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I. Shintaku · N. Kawagoe · S. Yutani · S. Hoshi
S. Orikasa · O. Yoshizumi · K. Itoh

Expression of the SART1 tumor rejection antigen in renal cell carcinoma

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Abstract We have previously described the SART1 gene, which encodes both the $SART1_{259}$ antigen expressed in the cytosol of the majority of squamous cell carcinomas and some adenocarcinomas and the $SART1_{800}$ antigen expressed in the nucleus of the majority of proliferating cells. The $SART1_{259}$ antigen is recognized by HLA-A24 and A26-restricted cytotoxic T lymphocytes (CTLs). The present study investigated the expression of these two antigens in renal cell carcinomas (RCCs) in order to identify an appropriate molecule for use in specific immunotherapy for RCC patients. These two antigens were detected in all RCC cell lines and cells of the primary cultures of the RCCs tested. Further, they were detectable in cells of the primary cultures of non-tumorous kidney tissues. In contrast to these cultured cells, $SART1_{259}$ was detectable in only a few uncultured RCC tissues (5/20, 25%) and was undetectable in non-tumorous kidney tissues. $SART1_{800}$ was also scarcely detectable in uncultured RCC tissues (3/20, 15%) and non-tumorous kidney tissues (4/20, 20%). HLA-A2402-restricted and tumor-specific CTL (KE4-CTL) used for the cloning of the SART1 gene showed significant levels of cytotoxicity to both the cells from the RCC cell line and the cells from the primary cultures of RCC tissues, but did not lyse any normal cells, including cells from the primary cultures of non-tumorous kidney tissues. The SART1-derived peptide at positions 690–698 induced HLA-A24 restricted CTLs cytotoxic to RCC cells from peripheral blood mononuclear cells (PBMCs) of RCC patients. Therefore, the SART1 peptide could be an appropriate molecule for use in peptide-based specific immunotherapy for RCC patients.

Key words SART1 antigen · Renal cell carcinoma · Cytotoxic T lymphocyte · Immunotherapy · Cancer vaccine

I. Shintaku (✉) · S. Hoshi · S. Orikasa
Department of Urology, Tohoku University,
School of Medicine, 1-1 Seiryomachi,
Aoba-ku, Sendai, 980-8574, Japan
e-mail: shintaku@uro.med.tohoku.ac.jp
Tel.: +81-22-717-7278, Fax: +81-22-717-7283

N. Kawagoe
Department of Urology,
Kurume University, School of Medicine, Kurume, Japan

S. Yutani · K. Itoh
Department of Immunology, Kurume University,
School of Medicine, Kurume, Japan

O. Yoshizumi
St. Mary Hospital, Kurume, Japan

Introduction

Renal cell carcinoma (RCC) and melanomas are the two major cancers that are usually resistant to conventional chemotherapy and radiation therapy, but partly respond to the various regimen of immunotherapies. Approximately one-fifth to one-fourth of patients with metastatic RCC and metastatic melanomas respond to various regimens of immunotherapy, including cytokine therapies and cellular therapies [8, 13, 24]. Many genes encoding tumor-rejection antigens that are recognized by cytotoxic T lymphocytes (CTLs) have been identified from the cDNA of melanomas in the past nine years [3, 6, 7, 14, 23, 29, 30]. Some of the peptides encoded by these genes are currently under clinical trials as cancer vaccines and have led to major tumor regression in some melanoma patients [18, 22, 25]. However, no tumor-rejection antigens nor peptides are available for use in specific immunotherapy for RCC patients. There are uncommon but well-documented reports of spontaneous regression of RCC [4] and the magnitude of lymphocyte infiltration in RCC is significantly higher than that of other cancers [1, 2]. Thus, specific immunotherapy with tumor-rejection antigens might also be applicable for RCC patients. We have recently described a SART1 gene that encodes for tumor antigens recognized by

HLA-A26-restricted CTLs [26] and also by HLA-A24-restricted CTLs [16]. This SART1 gene encodes both the 125 kD of $SART1_{800}$ antigen expressed in the nucleus of the majority of proliferating cells and the 43 kD of $SART1_{259}$ antigen in the cytosol of the majority of squamous cell carcinomas (SCCs) and some adenocarcinomas, but not in other types of cancers or any normal cells except for those of the testis. In this study, we have investigated the expression of SART1 antigens in RCC tissues, and report that the SART1-derived peptides could be appropriate molecules for use in specific immunotherapy for RCC patients.

