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Phase I Clinical Study of Survivin-Derived Peptide Vaccine for Patients with Advanced Gastrointestinal Cancers

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Abstract

Survivin is a member of the Inhibitor of Apoptosis Protein (IAP) family. It is expressed in fetal tissues but not in normal adult tissues. Since Survivin is over expressed in various types of tumor tissues as well as tumor cell lines, it is considered to be suitable as a target antigen for cancer vaccine therapy. We identified an HLA-A24-restricted antigenic peptide, SVN-2B (AYACNTSTL), derived from a splicing variant of Survivin-2B. In the present study, we carried out a phase I clinical study assessing the safety and efficacy of vaccination with the peptide in patients having advanced gastrointestinal cancer. Vaccinations with 0.1mg, 1.0mg, or 3.0mg doses of the SVN-2B peptide were given subcutaneously four times at 14-day intervals. In 20 patients who received at least one vaccination, grade 1 and grade 2 treatment-related adverse events were observed, including injection site extravasation (grade 2), injection site reaction (grade 1), skin induration (grade 1) and fever (grade 1). No severe adverse event was observed in any patient. Based on tumor size evaluated by computed tomography, eight of the 15 patients who completed the vaccination schedule were considered to have stable disease as assessed by the RECIST criteria. Analysis of peripheral blood lymphocytes using HLA-A24/peptide tetramers revealed the highest increase of SVN-2B-specific cytotoxic T lymphocyte frequency in the 1.0mg dose group. The present clinical study indicates that SVN-2B peptide vaccination is safe and can be considered a potent immunotherapy for HLA-A24-positive gastrointestinal cancer patients.

Keywords

Survivin, Cancer vaccine, Gastrointestinal cancer, Tetramer, Phase I trial

Abbreviations

IAP: Inhibitor of Apoptosis Protein, CTLs: Cytotoxic T lymphocytes, HLA: Human Leukocyte Antigen, CT: Computed Tomography, PBLs: Peripheral Blood Lymphocytes, AEs: Adverse Events, HIV: Human Immunodeficiency Virus, PD: Progressive Disease, SD: Stable Disease, IFN: Interferon

Introduction

Cytotoxic T lymphocytes (CTLs) can recognize MHC class I-bound peptides derived from tumor antigens in cancer cells. Following the first report of the identification of a human tumor antigen, melanoma antigen-1 (MAGE-1), in 1991 [1] a large number of antigenic peptides from various human cancers have been identified [2-7]. They have been employed in immunotherapy for cancer and clinical trials of peptide-based vaccine therapies have taken place [8-11].

We have identified a human leukocyte antigen (HLA)-A24-restricted antigenic peptide, SVN-2B (AYACNTSTL), which was derived from the exon 2B-encoded region of Survivin-2B, a splicing variant of Survivin [12]. Survivin is a member of the inhibitor of apoptosis protein (IAP) family with a single baculovirus IAP repeat domain [13]. It is expressed during fetal development but undetectable in terminally differentiated normal adult tissues. In contrast to normal tissues, Survivin and Survivin-2B are expressed in transformed cell lines and in most common cancers, including gastrointestinal cancer and pancreatic cancer [13,14]. We reported previously that SVN-2B peptide-specific CTLs were increased by

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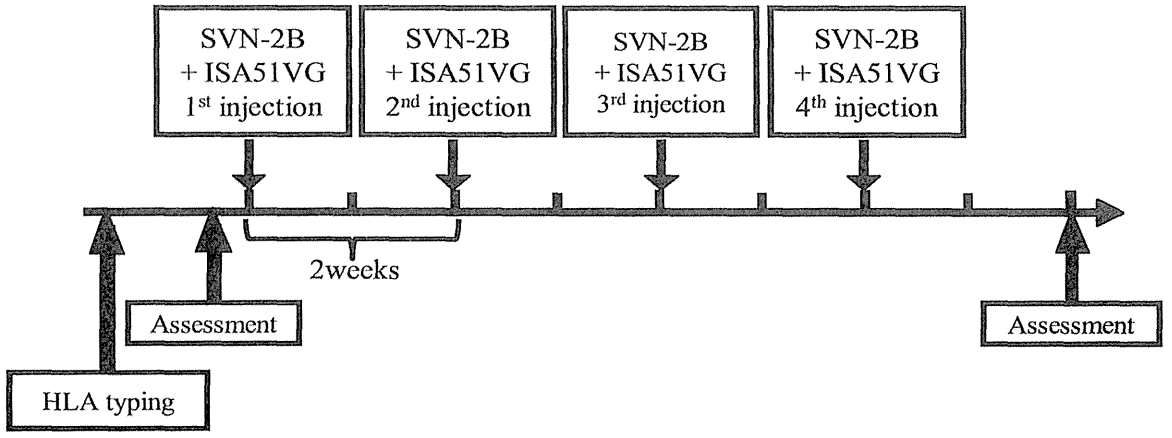

Figure 1: Protocols of the clinical study

The SVN-2B peptide at a dose of 0.1mg/1mL, 1mg/1mL, or 3mg/1mL was emulsified with Montanide ISA51VG at a volume of 0.8mL immediately before vaccination. The patients were then vaccinated subcutaneously (s.c.) four times at 14-day intervals. Tumor size and the immunological response were evaluated before treatment and at two weeks after the 4th vaccination.

stimulating peripheral blood lymphocytes (PBLs) of cancer patients with the peptide *in vitro* [15]. The induced CTLs showed specific cytotoxicity against HLA-A24-positive cancer cells [15-17]. We have carried out clinical trials of SVN-2B vaccination. The SVN-2B peptide was given subcutaneously to patients six times or more at biweekly intervals for colon, breast, oral cavity, and urinary bladder cancer patients [18-24]. There were no severe adverse effects and, clinically, certain patients showed reductions in tumor markers and tumor size as assessed by Computed Tomography (CT). In the present clinical study, we reevaluated the safety and efficacy of SVN-2B vaccination in accordance with good clinical practice guidelines and evaluated the optimal dose of the peptide.

Methods

Patient selection

The study protocol was approved by the Institutional Review Board of Sapporo Medical University. All patients gave informed consent before being enrolled. This study was conducted in accordance with the International Conference on Harmonisation E6 requirements for Good Clinical Practice and with the ethical principles outlined in the Declaration of Helsinki.

Patients enrolled in this study were required to conform to the following criteria: (1) to have histologically confirmed gastrointestinal, bile duct, or pancreatic cancer, (2) to be HLA-A*2402 positive, (3) to have Survivin-positive cancer tissue confirmed by immunohistochemical staining, (4) to be between 20 and 85 years old, (5) to have lesions measureable by CT at the time of registration, (6) to have a history of standard chemotherapy, (7) to have grade 0 or 1 in Eastern Cooperative Oncology Group (ECOG) performance status, and (8) to have no serious organ failure within 30 days at the time of registration.

Exclusion criteria included: (1) prior cancer therapy such as chemotherapy, radiation therapy or other immunotherapy within the previous 4 weeks, (2) presence of other cancers that might influence the prognosis, (3) administration of immunosuppressive drugs such as systemic steroid therapy, (4) severe cardiac insufficiency, acute infection, or hematopoietic failure, (5) uncontrollable diabetes or hypertension, (6) pregnancy or ongoing breast-feeding, and (7) unsuitability for the trial based on clinical judgment. In addition, patients with a high frequency of the peptide-specific CTLs at the time of registration were excluded since such patients were poor responders to the vaccine in our previous studies [23,24]. The number of the HLA-A24/SVN-2B peptide tetramer-positive CTLs per 10,000 CD8-positive T cells (CTLpre) was analyzed at the time of registration and patients who had a value of log10 (1+CTLpre) higher than 1.6 were excluded.

