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Serological Evaluation of Soluble CD44 in Renal Cancer

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In this study, we examined the feasibility of using elevated serum CD44 concentration as an indicator in renal cancer. We performed enzyme-linked immunosorbent assays using 63 sera obtained from 47 patients with renal cancer and 16 healthy controls and evaluated the clinico-pathological parameters. The concentration of soluble CD44 standard (sCD44std), indicating the concentration of all circulating CD44 isoforms, was significantly higher in renal cancer patients than in normal individuals (745 $\pm$170 ng/ml vs. 563$\pm$159 ng/ml, $P=0.001$). The concentration of soluble CD44 splice isoforms sharing exon v6 (sCD44v6) was also higher in the same patients (287$\pm$121 vs. 220$\pm$59, $P=0.056$). However, there were no correlations between the concentrations of sCD44std or sCD44v6 and clinico-pathological parameters such as grade, stage, histological type, tumor size and growth type. The ratio of sCD44std/sCD44v6 was higher in the rapid growth-type cancers than in the slow growth-type cancers (3.95$\pm$2.12 vs. 2.63$\pm$0.82, $P=0.014$). These findings suggested that the serum concentration of unknown soluble CD44 isoforms not sharing exon v6, which are present in sCD44std, increases in patients with rapid growth-type cancers. These findings indicated that sCD44std and sCD44v6 are not useful indicators of tumor burden and metastasis in patients with renal cancer, but that an unknown sCD44 isoform(s) plays a role in the biological behavior of the rapid growth-type cancers.

Key words: Soluble CD44 — Renal cancer

CD44 is a widely distributed cell surface glycoprotein which participates in various cell-cell or cell-matrix-cytoskeleton interactions, including lymphocyte activation[1,2] and tumor metastasis.[3,4] Many CD44 isoforms may be generated by the alternative splice mechanism.[5,6] Some CD44 variants sharing exon v6 were considered to confer a metastatic potential on rat pancreatic cell lines.[7]

Soluble forms of CD44 (sCD44) have been detected in the serum[8] and synovial fluid,[9] and the serum CD44 concentration correlated with tumor metastasis and tumor burden in patients with gastric and colon cancer.[10] Furthermore, sCD44 may influence the activation of T cells.[9]

Renal cell carcinoma (RCC) is the most frequent tumor of the adult kidney, and its behavior is unique. Spontaneous regression of the metastatic region is rarely seen after nephrectomy, and cytokines such as interferon-α, interferon-γ and interleukin (IL)-2 elicit a response in some renal cell carcinomas.[11] The immunological status of the patient may influence the tumor progression. We previously reported that renal cell carcinomas express a higher level of CD44 mRNA than normal kidney tissues.[12] However, the concentration of sCD44 in patients with renal cancer has not been examined in detail, and the relationship between sCD44 and their clinico-pathological features is unknown.

In this study, we examined the presence of two sCD44 isoforms (CD44 standard form (sCD44std) and CD44 variant form sharing exon v6 (sCD44v6)) in patients with renal cancer.

MATERIALS AND METHODS

Patients Serum sCD44std and sCD44v6 were measured in 47 patients with renal cancer treated in Tokushima University Hospital between 1988 and 1995, and in 16 healthy controls. Serum samples were taken before therapy and stored at -80°C.

Thirty-four (72%) of the patients were male, and the median age was 62 years (range, 23 to 82 years). Clinicopathological staging and grading were defined according to the General Rules for Clinical and Pathological Studies on Renal Cell Carcinoma by the Japanese Urological Association, the Japanese Pathological Society and the Japan Radiological Society. Five patients had stage I, 23 stage II, 3 stage III, and 16 stage IV disease. RCC grades 1, 2 and 3 were present in 23, 12 and 3, respectively. Nine patients were inoperable and were diagnosed clinically. Growth types were classified according to our previous report into rapid type when there were 2 or more of the 4 parameters of fever ($\geq$37.0°C) persisting for more than 3 days/week, erythrocyte sedi-

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mentation rate (ESR) exceeding 50mm/h, C-reactive protein (CRP) exceeding 1.0mg/dl and α_2-globulin exceeding 11.0%, or slow type when there was no fever, ESR was less than 20mm/h, CRP less than 0.5mg/dl and α_2-globulin less than 10.0%, or intermediate type for cases not classified into either of the above mentioned 2 types.[13] Slow, intermediate and rapid types were found in 23, 12 and 12 patients, respectively.