Material and methods

Samples and Western blot analysis

Renal cell carcinoma (RCC) tissues ($n = 20$) and non-tumorous renal tissues ($n = 20$) were obtained at the time of surgery at Kurume University Hospital and St. Mary Hospital (Kurume, Japan). A section of each sample was minced with scissors and kept at −80 °C until use. These samples were further used for primary culture with RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) for 10–15 days as reported previously [9]. Cells of the primary cultures of RCCs ($n = 10$) and primary cultures of non-tumorous kidney tissues ($n = 10$) were also used for the study. The 15 RCC cell lines used in this study were A-498, ACHN, Caki-1, Caki-2, KN39, KN41, KRC, KUR-11, MAMIYA, OSRC, OWR-10, RCC10RGB, TUHR4TKB, TUHR14TKB, and VMRC-RCW. These cell lines were incubated with RPMI 1640 medium supplemented with 10% FCS. The KE4 esophageal squamous carcinoma cell (SCC) line (HLA-A2402/A2601) from which the SART1 gene was cloned was used as a positive control, whereas uncultured PBMCs served as a negative control. For the Western blot analysis, samples were lysed with a buffer containing 10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.5% Triton X-100, 0.2 mM PMSF (Sigma Chem., St. Louis, Mo.), and 0.03 trypsin inhibitor units/ml aprotinin. The samples were then sonicated and centrifuged, and the supernatant was collected to use as the cytosol fraction. The precipitate was lysed with buffer consisting of 7.2 M urea, 1.6% Triton X-100, 0.8% dithiothreitol, and 2% lithium dodecyl sulfate. It was then centrifuged, and the supernatant was used as the nuclear fraction. The expression of $SART1_{259}$ in the cytosol and $SART1_{800}$ in the nucleus was investigated by Western blot analysis using the polyclonal anti-$SART1_{259}$ and $SART1_{800}$ antibodies, respectively, as reported previously [26].

Cytotoxic T lymphocytes and assays

HLA-A2402-restricted and tumor-specific CTL (KE4-CTL), which was used for cloning the SART1 gene and was established from an esophageal cancer patient (HLA-A2402/A2601) as previously described [16], was used in this study as a positive control for HLA-class I restricted CTLs. PBMCs from HLA-$A24^+$ healthy donors were incubated for 4–8 days with the RPMI 1640 medium supplemented with 10% FCS and 100 units/ml interleukin-2 (IL-2), and these lymphokine-activated killer (LAK) cells were used as a negative control. The RCC cell lines used in this study for positive and negative controls were HLA-$A24^+$ RCCs (TUHR14TKB, MAMIYA, VMRC-RCZ, KUR-11), and HLA-$A24^-$ RCC (OSRC), respectively. Cells of the primary cultures from RCCs and nontumorous kidney tissues were also provided for the study as target cells. A 6-h ^{51}Cr-release assay was used for the measurement of the cytotoxicity of CTLs in triplicate assays by the method reported previously [9]. The expression of HLA-class I or HLA-A24 antigens on tumor cells was studied by staining the cells with an anti-class I (w6/32) monoclonal antibody (mAb) recognizing a monomorphic region of the class I molecule or anti-HLA-A24 mAb recognizing a polymorphic region of HLA-A24 molecules (One Lambda, Inc., Canoga Park, Calif.), and the expression was measured by FACScan (Becton Dickinson, San Jose, Calif.) as reported previously [16].