Peptide preparation

The peptide SVN-2B with the sequence **AYACNTSTL** was prepared under good manufacturing practice conditions by PolyPeptide Laboratories San Diego (San Diego, CA, USA). The identity of the peptide was confirmed by mass spectral analysis, and the purity was shown to be more than 98% as assessed by high pressure liquid chromatography analysis. The peptide was supplied as a freeze-dried, sterile white powder. It was dissolved in 1.0 ml of physiological saline (Ohtsuka Pharmaceutical Co., Ltd., Tokyo, Japan) and stored at -80°C until just before use.

Patient treatment

This study was carried out as an open-label, randomized parallel group study at the Department of Surgery, Surgical Oncology and Science of Sapporo Medical University Hospital to evaluate the safety and efficacy of the SVN-2B peptide vaccine for patients who had advanced or recurrent gastrointestinal or pancreatic cancer (UMIN000008611). The patients were randomly assigned into the following three dosage groups: group 1 patients received 0.1mg, group 2 received 1.0mg and group 3 received 3mg. Each group included five patients. SVN-2B at a dose of 0.1mg/1mL, 1mg/1mL, or 3mg/1mL was emulsified with Montanide ISA51VG (Seppic, Paris, France) at a volume of 0.8mL immediately before vaccination. The patients were then vaccinated subcutaneously (s.c.) four times at 14-day intervals (Figure 1).

Toxicity evaluation

Patients were examined closely for signs of toxicity during and after vaccination. Adverse events (AEs) were recorded using CTCAE (version 4.03) criteria and graded for severity.

Clinical response evaluation

Physical and hematological examinations were conducted before and after each vaccination. Changes in tumor marker levels (CEA and CA19-9) were evaluated by comparison of the serum levels before the first vaccination and those after the fourth vaccination. Tumor size was evaluated by CT scans before treatment and at two weeks after the fourth vaccination (Figure 1). The antitumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST: version 1.1) guideline [25]. Briefly, a complete response (CR) was defined as complete disappearance of all measurable and evaluable disease. A partial response (PR) was defined as a >=30%

Table1: Profiles of patients in the full analysis set for safety assessment (N=20)

Clinical variables		0.3 mg (n=7)	1.0 mg (n=7)	3.0 mg (n=6)	Total (n=20)
Gender	Men: Women	2:5	5:2	3:3	10:10
Age	Median (min-max)	69.5 (53-80)	63.0 (51-84)	64.4 (41-66)	65.1(41-84)
Type of cancer	Pancreatic cancer	5	2	5	12
	Colon cancer	2	3	1	6
	Gastric cancer	0	1	0	1
	Bile duct cancer	0	1	0	1
Metastasis	(positive: negative)	7:0	5:2	5:1	17:3
Prior surgery	(positive: negative)	4:3	5:2	3:3	12:8
Prior radiation therapy	(positive: negative)	2:5	2:5	3:3	7:13
Prior chemotherapy	(positive: negative)	6:1	6:1	6:0	18:2
ECOG PS	(0:1)	1:6	1:6	2:4	4:16
Treatment-related AEs					
Fever	Grade 1	1			1
Injection site extravasation	Grade 2			1	1
Injection site reaction	Grade 1	1		1	2
Skin induration	Grade 1	1	1		2

decrease from baseline in the size of all measurable lesions (sum of maximal diameters). Progressive disease (PD) was defined as an increase in the sum of maximal diameters by at least 20% or the appearance of new lesions. Stable disease (SD) was defined as the absence of criteria matching those for CR, PR or PD. Patients who received fewer than four vaccinations were excluded from clinical response evaluations in this study.

In vitro stimulation of PBLs

PBLs were isolated by Ficoll-Conray density gradient centrifugation using Lymphoprep (AXIS-SHIELD, Oslo, Norway). They were then frozen and stored at -80°C. The frozen PBLs were thawed and incubated in the presence of 40μg/mL SVN-2B in AIM-V medium (Life Technologies, Carlsbad, CA, USA) containing 10% human serum at room temperature. Next, interleukin-2 was added at a final concentration of 50 U/mL 1 hour, 2 days and 4 days after addition of the peptide. On day 7 of culture, the PBLs were analyzed by tetramer staining assay and ELISPOT assay.

Tetramer staining

FITC-labeled HLA-A*2402/human immunodeficiency virus (HIV)-derived peptide (RYLRDQQLL) and PE-labeled HLA-A*2402/SVN-2B peptide tetramers were purchased from MBL, Inc. (Nagoya, Japan). For flow cytometric analysis, PBLs, which were stimulated *in vitro* as above, were stained with the FITC-labeled tetramer and PE-labeled tetramer at 37°C for 20 min, followed by staining with a PC5-labeled anti-CD8 monoclonal antibody (Beckton Dickinson Biosciences, San Jose, CA, USA) at 4°C for 30 min. The cells were washed twice with PBS before fixation in 1% formaldehyde. Flow cytometric analysis was performed using FACSCalibur and CellQuest software (Beckton Dickinson Biosciences). The frequency of CTL precursors was calculated as the number of HLA-A24/SVN-2B tetramer-positive cells per 10,000 CD8-positive cells.

ELISPOT assay

ELISPOT plates were coated sterilely overnight with an IFN-γ capture antibody (Beckton Dickinson Biosciences) at 4°C. The plates were then washed once and blocked with AIM-V medium containing 10% human serum for 2 h at room temperature. CD8-positive T cells separated from patients' PBLs ($5x10^3$ cells/well), which were stimulated *in vitro* as above, were then added to each well along with HLA-A24-transfected CIR cells (CIR-A24) ($5x10^4$ cells/well) preincubated with SVN-2B (10ng/mL, 100ng/mL, 10μg/mL) or the HIV peptide (RYLRDQQLL) as a negative control. After incubation in a 5% CO_2 humidified chamber at 37°C for 24 h, the wells were washed vigorously five times with PBS and incubated with a biotinylated anti-human IFN-γ detection antibody (Beckton Dickinson Biosciences) and horseradish peroxidase-conjugated avidin. Spots were visualized and analyzed using KS ELISPOT (Carl Zeiss, Jena, Germany).

Immunohistochemistry

Immunohistochemical study of the HLA class I expression in the patients' primary cancer tissues was done with anti-HLA class I heavy chain monoclonal antibody EMR8-5 according to the standard methods described previously [26].

Statistical analysis

All statistical analyses were done using SAS Version 9.3 and JMP Version 11.0 (SAS Institute, Inc.). For the tetramer assay, statistical analysis was performed using a one-sided t-test. Statistical analysis of ELISPOT assay was performed using the student t-test.

Results

Patient profiles

From August 2012 to May 2013, 38 patients were assessed for eligibility and 21 patients were initially enrolled in this trial (Figure 2). However, one patient was withdrawn before the first vaccination due to deterioration of the systemic condition. Twenty patients who received at least one vaccination were evaluated for safety as a full analysis set (FAS). Five patients discontinued halfway through the protocol due to progression of the disease. None of the interruptions was due to treatment-related AEs. Fifteen patients received the complete regimen including four vaccinations and were evaluated for efficacy of the vaccine (Figure 2). The patient profiles are shown in Table 1. The primary malignant tumors of the 20 patients were 12 pancreatic cancers, 6 colon cancers (including 2 appendix cancers), 1 gastric cancer and 1 bile duct cancer.

