analysis revealed that HLA class I expression, TNM stage and tumor grade were significant factors influencing disease specific survival of patients with RCC (see table). Multivariate analysis revealed that HLA class I expression was the only significant and independent factor that affected the disease specific survival (see table). The 5-year survivals were 95.5% and 61.1% in the HLA class I positive and down-regulated arms, respectively. Patients with HLA class I positive RCC had significantly longer disease specific survival than those with down-regulated expression (log rank $p = 0.0145$) (fig. 2).

Results of Cox regression analysis for disease specific survival

	Univariate Analysis		Multivariate Analysis	
	Risk Ratio Label (95% CI)	p Value	Risk Ratio Label (95% CI)	p Value
TNM stage	1.84 (1.25–2.90)	0.0014	1.74 (0.99–3.18)	0.0548
Fuhrman grade	2.32 (1.22–4.73)	0.0094	1.99 (0.62–6.98)	0.2501
Tumor size	1.08 (0.99–1.18)	0.0887	1.05 (0.86–1.25)	0.5946
HLA class I	0.13 (0.04–0.33)	<0.0001	0.21 (0.04–0.86)	0.0294