Peptides and cytotoxic T lymphocyte induction

In 11 RCC patients PBMCs were provided for experiments on CTL induction, and the TNM classifications of these patients were based on the UICC classification [11]. For CTL induction, the $SART1_{690\text{-}698}$ (EYRGFTQDF) peptide that was recognized by the HLA-A24-restricted KE4-CTLs and was also capable of inducing HLA-A24-restricted CTLs [16] was used in this study. This peptide was kindly provided by Dr. Kanaoka (Research Division of Sumitomo Pharmaceutical Co., Osaka, Japan), and the purity was >95%. The methods for CTL induction using this peptide have been described previously [16]. Briefly, PBMCs (2×10^6) from RCC patients were incubated with 10 μM peptide in one well of a 24-well plate containing 2 ml culture medium (45% RPMI-1640 medium, 45% AIM-V medium (GIBCO BRL, Walkersville, Mass.], and 10% fetal calf serum (FCS) with 100 units/ml interleukin-2 (IL-2) [Shionogi Pharm. Co., Osaka, Japan]). After being cultured for 7 and 14 days, the cells were harvested, washed and re-incubated with the irradiated (30 gray) autologous PBMCs acting as antigen-presenting cells that had been pre-incubated with the same peptide at the same dose for 2 h. After 21 days, these cells were further cultured in a 96-well U-bottom microculture plate in the presence of feeder cells (irradiated HLA-$A24^+$ allogenic PBMCs from healthy donors, 3×10^5 cells/well) that had been prepulsed with the $SART1_{690\text{-}698}$ peptide in order to obtain large number of effector cells. Seven to 10 days later, the expanded cells were transferred to wells of a 24-well plate and incubated in the presence of IL-2 alone for an additional 21–35 days. The surface phenotypes and CTL activity of these cells were then tested for their cytotoxicity against ^{51}Cr-labeled target cells at different effector-to-target cell (E/T) ratios in triplicate assays. The surface phenotype of the CTL line and sublines was investigated by an immunofluorescence assay with anti-CD3 and anti-CD8 mAb [21]. For the inhibition of CTL activity, 20 μg/ml of anti-class I (W6/32, IgG2a), anti-CD8 (Nu-$T_{S/C}$, IgG2a), anti-class II (H-DR1, IgG2a), and anti-CD4 (NU-$T_{H/I}$, IgG1) mAb were used as reported previously [32]. Anti-CD14 (H14, IgG2a) mAb were used as an isotype-matched control. A two-tailed Student's *t*-test was employed for the statistical analyses in this study.

Results

Expression of SART1 antigen

The expression of the SART1 antigens on malignant and normal kidney cells and tissues was investigated by Western blot analysis. Representative results are shown in Fig. 1, and a summary is given in Table 1. The $SART1_{259}$ and $SART1_{800}$ antigens were expressed in both the cytosol and nuclear fractions of all the RCC cell lines tested ($n = 15$), respectively. In contrast, $SART1_{259}$ was detectable in a few of the uncultured RCC tissues (5/20, 25%) and was undetectable in any non-tumorous kidney tissues tested. The $SART1_{800}$ was also scarcely detectable in uncultured RCC tissues (3/20, 15%) and non-tumorous kidneys (4/20, 20%). To understand better the different levels of expression between cell lines and uncultured tissues, we studied the

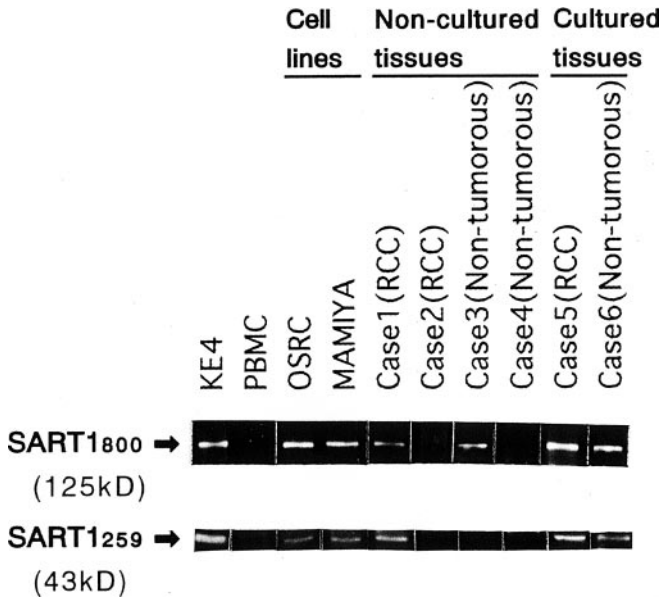

Fig. 1 Expression of the $SART1_{259}$ and $SART1_{800}$ antigens at the protein level was investigated by Western blot analysis using anti-$SART1_{259}$ and anti-$SART1_{800}$ polyclonal antibodies as previously described [26]. Cytosol and nuclear fractions were both investigated. Representative results are shown in this figure. Peripheral blood mononuclear cell (*PBMC*), a negative control; KE4, a positive control; OSRC and MAMIYA, renal cell carcinoma (*RCC*) cell lines, non-tumorous kidney cells