Safety

Peptide vaccination was well tolerated in all patients. The treatment-related AEs are listed in Table 1. They included injection site extravasation (grade 2), injection site reaction (grade 1), skin induration (grade 1) and fever (grade 1). No serious toxicity-associated adverse event was observed during or after the vaccination.

Clinical responses

Table 2 summarizes the clinical outcomes of the 15 patients who received the complete regimen. CT evaluation of tumor size showed that 8 patients had SD and 7 patients PD, although none had PR or

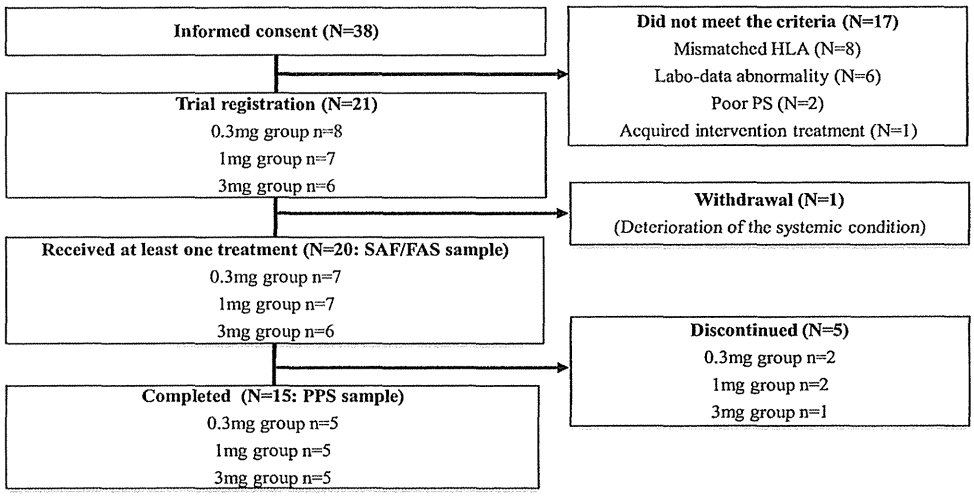

Figure 2: Enrollment of patients

Thirty-eight patients were assessed for eligibility and 21 were initially enrolled in this trial. One patient was withdrawn before the first vaccination due to deterioration of the systemic condition. Twenty patients who received at least one vaccination were evaluated for safety as the full analysis set. Five patients discontinued halfway through the protocol due to progression of the disease. Fifteen patients received the complete regimen and were evaluated for efficacy of the vaccine as the per protocol set. SAF: Safety Analysis Set, FAS: Full Analysis Set, PPS: Per Protocol Set, HLA: Human Leukocyte Antigen, PS: Performance Status.

Table 2: Profiles and clinical outcomes of patients who completed the regimen

Clinical Background						Immunological Response		Antitumor Response		
Dose	Age	Gender	Origin	Status	HLA class I	Tetramer increase	ELISPOT increase	RECIST	CEA	CA19-9
0.3 mg	63	Woman	Pancreas	Inoperable	+	35	-17	**SD**	**Decreased**	**Decreased**
0.3 mg	69	Woman	Pancreas	Inoperable	+	5	-31	**SD**	WNL	Increased
0.3 mg	53	Woman	Pancreas	Post-op	-	7	6	PD	Increased	Increased
0.3 mg	68	Man	Pancreas	Post-op	+	8	17	PD	Increased	Increased
0.3 mg	78	Man	Colon	Post-op	+	-4	2	PD	Increased	Increased
1.0 mg	61	Man	Pancreas	Inoperable	+	21	-1	**SD**	Increased	Increased
1.0 mg	84	Woman	Colon	Post-op	+	28	14	**SD**	Increased	Increased
1.0 mg	69	Man	Stomach	Post-op	+	7	26	**SD**	Increased	Increased
1.0 mg	59	Man	Colon	Post-op	+	29	16	PD	Increased	Increased
1.0 mg	62	Man	Colon	Post-op	+	15	2	PD	Increased	WNL
3.0 mg	41	Woman	Pancreas	Post-op	+	12	158	**SD**	WNL	**Stable**
3.0 mg	66	Man	Pancreas	Inoperable	+	9	19	**SD**	**Decreased**	Increased
3.0 mg	64	Man	Pancreas	Post-op	+	2	-16	**SD**	WNL	**Decreased**
3.0 mg	50	Man	Pancreas	Post-op	+	9	21	PD	WNL	Increased
3.0 mg	64	Woman	Pancreas	Inoperable	+	0	10	PD	Increased	Increased

Post-op: Post-Operative, SD: Stable Disease, PD: Progressive Disease, WNL: Within the Normal Limit

CR. The disease control rate was 53.3%. Among the 8 patients who were defined as having SD, the CEA levels and the CA19-9 levels were decreased or at least stable during vaccination in 2 patients and 3 patients, respectively. The CEA levels stayed within the normal range (0~5.9ng/ml) throughout the study in 4 patients, and the CA19-9 level stayed within the normal range (0~37 U/ml) in one patient. It was noted that all three patients who had undergone immunotherapy before the registration had PD. Moreover, the result for one patient who had HLA class I-negative cancer tissue was also PD.

Tetramer assay and ELISPOT assay

We investigated whether the SVN-2B peptide vaccination could actually induce specific immune responses in the enrolled patients. The peptide-specific CTL frequencies in PBLs before the first vaccination (CTLpre) and after the fourth vaccination (CTLpost) were assessed using the HLA-A24/SVN-2B tetramer, and the tetramer increase (CTLpost-CTLpre) was calculated (Table 2). The HLA-A24/HIV peptide (RYLRDQQLL) tetramer was used as a negative control. SVN-2B-specific CTL frequencies were increased after the vaccination in all patients except two who had undergone immunotherapy before the registration. We compared the tetramer increases between the PD group (non-responders) and SD group (responders). The mean tetramer increase of the SD group was higher than that of the PD group (Figure 3A), although there was no statistical significance