The healthy controls consisted of 13 males and 3 females, with a median age of 38 years (range, 23 to 55 years).

Enzyme-linked immunosorbent assay (ELISA) Serum levels of sCD44std and sCD44v6 were measured using the sCD44std ELISA and sCD44var(v6) ELISA (Bender MedSystems, Vienna, Austria). All tests were performed as double tests according to the manufacturer's instructions. The sCD44std ELISA kit theoretically detects the total number of different isoforms and the sCD44v6 ELISA kit detects sCD44 isoforms sharing exon v6.

Statistical analysis The significance of differences was determined using the χ^2 test, Fisher's exact probability test, Spearman rank correlation and Student's t test. P values below 0.05 were considered to indicate significant differences.

RESULTS

Serum levels of sCD44std were significantly higher in patients with renal cancer than in healthy controls (745 $\pm$170 ng/ml vs. 563$\pm$159 ng/ml, $P=0.001$). The serum levels of sCD44v6 were also higher in patients than in healthy controls, but not significantly (287$\pm$121 ng/ml vs. 220$\pm$59 ng/ml, $P=0.056$). We found no difference in the mean levels of sCD44std or sCD44v6 with the stage, histological type, grade, growth type or size of the tumor. However, the mean level of sCD44v6 was higher in the slow growth type (319$\pm$115 ng/ml) than in the intermediate (292$\pm$141 ng/ml) and rapid types (227$\pm$93 ng/ml) (slow vs. rapid, $P=0.024$).

The ratio of sCD44std/sCD44v6 was higher in the rapid growth type (3.95$\pm$2.12) than in the intermediate (2.77$\pm$1.26) and slow growth types (2.63$\pm$0.82) (control (2.34$\pm$0.62) vs. rapid, $P=0.031$; slow vs. rapid, $P=0.014$)) (Table I).

Although neither sCD44std nor sCD44v6 was positively correlated with any of the 4 parameters of growth type, the ratio of sCD44std/sCD44v6 showed a positive correlation with 2 parameters, CRP (regression=0.43, $P=0.004$) and ESR (regression=0.33, $P=0.038$).

DISCUSSION

We examined the concentrations of sCD44std and sCD44v6 in patients with renal cancer by ELISA. Both serum sCD44std and sCD44v6 levels were higher in patients with renal cancer than in healthy controls. Neither the concentration of sCD44std nor that of sCD44v6 correlated with the clinico-pathological features of stage, grade, size of tumor and growth type.

Several investigators, most of whom assayed sCD44std, observed higher serum levels of sCD44 isoforms in various cancers than in healthy controls or in

Table I. Mean Levels of sCD44std, sCD44v6 and the Ratio of sCD44/sCD44v6 Grouped by Clinico-pathological Features, Expressed as Mean$\pm$SD

	No. of cases	sCD44std (ng/ml)	sCD44v6 (ng/ml)	sCD44std/sCD44v6
Control	16	563$\pm$159	220$\pm$59	2.34$\pm$0.62
Renal cancer	47	745$\pm$170 *	287$\pm$121	3.01$\pm$1.46
Growth type				
slow	23	770$\pm$152	319$\pm$115	2.63$\pm$0.82
intermediate	12	679$\pm$185	292$\pm$141 *	2.77$\pm$1.26 *
rapid	12	765$\pm$186	227$\pm$94	3.95$\pm$2.12
Stage				
I–II	28	728$\pm$170	294$\pm$128	2.86$\pm$1.48
III–IV	19	771$\pm$173	278$\pm$113	3.22$\pm$1.45
Grade				
1	23	755$\pm$191	288$\pm$119	2.95$\pm$1.51
2	12	738$\pm$132	264$\pm$133	3.44$\pm$1.59
3	3	865$\pm$241	278$\pm$94	3.51$\pm$2.08
Size (cm)				
<5	17	757$\pm$201	294$\pm$134	2.91$\pm$1.68
≥ 5	25	751$\pm$149	293$\pm$114	2.97$\pm$1.29