Table 1 Expression of the $SART1_{259}$ and the $SART1_{800}$ antigens (*RCC* renal cell carcinoma)

Samples	$SART1_{259}$ (%) (Cytosol)	$SART1_{800}$ (%) (Nucleus)
RCC cell lines	15/15 (100)	15/15 (100)
Uncultured RCC tissues	5/20 (25)	3/20 (15)
Uncultured non-tumorous kidney tissues	0/20 (0)	4/20 (20)
Primary cultured RCC cells	10/10 (100)	10/10 (100)
Primary cultured non-tumorous kidney cells	10/10 (100)	10/10 (100)

expression of these antigens in cells of primary cultures of RCC tissues, since uncultured RCC samples usually consist of a mixture of tumor cells, lymphocytes, and dead cells as reported previously [9, 17]. We also provided non-tumorous kidneys for primary culture as a control, since the $SART1_{800}$ nuclear antigen is also detectable in proliferating normal cells [26] and non-tumorous kidneys contain normal epithelial cells capable of proliferating in culture for several weeks as reported [9, 17]. The $SART1_{259}$ and $SART1_{800}$ antigens were detectable in both the cytosol and nucleus of all these cells from the primary cultures of RCC tissues ($n = 10$) and non-tumorous kidney tissues ($n = 10$), respectively. All these results suggest that these two SART1 antigens are self antigens detectable in both RCC cells and normal kidney epithelial cells that are under proliferation.

Recognition of renal cell carcinoma cells by HLA-A2402-restricted cytotoxic T lymphocytes

In order to investigate whether RCC cells are recognized by HLA class I-restricted CTLs recognizing the $SART1_{259}$ antigen, the following kinds of RCC cells were tested for their susceptibility to lysis by KE4-CTLs by a 6-h ^{51}Cr-release assay at three different E/T ratios: HLA-$A24^+$ RCC cells (MAMIYA, TUHR14TKB, VMRC-RCZ) or HLA-$A24^-$ RCC cells (OSRC). KE4 tumor cells served as a positive control, whereas VA13, K562 (susceptible for natural killer cell-mediated lysis), and HLA-$A24^+$ phytohemagglutinin (PHA)-activated PBMCs served as negative controls. All the HLA-$A24^+$ tumor cells (MAMIYA, VMRC-RCZ, TUHR14TKB, and KE4 tumor cells) were susceptible to lysis by the KE4-CTLs, with higher susceptibility being observed in MAMIYA and VMRC-RCZ (Fig. 2a). In contrast, none of the three negative controls (OSRC, VA13, PHA-blast cells) were susceptible to lysis. The expression levels of the HLA-A24 molecules of these four tumor cells, however, were not significantly different from each other. Specifically, the mean fluorescein intensities of MAMIYA, VMRC-RCZ, TUHR14TKB, and KE4 were 138, 132, 121, and 109 respectively. We then tested the susceptibility of cells from the primary culture of RCC tissue and those from non-tumorous kidney tissue to lysis by the KE4-CTLs, since both kinds of cells had expressed the SART1 antigens. LAK cells from an HLA-$A24^+$ healthy donor were used as positive effector cells, as these cells had been shown to be equally susceptible to LAK cell-mediated lysis in a previous study [9]. The KE4-CTLs showed significant levels of cytotoxicity to both the cells from the RCC cell line and from the primary cultures of RCC tissues, but failed to show cytotoxicity to the cells of non-tumorous kidney tissues. In contrast, the LAK cells showed significant and equal levels of cytotoxicity to both the cells from primary culture of RCC tissue and those of non-tumorous kidney tissue (Fig. 2b).