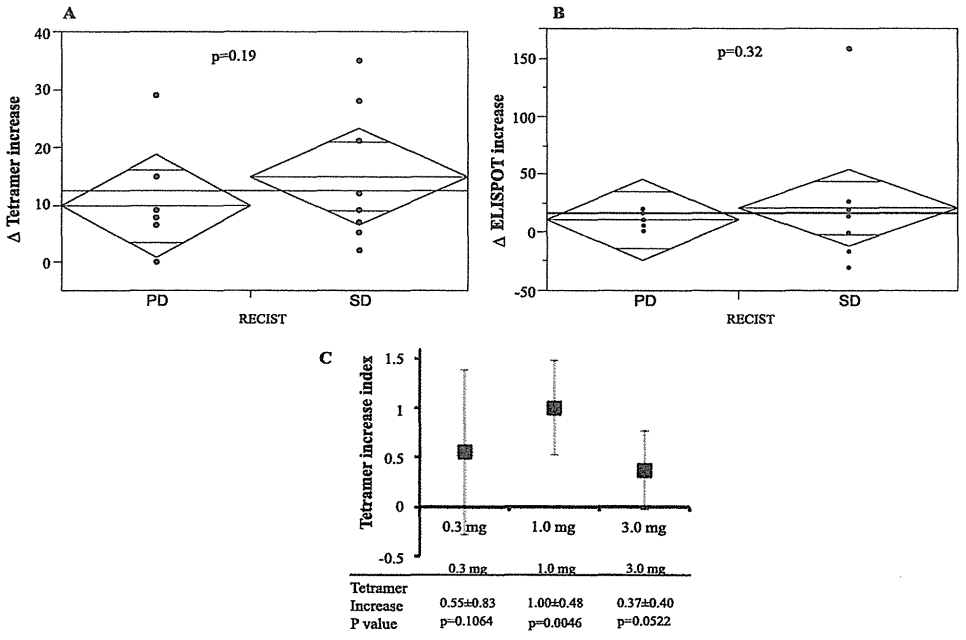

Figure 3: Tetramer assay and ELISPOT assay

(A) The tetramer increase (CTLpost-CTLpre) was calculated from the peptide-specific CTL frequency in PBLs before the first vaccination (CTLpre) and after the fourth vaccination (CTLpost) using the HLA-A24/SVN-2B tetramer. The mean tetramer increases of the PD (non-responders) and SD groups (responders) were compared. (B) The ELISPOT increase was calculated from the numbers of the peptide-specific IFN-γ spots before the first vaccination and after the fourth vaccination. The mean ELISPOT increases of the PD and SD groups were compared. (C) The mean tetramer increase index was calculated according to the following formula: Tetramer increase index=Log10(1+CTLpost)-Log10(1+CTLpre). The mean tetramer indices of the three groups (0.1mg dose, 1.0mg dose, and 3.0mg dose) were compared.

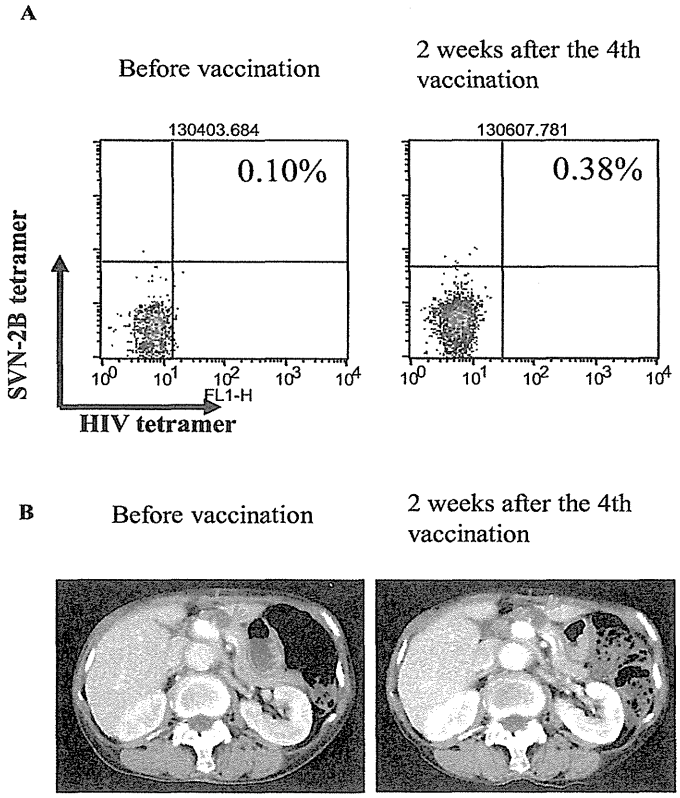

Figure 4: Tetramer assay and CT scan images of the patient with a metastatic pancreatic tumor

An 84-year-old woman with primary colon cancer and metastatic pancreatic tumor. (A) Tetramer assay before vaccination (left panel) and 2 weeks after the 4th vaccination (right panel). (B) CT scan images before vaccination (left panel) and 2 weeks after the 4th vaccination (right panel). The arrowhead indicates the metastatic pancreatic tumor. The tumor grew slightly from 16 mm to 17mm during the 8 weeks of the study.

(p=0.19). To determine the optimal dose of the peptide to induce specific CTLs in patients, the mean tetramer increase indices of the three groups (0.1mg dose, 1.0mg dose, and 3.0mg dose) were compared (Figure 3C). It was found that 1.0mg was the most effective dose for the induction of peptide-specific T cells after the fourth vaccination (p=0.0046).

We also analyzed the peptide-specific IFN-γ responses of CD8-positive T cells by ELISPOT assay. The HIV peptide (RYLRDQQLL) was used as a negative control. The numbers of peptide-specific IFN-γ spots before the first vaccination and after the fourth vaccination were counted, and the ELISPOT increase was calculated (Table 2). There was no significant difference in the mean ELISPOT increase between the SD group and PD group (Figure 3B).

Overall, this study suggests that the immunological response of the vaccine is well represented by tetramer assay rather than ELISPOT assay and that the immunological responses, at least in some patients, appropriately reflect the antitumor responses.

A Case Study

An 84-year-old woman who had primary colon cancer and metastatic liver and pancreatic tumors received the 1.0 mg dose of the SVN-2B vaccine. CT images and tetramer staining data are shown in Figure 4. In this case, the clinical response was defined as SD, and the peptide-specific CTL frequency was increased after the vaccination (Figure 4A). The metastatic pancreatic tumor barely changed from 16 mm to 17 mm during the 8 weeks of the study (Figure 4B). She continued the vaccination after the study. After 6 months, the pancreatic tumor size had increased by 31%, and a new lesion appeared in the caudate lobe of the liver. The time to progression was 267 days. There was no treatment-related AE and she could maintain high quality of daily life for almost one and a half years.

Discussion

The present study demonstrated the safety and clinical efficacy of the survivin-2B peptide vaccine for patients with advanced gastrointestinal cancer. However, the efficacy of vaccination with the SVN-2B peptide plus oil adjuvant Montanide ISA51VG was limited and not sufficient to elicit overt clinical responses. It is obvious that superior clinical and immunological responses are necessary for cancer immunotherapy. It should be considered that vaccination in combination with immunostimulatory adjuvants or cytokines may lead to greater immune and clinical responses. We have reported that type I interferon (IFN) can enhance the antitumor and immunological responses of the peptide vaccine [19,20]. On the basis of the results in this phase I study, we have started a phase II study of the SVN-2B peptide vaccine in combination with IFN-γ.

Immunomonitoring revealed that the tetramer increases were well correlated with antitumor responses as compared with ELISPOT analysis. Therefore, we used the tetramer increase as an index of vaccine-specific immune responses and determined the optimal peptide dose. A significantly higher frequency of tetramer-positive CTLs was induced in the 1mg dose group. However, the optimal dose may vary depending on conditions such as the vaccination interval and combination with distinct adjuvants and/or cytokines, and may have to be reevaluated in combination with IFN. It is enigmatic why the 3mg dose vaccination caused less induction of the peptide-specific CTLs. It was reported previously that persistent vaccine depots could induce sequestration, dysfunction and depletion of antigen-specific CTLs [27]. That may explain, at least in part, the mechanism of the bell-shaped dose effect of the antigenic peptide.

Three patients with a history of immunotherapy such as a dendritic cell vaccine and certain peptide vaccine failed to respond to the SVN-2B peptide vaccine clinically and immunologically. It is possible that their cancers may have had immunoescape phenotypes, thereby maintaining resistance to the vaccine as well as the prior immunotherapy. Alternatively, prior immunotherapy might have affected the immune system, thereby inducing tolerance against the vaccination. In any case, a history of immunotherapy was considered to be a predictive factor for a worse response, and was therefore added to the exclusion criteria in the ongoing phase II clinical study.

In conclusion, we demonstrated the safety and clinical efficacy of the SVN-2B peptide vaccine for patients with advanced gastrointestinal cancer, although clinical interpretation of the results was limited due to this being a phase I study with a small number of patients. At present, a phase II study (UMIN000012146) of the SVN-2B peptide vaccine for advanced pancreatic cancer is ongoing in combination with IFN-γ.