* $P<0.05$ by Student's t test.

patients with inflammatory diseases such as viral infection, sepsis and chronic rheumatoid arthritis.[10, 14] However, the concentration of sCD44 isoforms differed with the type of cancer. Guo *et al.* reported that the serum sCD44std level was elevated in patients with gastric and colon cancer, and that it was correlated with tumor metastasis and burden.[10] Also the serum sCD44std level was elevated in patients with malignant lymphoma before treatment, and the serum CD44v6 level was slightly elevated. Furthermore, the serum CD44 level in malignant lymphoma correlated with the response to treatment.[14] On the other hand, Kainz *et al.* reported that the concentration of sCD44v6 was significantly higher in patients with cervical cancer, but the concentration of sCD44std was not; there were no significant differences in the concentration of sCD44std depending on stage, histological type or lymph node involvement.[15] In ovarian cancer too, the concentrations of sCD44std, sCD44v5 and sCD44v6 were not associated with the presence of tumors.[16]

The origin and mechanism of sCD44 isoform production are not clear, but shedding may be an important means of producing sCD44 isoforms.[17] Therefore, the quantitative and qualitative differences of CD44 molecules in various cancer cells and the regulatory mechanism of shedding may be reflected in the differential expression of sCD44 isoforms. However, the reason why there was no correlation between the concentration of sCD44std and sCD44v6 and clinico-pathological parameters in renal cancer, particularly with stage and tumor size, remains unknown. Recently, Ristamäki *et al.* suggested that cytokines have a role in sCD44 shedding.[14] Sliutz *et al.* suggested that the serum CD44std production in hematopoietic cells outweighs the production in ovarian cancer cells and this is one reason why the elevated concentration of sCD44std did not correlate with the clinico-pathological parameters in ovarian cancer.[16] Therefore, the origin of sCD44 isoforms in sera from renal cancer patients and the role of cytokines in the mechanism of their production must be studied.

Based on our preliminary study, the concentrations of sCD44std and sCD44v6 were not considered to be useful specific markers or predictors in renal cancer. However, the ratio of sCD44std/sCD44v6 was significantly higher in patients with rapid growth-type tumors than in those with slow growth-type tumors. Therefore, the distribution of sCD44 isoforms differs between rapid and slow growth-type tumors, and an sCD44 isoform(s) not sharing exon v6 is increased in the serum of patients with rapid growth-type renal cancer.

The rapid growth-type renal cell carcinomas cause a systemic inflammatory reaction manifested as fever, accelerated erythrocyte sedimentation rate, and elevated CRP and α_2-globulin levels.[18] Various cytokines such as IL-6, IL-1β and tumor necrotic factor-α may be responsible for these reactions.[13] IL-6 was shown to be involved in the rapid growth-type renal cell carcinoma in previous studies.[13, 19] In this study, the ratio of sCD44std/sCD44v6 in renal cancer was positively correlated to both CRP and ESR. Therefore, not only the elevated serum level of sCD44 isoforms, but also the altered distribution of sCD44 isoforms in renal cancer may be influenced by certain cytokines.

We observed a quantitative increase in CD44 mRNA by northern blotting and a high level of expression of the CD44 isoform sharing exon v10 in renal cancer by means of reverse transcription-polymerase chain reaction followed by Southern blotting.[12] We observed high-level expression of CD44 variants sharing exon v10 in the rapid growth type of renal cell carcinoma, though with a small sample size, in our previous study.[20] Therefore, the sCD44 isoforms sharing exon v10 in patients with renal cancer should be studied.

In conclusion, the ratio of sCD44std/sCD44v6 in patients with rapid growth-type renal cancer was higher than that in patients with the slow growth type. Further studies are required to identify the increased isoforms in serum from patients with the rapid growth type, in order to clarify the role of sCD44 isoforms and their relationship to cytokines.

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