Induction of cytotoxic T lymphocytes by the SART1 peptide

The PBMCs from HLA-$A24^+$ patients with RCC ($n = 11$) were incubated with 10 μM of the $SART1_{690–698}$ (EYRGFTQDF) peptide followed by a test of their CTL activity. Profiles of these patients are shown in Table 2. The PBMCs from four out of the 11 patients (termed as group 1 in Table 2) produced significantly higher amounts of IFN-γ by recognition of the HLA-$A24^+$ RCC cells (TUHR14TKB) than by recognition of HLA-$A24^-$ RCC cells (OSRC) when stimulated three times with the peptide followed by a test of the activity at day 21 at an E/T ratio of four. Representative results of cases 1 and 2 are shown in Fig. 3a. The PBMCs from four patients (group 2) produced similar levels of IFN-γ in response to all four kinds of target cells, while those

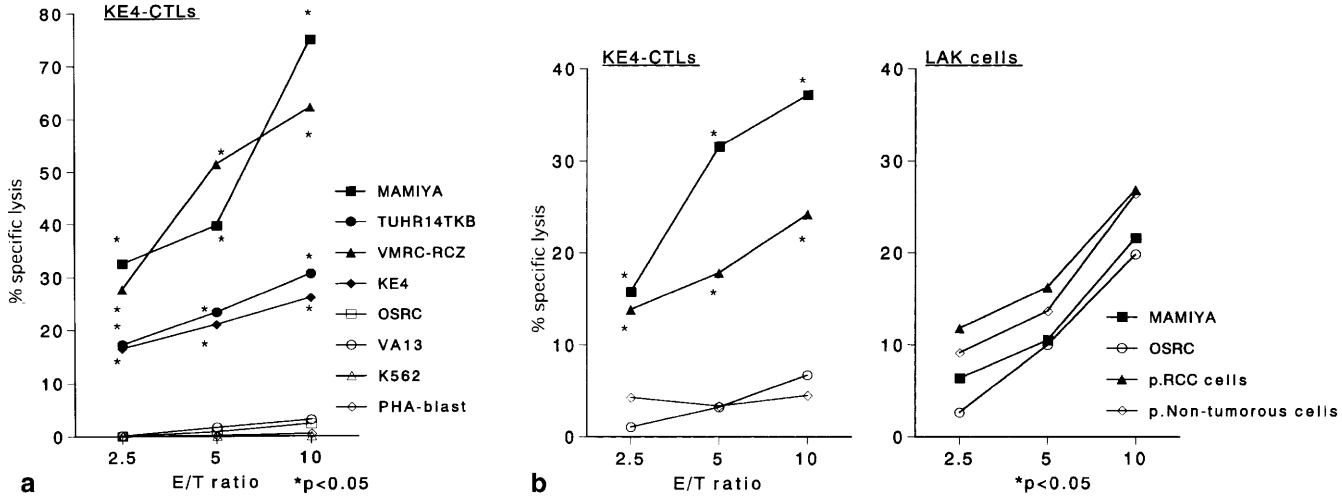

Fig. 2a, b The cytotoxicity of HLA-24-restricted KE4-CTLs against RCC cells. KE4-CTLs were tested against various target cells by a 6-h ^{51}Cr- release assay at three different E/T ratios. **a** The target cells were HLA-A24^{+} RCC cells (MAMIYA, TUHR14TKB, VMRC-RCZ) and KE4 tumor cells (a positive control), HLA-A24^{-} RCC cells (OSRC), HLA-24^{-} fibroblast cells (VA13), K562 tumor cells, and PHA blastoid cells (PHA-blast) (a negative control). Values represent the mean of triplicate assays. A two-tailed Student's *t*-test was employed for statistical analysis throughout this study. **b** The target cells were HLA-A24^{+} RCC cells (MAMIYA) as a positive control, HLA-A24^{-} RCC cells (OSRC) as a negative control, cells from the primary cultures of HLA-24^{+} RCC tissues (p.RCC cells in the figure) and those of HLA24^{+} non-tumorous kidney tissues (p.Non-tumorous cells in the figure)

from three patients (group 3) did not proliferate well under the employed conditions. Although the percentages of CD3^{+}CD8^{+} cells were somewhat higher in the PBMCs of group 1 after stimulation with the peptide, the other clinical markers were not significantly different among these three groups. Some of the results are shown in Table 2.