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RESEARCH Open Access

Prognostic value of HLA class I expression in patients with colorectal cancer

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Abstract

Background: Prognostic factors are useful for determination of the therapeutic strategy and follow-up examination after curative operation in cancer treatment. The immunological state of the host can influence the prognosis for cancer patients as well as the features of the cancer. Human lymphocyte antigen (HLA) class I molecules have a central role in the anti-cancer immune system. Therefore, we focused on the HLA class I expression level in cancer cells to investigate its prognostic value in patients with colorectal cancer.

Methods: We reviewed the clinical pathology archives of 97 consecutive patients with stage II colorectal cancer who underwent curative operation at the Sapporo Medical University, Japan, from February 1994 to January 2005. Fifty-six high-risk patients had adjuvant chemotherapy. The cancer cell membrane immunoreactivity level for HLA class I expressed by EMR8-5 was classified into three categories (positive, dull, and negative). In this study, the cases were divided into two groups: "positive" and "dull/negative". HLA class I expression level and clinicopathological parameters were evaluated with the Pearson χ^2 test. Survival analysis was assessed by the Kaplan-Meier methods, and the differences between survival curves were analyzed using the log-rank test.

Results: Immunohistochemical study of HLA class I revealed the following. There were 51 cases that were positive, 40 were dull, and six negative. The HLA class I expression level had no significant correlation with other clinicopathological parameters, except for gender. Univariate and multivariate analyses related to disease-free survival (DFS) revealed that tumor location, HLA expression level, and venous invasion were significant independent prognostic factors ($P < 0.05$). The 5-year DFS rates in HLA class I positive group and in the dull/negative group were 89% and 70%, respectively. For high-risk patients with adjuvant chemotherapy, the 5-year DFS rates in the HLA class I positive group and in the dull/negative group were 84% and 68%, respectively. For low-risk patients without the chemotherapy, the
5-year DFS rates in the HLA class I positive group and in the dull/negative group were 100% and 71%, respectively.

Conclusions: Our study concluded that the HLA class I expression level might be a very sensitive prognostic factor in colorectal cancer patients with stage II disease.

Keywords: HLA class I, Colorectal cancer, Prognostic factor, Relapse, Disease-free survival

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Background

Recently, the number of patients with colorectal cancer is increasing. Colorectal cancer is the third most common cancer and the fourth most frequent cause of cancer death worldwide [1]. In Japan, colorectal cancer is the third leading cause of cancer-related death.

The prognosis is related to many histopathological and clinical parameters, with the most important prognostic factor affecting survival for patients undergoing curative operation being the presence or absence of regional lymph node involvement [2]. Therefore, it is generally recommended that patients with stage III colorectal cancer, which includes regional lymph node metastases, should undergo adjuvant chemotherapy. However, controversy still exists regarding the necessity of adjuvant chemotherapy for node-negative patients with stage II disease [3]. The QUASAR trial demonstrated that adjuvant chemotherapy with fluorouracil/leucovorin (FU/LV) could improve survival of patients with stage II colorectal cancer, although the absolute improvements were small [4]. Pooled analysis (IMPACT B2) of randomized trials comparing groups with adjuvant chemotherapy receiving FU/LV and those with surgery alone demonstrated that there was no significant difference in event-free and overall survival [5]. Meanwhile, O'Connor et al. reported that no 5-year survival benefit from adjuvant chemotherapy was observed for patients with stage II disease, although a benefit was observed for those with stage III disease [6]. In the present situation, adjuvant chemotherapy is conducted for patients categorized into a high-risk group among those with stage II disease on the basis of various histopathological or clinical parameters such as poorly differentiated histology, lymphovascular invasion, perineural invasion, T4 tumor stage, bowel obstruction or perforation, and an elevated preoperative plasma level of carcinoembryonic antigen (CEA) [7]. These parameters are indicated in some guidelines such as the National Comprehensive Cancer Network (NCCN), European Society for Medical Oncology (ESMO), etc. [8], although they are not based on conclusive evidence.

The immune system discriminates between self and nonself, targeting, for example, cancer cells. However, cancer cells can escape from the immune system and grow, metastasize, and finally cause death. One mechanism of the immune escape by cancer development is the downregulation of human lymphocyte antigen (HLA) class I molecules, which are cancer antigen-presenting molecules for cytotoxic T lymphocytes (CTLs) [9-12]. The immune state is of great importance in the prognosis of cancer patients. Therefore, we focused on the HLA class I expression level in cancer cells to investigate its prognostic value in patients with colorectal cancer. Since most anti-HLA class I antibodies recognize the allele-specific native structure of HLA class I molecules, these antibodies have been unable to react with denatured HLA class I molecules in formalin-fixed paraffin-embedded tissue sections. However, we created a novel monoclonal pan-HLA class I antibody, EMR8-5, suitable for the immunostaining of formalin-fixed tissue specimens [13]. Therefore, we are now able to retrospectively investigate HLA class I expression levels in cancer specimens that were surgically resected and stored for a long time.

In this study, we investigated the prognostic value of HLA class I expression in patients with stage II colorectal cancer.

Methods

Patients

The study was approved by the Clinical Institutional Ethical Review Board of the Medical Institute of Bioregulation, Sapporo Medical University, Japan. We reviewed the clinical pathology archives of 97 consecutive patients with stage II (TNM classification [UICC]) colorectal cancer (61 men and 36 women; age range: 31–83 years) who underwent curative operation, defined as the removal of all of the tumoral masses, the absence of microscopic residual tumors, histology-negative resection margins, and lymphadenectomy extended beyond the involved nodes at the postoperative pathologic examination, at the Sapporo Medical University Hospital, Sapporo, Japan, from February 1994 to January 2005. Written informed consent was obtained from each patient according to the guidelines of the Declaration of Helsinki. Fifty-six patients with poorly differentiated histology or positive lymphovascular invasion had adjuvant chemotherapy. These patients were randomly assigned to receive 5-FU plus daily divided dose cisplatin (5-FU, 320 mg/m^2 daily for 21 days; CDDP, 3.5 mg/m^2 daily for 21 days) followed by oral 5-FU (200 mg/body daily for 2 years) or oral 5-FU therapy (200 mg/body daily for 2 years) exclusively as randomized trial [14]. No patients with rectal cancer had radiotherapy. Patients whose medical reports were incomplete were excluded. The median follow-up time was 54 months. Patients' characteristics were assessed by tumor stage (stage IIA, stage IIB, and stage IIC), age, gender, tumor size, tumor location, histological type, and lymphovascular invasion.

Antibody

The monoclonal anti-pan-HLA class I antibody EMR8-5 was established at our laboratory [13]. This mouse mAb (currently commercially available from Hokudo Co., Ltd., Japan) reacts with extracellular domains of HLA-A*2402, A*0101, A*1101, A*0201, A*0207, B*0702, B*0801, B*1501, B*3501, B*4001, B*4002, B*4006, B*4403, Cw*0102, Cw*0801, Cw*1202, and Cw*1502 [15]

and shows strong reactivity in Western blots and conventional light microscopic analysis of formalin-fixed, paraffin-embedded sections.

Immunohistochemistry

Immunohistochemical staining with the antibody was performed on formalin-fixed, paraffin-embedded tissues after steam heat-induced epitope retrieval. Subsequent incubations with a secondary biotinylated antibody, avidin-conjugated peroxidase complex, and chromogen were carried out on a Ventana NexES (Ventana Medical Systems, Inc., Tucson, AZ) [16]. Slides were then counterstained with hematoxylin, rinsed, dehydrated through graded alcohols into nonaqueous solution, and coverslipped with mounting media. Positive reactivity to EMR8-5 was confirmed by staining of vascular endothelial cells and lymphocytes in sections of tumor specimens [15].

Evaluation of HLA class I expression

The cancer cell membrane immunoreactivity level for HLA class I expressed by EMR8-5 was classified into three categories (positive, dull, and negative). Positive was defined as complete and heterogeneous membrane staining in more than 80% of the tumor cells (Figure 1a). Dull was defined as faint, incomplete, and heterogeneous membrane staining in 20% ~ 80% of the tumor cells (Figure 1b). Negative was defined as membrane staining in less than 20% of the tumor cells (Figure 1c). All specimens were reviewed independently using light microscopy in at least five areas at × 200 magnification by two investigators who were blinded to the clinicopathological data (TT and YI).

Statistical analysis

We investigated the relationships between HLA class I expression levels and the other parameters (age, gender, tumor location, tumor size, depth, histological type, lymphovascular invasion, budding, number of lymph nodes analyzed after surgery (<12), HLA class I expression level, and adjuvant chemotherapy) and clinical outcome (disease-free survival: DFS). Some of these parameters (depth, histological type, lymphovascular invasion, budding, number of lymph nodes analyzed after surgery (<12)) were recommended as potential prognostic factors for curatively resected colorectal cancer by ESMO guidelines [8] or NCCN Guidelines Version 2 (2014). Statistical analysis was performed using SPSS Statistics 17.0. Deviation between the HLA class I expression level and clinicopathological parameters was evaluated with the Pearson χ^2 test. Survival analysis was assessed by the Kaplan-Meier method, and the differences between survival curves were analyzed using the log-rank test. To evaluate the correlations between the survival rate and clinicopathological parameters, univariate and multivariate regression analyses according to the Cox proportional hazards regression model were used. A P value <0.05 was considered to indicate statistical significance.

Results

HLA class I expression level and patient characteristics in patients with stage II colorectal cancer

Immunohistochemical study of HLA class I in cancer cells revealed the following. There were 51 cases (53%) that were positive, which was defined as complete and heterogeneous membrane staining in more than 80% of the tumor cells, as well as 40 (41%) that were dull, which was defined as faint, incomplete, and heterogeneous membrane staining in 20% ~ 80% of the tumor cells, and six (6%) that were negative, which was defined as membrane staining in less than 20% of the tumor cells. In this study, the cases were divided into two groups, those that were "positive" ($n = 51$) and those that were "dull and negative" ($n = 46$). The relationships between HLA class

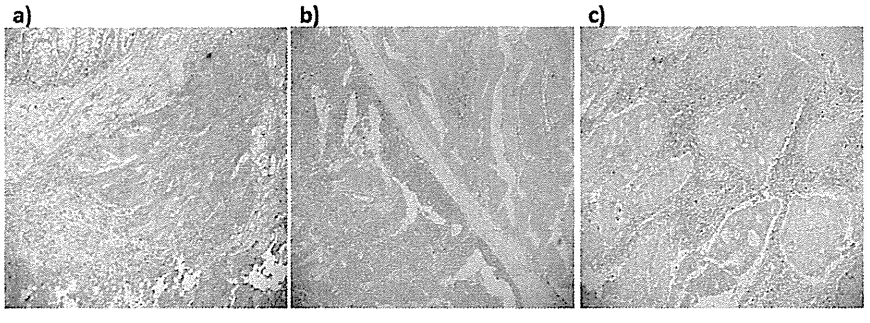

Figure 1 Representative picture of immunostaining with the antibody EMR8-5. The cancer cell membrane immunoreactivity level for HLA class I, which was expressed by EMR8-5, was classified into three categories (positive, dull, and negative). Positive was defined as complete and heterogeneous membrane staining in more than 80% of the tumor cells. Dull was defined as faint, incomplete, and heterogeneous membrane staining in 20% ~ 80% of the tumor cells. Negative was defined as membrane staining in less than 20% of the tumor cells. **(a)** Positive, **(b)** dull, and **(c)** negative.

I expression level and patients' characteristics, i.e., tumor stage (stage IIA, stage IIB, and stage IIC), age, gender, tumor size, tumor location, histological type, and lymphovascular invasion, were assessed. The HLA class I expression level had no significant correlation with other clinicopathological parameters, except for gender (Table 1).

Prognostic factors in patients with stage II colorectal cancer

Univariate analysis related to DFS revealed that the tumor location ($P = 0.01$) and HLA class I expression level ($P = 0.02$) might be significant prognostic factors among age, gender, tumor location, tumor size, depth, histological type, lymphovascular invasion, budding, number of lymph nodes analyzed, HLA class I expression level, and adjuvant chemotherapy. It also suggested that venous invasion might be a prognostic factor ($P = 0.05$). Moreover, multivariate analysis revealed that tumor location, HLA expression level, and venous invasion were significant independent prognostic factors ($P < 0.05$) (Table 2).

HLA class I expression and 5-year DFS

Univariate and multivariate analyses revealed that the HLA class I expression level might be a useful prognostic factor related to DFS. Therefore, survival analysis was conducted using the Kaplan-Meier method. The 5-year DFS rates in the HLA class I positive group and in the dull and negative (dull/negative) group were 89% and 70%, respectively ($P = 0.01$) (Figure 2).

HLA class I expression and adjuvant chemotherapy

Fifty-six stage II colorectal cancer patients with poorly differentiated histology or positive lymphovascular invasion had adjuvant chemotherapy. For patients with this

Table 1 HLA class I expression levels and characteristics of the patients (stage II colorectal cancer)

	Positive ($n = 51$; 53%)	Dull and negative ($n = 46$; 47%)	Total ($n = 97$)	p value
Stage				0.54
Stage IIA	46 (90)	42 (91)	88	
Stage IIB	2 (4)	0 (0)	2	
Stage IIC	3 (6)	4 (9)	7	
Age (years)				0.11
Mean ± SD	64 ± 9.7	60 ± 12.3		
Range	42 ~ 80	31 ~ 83		
Gender—no. of patients (%)				0.03
Male	27 (53%)	34 (74%)	61	
Female	24 (47%)	12 (26%)	36	
Diameter of primary tumor (mm)—no. (%)				0.87
≦30	11 (22%)	12 (26%)	23	
31–50	21 (41%)	17 (37%)	38	
≧51	19 (37%)	17 (37%)	36	
Location—no. of patients (%)				0.84
Right	16 (31%)	13 (28%)	29	
Left	15 (30%)	16 (35%)	31	
Rectum	20 (39%)	17 (37%)	37	
Histological type—no. (%)				0.23
Well/mod	48 (94%)	40 (87%)	88	
Por/muc	3 (6%)	6 (13%)	9	
Lymphatic invasion—no. of patients (%)				0.55
Negative	45 (88%)	40 (87%)	85	
Positive	6 (12%)	6 (13%)	12	
Venous invasion—no. of patients (%)				0.33
Negative	44 (86%)	42 (91%)	86	
Positive	7 (14%)	4 (9%)	11	

Table 2 Univariate and multivariate analyses related to disease-free survival in 97 colorectal cancer patients