To confirm the CTL activity, PBMCs from the two patients of group 1 (cases 1 and 3) were further incubated in the presence of feeder cells and IL-2 followed by a test of their cytotoxicity at 18–23 days of re-culture. These PBMCs showed significant levels of cytotoxicity to KE4 or MAMIYA tumor cells, but not to OSRC or HLA-A24^{+} PHA-blastoid cells. Representative results of case 3 are shown in Fig. 3b. In contrast, LAK cells from a HLA-A24^{+} healthy donor showed modest and equal levels of cytotoxicity to these two tumor cells (Fig. 3b). This CTL activity was inhibited by the anti-CD8, anti-HLA-class I mAb, but not the anti-CD4, anti-HLA-Class II, or irrelevant (CD14) mAb (Fig. 3c). These results suggest that this SART1 peptide has the ability to induce HLA-A24-restricted and tumor-specific cytotoxicity from the PBMCs of HLA-A24^{+} RCC % of CD8^{+}d. patients.

Discussion

This study showed that both the SART1$_{259}$ and SART1$_{800}$ antigens were detectable in the cytosol and nuclear fractions of all the RCC cell lines and cells from

Table 2 Characteristics of RCC patients and a summary of CTL induction by the SART1$_{690–698}$ peptide (*IFN* interferon)

Group (Cell growth/CTL induction)	Case	Age	Sex	PS[a]	Stage (TNM)[b]	Treatment[b]	n-fold increase[c]	% of CD8^{+}[d]
Group 1 (+/+)	1	44	M	0	pT3a	none	1.6	6.5
	2	72	F	1	pT3a	none	2.1	6.3
	3	56	F	0	pT2	IFN	17.0	31.0
	4	67	F	0	pT3a	IFN	4.1	51.3
Group 2 (+/−)	5	69	F	0	pT3a	IFN	1.6	4.8
	6	61	F	0	pT2	none	2.6	2.3
	7	56	F	0	pT1	IFN	2.7	9.7
	8	70	M	0	pT3a	IFN	2.7	6.2
Group 3 (−/−)	9	56	F	0	pT2	IFN	0.5	–
	10	44	M	0	pT1	none	0.3	–
	11	67	F	1	T3b	IFN	0.7	–

[a] Performance status
[b] Stage (TNM) and treatment at the time of blood sampling
[c] Level of proliferation of the peptide-stimulated PBMCs were calculated as n-fold increase of yielded number of cells at day 21 of culture as compared to the applied number of PBMCs
[d] Percentages of CD^{3+}CD^{8+} T cells were measured at day 21 of culture