Variables	Univariate		Multivariate	
	Hazard ratio	*P* value	Hazard ratio	*P* value
Age	0.98 (0.94–1.02)	0.38		
Gender (F)	1.42 (0.50–4.04)	0.51		
Tumor location (colon vs rectum)	4.23 (1.49–12.01)	0.01	4.11 (1.42–11.91)	0.009
Tumor size (≦5 cm)	0.64 (0.24–1.73)	0.38		
Tumor invasion (SI)	0.52 (0.12–2.28)	0.39		
Differentiation (por or muc)	1.50 (0.20–11.35)	0.70		
Lymphatic invasion (ly0, 1 vs ly2, 3)	1.10 (0.25–4.83)	0.90		
Venous invasion (v0, 1 vs v2, 3)	3.10 (1.00–9.56)	0.05	3.85 (1.15–12.92)	0.03
Budding	0.52 (0.19–1.41)	0.20		
Number of lymph nodes analyzed (<12)	1.32 (0.51–3.43)	0.57		
HLA expression level (dull or negative)	3.86 (1.26–11.85)	0.02	5.36 (1.68–17.11)	0.005
Adjuvant chemotherapy (no)	0.82 (0.30–2.22)	0.70		

chemotherapy, the 5-year DFS rates of those with HLA class I positive expression and those with dull/negative expression were compared. The 5-year DFS rates in the HLA class I positive group and in the dull/negative group were 84% and 68%, respectively (Figure 3). The 5-year DFS in patients with HLA dull/negative expression was lower than that of those with HLA positive expression, although there was no significant difference ($P = 0.10$). On the other hand, no patient with HLA class I positive expression without chemotherapy relapsed, whereas 29% of those with HLA dull/negative expression relapsed. For those without adjuvant chemotherapy, there was a significant difference in 5-year DFS between patients with HLA class I positive expression and dull/negative expression ($P = 0.03$) (Figure 4).

Discussion

Prognostic factors are useful for determination of the therapeutic strategy and follow-up examination after curative operation in cancer treatment. There are various reports of clinical and pathological prognostic factors. However, there are few immunological prognostic factors. The immunological state of the host can influence the prognosis for cancer patients as well as the features of the cancer.

HLA class I molecules have a central role in the anticancer immune system, especially as cancer antigen-presenting molecules for CTLs [13]. CTLs can recognize antigenic peptides presented on the cell surface by HLA

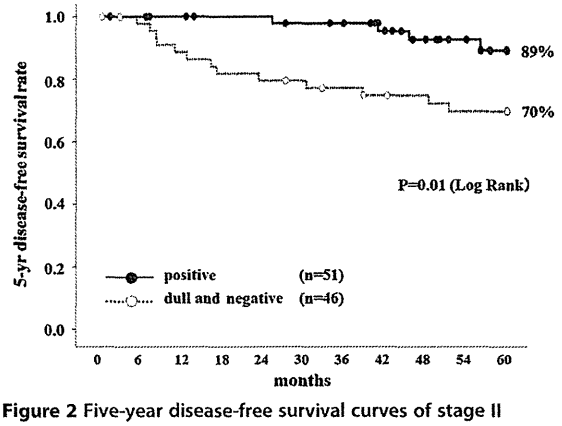

Figure 2 Five-year disease-free survival curves of stage II colorectal cancer patients. The 5-year DFS rates in the HLA class I positive group (*black circle*) and in the dull and negative group (*white circle*) were 89% and 70%, respectively. Patients with HLA class I positive expression had a significantly higher DFS rate than that of those with HLA class I dull and negative expression ($P = 0.01$).

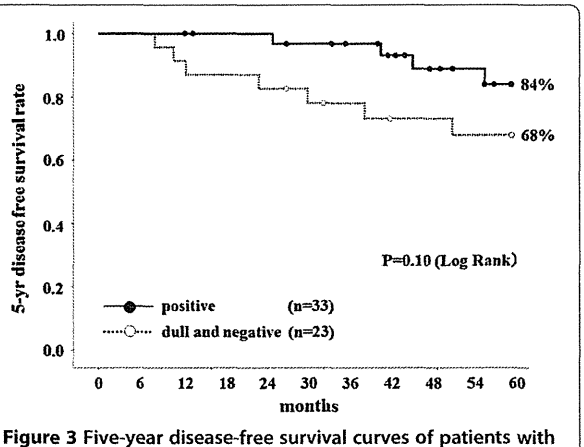

Figure 3 Five-year disease-free survival curves of patients with adjuvant chemotherapy. The 5-year DFS rates of patients with HLA class I positive expression (*black circle*) and with dull and negative expression (*white circle*) were compared. The 5-year DFS in patients with HLA dull and negative expression was decreased more than that of those with HLA positive expression, although there was no significant difference ($P = 0.10$).

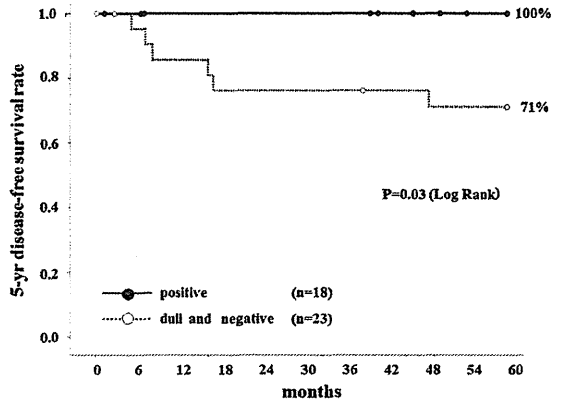

Figure 4 Five-year disease-free survival curves of patients without adjuvant chemotherapy. No patient with HLA class I positive expression (*black circle*) relapsed. Twenty-nine percent of patients with HLA dull and negative expression (*white circle*) relapsed. For patients without adjuvant chemotherapy, there was a significant difference in the 5-year DFS between patients with HLA class I positive expression and those with dull and negative expression ($P = 0.03$).

class I molecules and kill target cells such as cancer cells. However, cancer cells can escape from the immune system by downregulation of HLA class I molecules, secretion of immunosuppressive cytokines, and infiltration of immunosuppressive cells [9-13]. One mechanism of recurrence after curative operation might be immune escape by micrometastatic cancer cells. Therefore, we focused on HLA class I molecules, key molecules in the immune system, to investigate the possibility of new immunological prognostic factors. This investigation was enabled through the use of the novel monoclonal pan-HLA class I antibody EMR8-5 [13], which is suitable for the immunostaining of surgically resected, formalin-fixed tissue specimens stored for a long time.

In this study, we investigated the HLA class I expression level and the prognoses of stage II colorectal cancer patients who underwent curative operation. In patients with stage II cancer, there was a significant difference in 5-year DFS between HLA class I positive patients and dull/negative patients ($P = 0.01$). Patients with HLA class I positive expression had a higher 5-year overall survival (OS) rate than those with HLA class I dull/negative expression, although there was no significant difference ($P = 0.29$) (data not shown). In addition, univariate and multivariate analyses revealed that the HLA class I expression level might be a significant independent prognostic factor. These data suggested that the HLA class I expression level might be a useful prognostic factor, particularly as a predictive factor for relapse, in stage II colorectal cancer. The reason why there was no significant difference in OS for stage II colorectal cancer patients is speculated to be that the beneficial treatments after recurrence might have more influence on OS than the immunological state in the living body such as the HLA class I expression level.