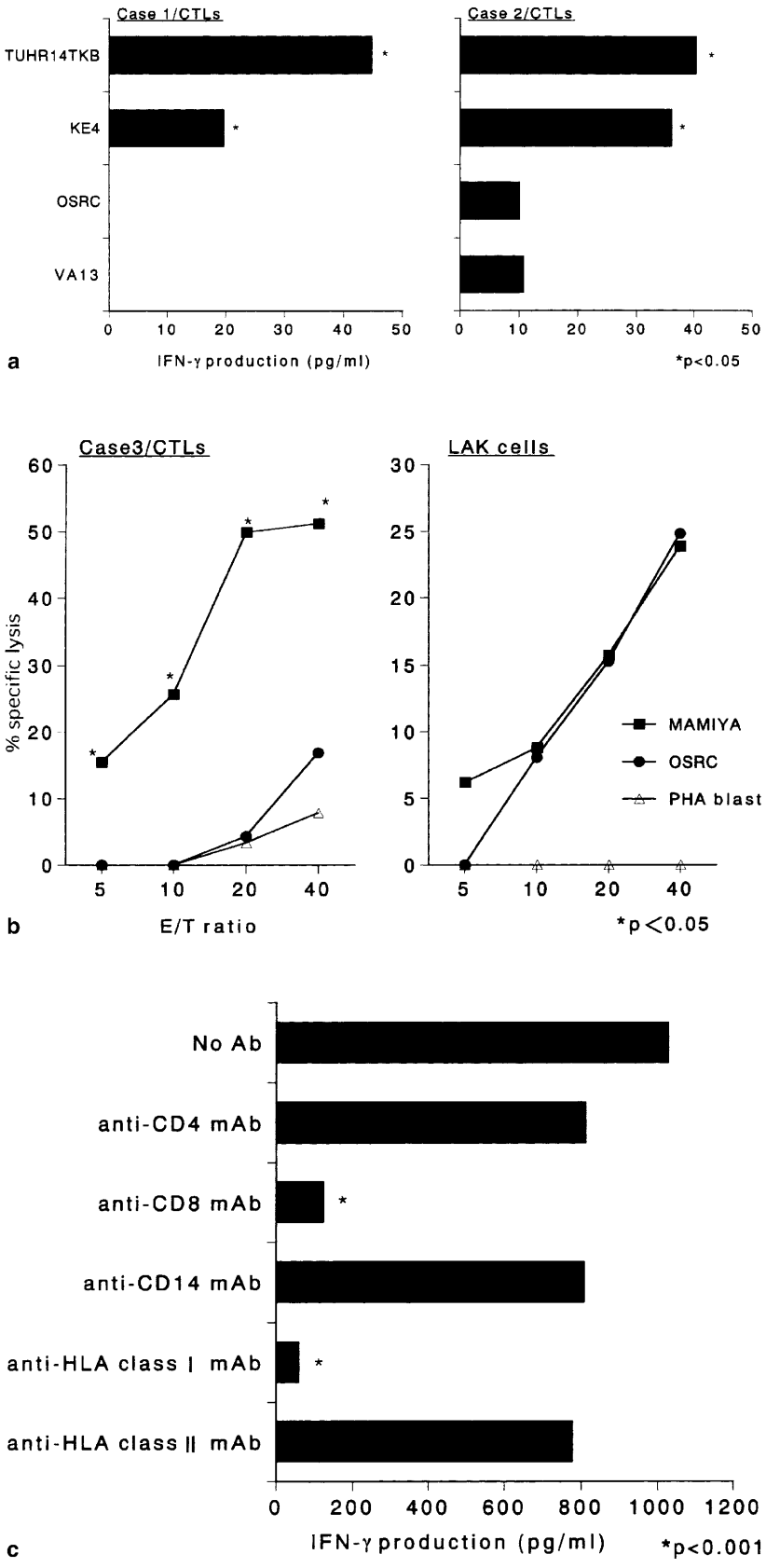

Fig. 3a–c Induction of CTL activity by the peptide. **a** HLA-A24$^+$ RCC cells (TUHR14TKB), HLA-A24$^-$ RCC cells (OSRC), and VA13 (HLA-A24$^-$) were tested for the ability to stimulate IFN-γ production at an E/T ratio of four by the PBMCs of RCC patients that were stimulated with $SART1_{690–698}$ peptide (10 μM) three times in vitro. Values represent the mean of the triplicate assays. **b** The cytotoxicity of these PBMCs was measured by a 6-h ^{51}Cr-release assay after expansion in vitro. LAK cells from a HLA-24$^+$ healthy donor were used as a control. The target cells were HLA-A24$^+$ RCC cells (MAMIYA) as a positive control, HLA-A24$^-$ RCC cells (OSRC) and PHA blastoid cells (PHA-blast) as a negative control. **c** For the inhibition assay, these PBMCs showing HLA-24-restricted CTLs after stimulation by $SART1_{690–698}$ were tested for their ability to produce IFN-γ in HLA-A24$^+$ RCC cells (MAMIYA) at an E/T ratio of two in the presence of 20 μg/ml anti-HLA-class I (W6/32), anti-CD8 (Nu-$T_{S/C}$), anti-HLA-class II (H-DR1), anti-CD4 (Nu-$T_{H/I}$), or anti-CD14 (H14) mAb. Values represent the mean of the triplicate assays

the primary culture of RCC tissues and non-tumorous kidney tissues, respectively. Thus, these antigens are self antigens expressed in both proliferating kidney epithelial cells and RCC cells. The results for $SART1_{800}$ are in agreement with our observations regarding other cancers [15, 19, 26]. Specifically, $SART1_{800}$ is detectable in the nucleus of all proliferating cells, including malignant cells, normal fibroblast cells, the COS7 kidney epithelial cell line, and PHA-activated T cells. In contrast to $SART1_{800}$, the expression of $SART1_{259}$ cytosol antigen varied among the cancers both at the cell-line level and in fresh samples. For example, it was expressed in tumor cell lines from the majority of head and neck SCCs, 60% of esophageal SCCs, 40–50% of lung SCCs and adenocarcinomas [26], but was not expressed at all in breast cancers [15], melanoma, or leukemia cells [26]. It was also undetectable in any normal cells, including fibroblasts or COS7 cells, except for those of the testis. Other tumor-rejection antigens are also expressed in the testis, including the MAGE family, GAGE family, and SART3 antigen [5, 28, 32]. Although the reason for this unique expression pattern in the kidney is presently unknown, it may be due to the vigorous proliferation of cells from the RCC and normal kidneys, since the testis contains spermatogonia and the other types of constitutively proliferating cells [12].

The $SART1_{259}$ antigen was detectable in only 25% of uncultured RCCs tested and undetectable in any non-tumorous kidney tissues tested. Similarly, $SART1_{800}$ was scarcely detectable in these uncultured samples. In contrast to RCC tissues, both antigens were detectable in more than 50% of uncultured tumor tissues from head and neck, esophageal, and lung cancers [26]. One explanation for this discrepancy could be the contamination of dead cells or infiltrating lymphocytes to RCC tissues, as previously reported [9, 17]. These uncultured RCC samples would contain too small a number of tumor cells to be detected by the polyclonal Ab under the employed conditions. This assumption is supported by the results of the experiments on cells of the primary cultures from RCC tissues.

This study suggests that these SART1 antigens play an important role in the cellular proliferation of normal and malignant kidney epithelial cells. Further studies on the biological function of SART1 antigens are needed in order to provide the SART1 peptides for peptide-based specific immunotherapy of RCC patients. In addition, the involvement of CTLs recognizing SART1 antigen in auto-immune renal diseases should be explored because tumor-rejection antigens seem to be some of the target molecules involved in autoimmune diseases [20, 31].

Although SART1 is expressed in PHA-activated T cells, the KE4-CTLs failed to lyse PHA-activated normal T cells. Similarly, the cells of the primary culture of non-tumorous kidney tissues were not susceptible to lysis by the KE4-CTLs. In contrast, LAK cells showed cytotoxicity to both the cells of the primary cultures of RCC and those of non-tumorous kidney, in agreement with our previous observations [9]. These results suggest that proliferating normal kidney cells, though sensitive to lysis by LAK cells, are resistant to lysis by HLA-class I-restricted CTLs reacting to SART1 peptides. In contrast to normal kidney cells, cells of normal melanocyte cell lines are susceptible to lysis by HLA-A2-restricted and melanoma-specific CTLs and also the MART-1 peptide-induced CTLs [27]. From a clinical point of view, the resistance of proliferating normal kidney cells to lysis by HLA-class I-restricted CTLs raise the possibility of SART1 peptide-based specific immunotherapy for RCC patients. Severe adverse effects of renal dysfunction in response to CTL-mediated kidney cell lysis have not been reported in the clinical trials with RCC patients with either adoptive immunotherapy of LAK cells or immunotherapy with IL-2 [8, 24]. Similarly, severe adverse effects in response to CTL-mediated melanocyte lysis have not been reported in melanoma vaccine trials as far as searched at the literature level [18, 22, 25].

The present study showed that the SART1 peptide could induce HLA-A24-restricted CTLs to RCC cells in PBMCs from four of 11 $HLA\text{-}A24^+$ RCC patients tested. These results indicate that CTL precursors reacting to SART1 epitopes on RCC cells are detectable in the circulation of RCC patients. However, the present results do not deny the possible presence of CTL precursors in the remaining seven patients, since the PBMCs were stimulated only three times in this experiment. Other conditions could possibly have induced CTL activity in these seven patients, including more frequent stimulation with the peptide, the use of dendritic cells as antigen-presenting cells, or the use of purified $CD8^+$ cells as effector cells. Although the percentages of $CD8^+$ T cells were somewhat higher in the former patients, there were no obvious differences in the patient profiles between these four and the other seven patients. Further studies might be needed to resolve this issue.

Summary

This study showed that the SART1 peptide might be an appropriate molecule for use in specific immunotherapy for $HLA\text{-}A24^+$ RCC patients. The HLA-A24 allele is found in 60% Japanese, 20% Caucasians, and 12% Africans [10]. The SART1 peptide at positions 736–744 has the ability to induce HLA-A26-restricted CTLs from cancer patients as reported elsewhere [26]. The HLA-A26 allele is found in 22% Japanese, 17% Caucasians, and 16% Africans [10]. Therefore, these SART1 peptides could be an appropriate vaccine candidate for use in peptide-based specific immunotherapy for a relatively large number of potential RCC patients.

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