We have also reported that the HLA class I expression level might be a prognostic factor for other cancers such as osteosarcoma, clear cell renal cell carcinoma, and bladder cancer [15-19]. Tsukahara et al. reported that patients with osteosarcoma highly expressing HLA class I had significantly better OS and DFS than those with HLA class I-negative osteosarcoma [15]. Thus, there might be a difference in the impact of the HLA class I expression level on OS or DFS depending on the cancer. Although most reports, including our study, suggested that downregulation of HLA class I expression level was associated with a poor prognosis, Madjd Z et al. reported that total loss of HLA class I was an independent indicator of good prognosis in breast cancer [20]. They considered that the loss of HLA class I might make the tumors more susceptible to natural killer (NK) killing and result in a better prognostic outcome. It is due to the presence of HLA class I allele-specific killer cell inhibitory receptors (KIRs) on the surface of NK cells. Thus, in the absence of HLA class I expression, this KIRs-mediated inhibitory signaling is lost, resulting in the activation of NK cytolytic effector functions [21]. NK cell-mediated cytotoxicity is regulated by a delicate balance between activating and inhibitory signals. So, the prognostic influence brought by the HLA class I expression level might depend on the various cancer immune circumstances.

Surgery alone has relatively favorable results in colorectal cancer patients with stage II disease; hence, any advantage conferred by adjuvant chemotherapy after the curative operation is likely to be small. However, in real life in Japan, approximately 13% of patients with stage II colorectal cancer are found to have recurrence. The seventh edition of the American Joint Committee on Cancer (AJCC) Staging Manual divides stage II into three groups: stage IIA (T3N0), stage IIB (T4aN0), and stage IIC (T4bN0). There is a report that the prognoses for the stage IIB and IIC subgroups are worse than those of some stage III patients [22]. Therefore, stage II patients could be divided into high- and low-risk populations. We should select high-risk stage II patients and give adjuvant chemotherapy to prevent recurrence by micrometastases only to those patients who can obtain a significant benefit from it. The NCCN Guidelines Version 2 (2014) recommended the following risk factors for recurrence: number of lymph nodes analyzed after surgery (<12), poorly differentiated histology, lymphatic/vascular invasion, bowel obstruction, perineural invasion, localized perforation, and close, indeterminate, or positive margins. The ESMO consensus guideline recommended

the following factors: lymph node sampling <12, poorly differentiated tumor, vascular or lymphatic or perineural invasion, T4 stage, and clinical presentation with intestinal occlusion or perforation [8]. In this study, patients with poorly differentiated tumors or moderate and severe lymphovascular invasion were considered to be high-risk stage II patients and underwent adjuvant chemotherapy. We investigated the 5-year DFS in stage II patients with and without adjuvant chemotherapy, respectively. Patients with HLA class I positive expression had a higher DFS rate than those with HLA class I dull/negative expression under both settings. In addition, for low-risk patients without chemotherapy, all patients with HLA class I positive expression did not relapse, although 29% of those with HLA class I dull/negative expression relapsed. These data might make certain of the prognostic value of HLA class I expression for relapse.

Conclusions

The HLA class I expression level might be a very sensitive prognostic factor in colorectal cancer patients with stage II disease.

Abbreviations

DFS: Disease-free survival; CEA: Carcinoembryonic antigen; CTLs: Cytotoxic T lymphocytes; ESMO: European Society for Medical Oncology; NCCN: National Comprehensive Cancer Network; NK: Natural killer; KIRs: Killer cell inhibitory receptors; AJCC: American Joint Committee on Cancer.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YI and TT reviewed all specimens stained with the antibody EMR8-5. TM and TF managed the database of colorectal cancer patients. NT performed the statistical analysis. MM and TT carried out the immunohistochemical staining. NS and KH participated in the design and coordination of this study and helped to draft the manuscript. All authors read and approved the final manuscript.

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MINIREVIEWS

Comprehensive review of post-liver resection surgical complications and a new universal classification and grading system

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Author contributions: Ishii M and Mizuguchi T contributed equally to this study; Ishii M, Mizuguchi T, Harada K and Hirata K designed the review; Ota S and Meguro M collected and reviewed the literature; Ueki T, Nishidate T and Okita K collected and managed the clinical data.

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Abstract

Liver resection is the gold standard treatment for certain liver tumors such as hepatocellular carcinoma and metastatic liver tumors. Some patients with such tumors already have reduced liver function due to chronic hepatitis, liver cirrhosis, or chemotherapy-associated steatohepatitis before surgery. Therefore, complications due to poor liver function are inevitable after liver resection. Although the mortality rate of liver resection has been reduced to a few percent in recent case series, its overall morbidity rate is reported to range from 4.1% to 47.7%. The large degree of variation in the post-liver resection morbidity rates reported in previous studies might be due to the lack of consensus regarding the definitions and classification of post-liver resection complications. The Clavien-Dindo (CD) classification of post-operative complications is widely accepted internationally. However, it is hard to apply to some major post-liver resection complications because the consensus definitions and grading systems for post-hepatectomy liver failure and bile leakage established by the International Study Group of Liver Surgery are incompatible with the CD classification. Therefore, a unified classification of post-liver resection complications has to be established to allow comparisons between academic reports.

Key words: Complication; Liver failure; Bile leakage; Renal failure; Ascites; Coagulation disorder; Surgical site infection

Core tip: The large degree of variation in the post-liver resection morbidity rates reported by previous studies might be due to a lack of consensus regarding the definitions and classification of post-liver resection complications. The Clavien-Dindo classification of postoperative complications is widely accepted internationally. However, it is difficult to apply to some major post-liver resection complications. Therefore, a unified classification of post-liver resection complications has to be established to allow comparisons between academic reports.

Ishii M, Mizuguchi T, Harada K, Ota S, Meguro M, Ueki T, Nishidate T, Okita K, Hirata K. Comprehensive review of post-liver resection surgical complications and a new universal classification and grading system. *World J Hepatol* 2014; 6(10): 745-751 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i10/745.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i10.745

INTRODUCTION

Liver resection has become a safe operation, and its mortality rate is now almost zero, which is much lower than the rate seen a decade ago[1-3]. Liver resection is the best curative option for patients with certain types of liver cancer such as hepatocellular carcinoma[4,5] and metastatic liver cancer[6], as it is cost effective and results in a shorter period of disease-related suffering. To reduce the invasiveness of surgery, laparoscopic procedures have been widely adopted for various types of liver resection[2,7-9]. Preliminary clinical studies have demonstrated that compared with open surgery laparoscopic liver resection results in fewer surgical complications, less intraoperative bleeding, and shorter hospital stays whilst achieving similar oncological outcomes[2,10].

Although the mortality rates described by previous studies were similar, the reported post-liver resection morbidity rates varied markedly due to the use of different definitions for each complication. In fact, the overall morbidity rate of open liver surgery has been reported to range from 4.1% to 47.7%[2,11]. Dindo *et al*[12] attempted to unify the definitions of post-liver resection surgical complications by developing their own grading system (Table 1), which has been widely accepted according to surgical academic reports. However, a classification of the complications seen after hepatobiliary surgery produced by the International Study Group of Liver Surgery (ISGLS)[13] was incompatible with the definitions outlined in Clavien's classification. For example, cases that involve surgical or radiological interventions performed under general anesthesia (categorized as IIIb under the Clavien-Dindo classification) are rarely seen in the clinical setting. Furthermore, patients who suffer organ failure usually exhibit multiple complications, and thus, it is difficult to identify a single cause of the organ failure.

Therefore, we reviewed the definitions of post-liver resection surgical complications and have developed a simple grading and classification system to allow academic reports to be compared.

POST-HEPATECTOMY LIVER FAILURE

Liver failure is the most serious complication after liver resection and can be life-threatening[14,15]. The etiologies of post-hepatectomy liver failure (PHLF) include a small remnant liver[16], vascular flow disturbance[17], bile duct obstruction[15], drug-induced injury[18], viral reactivation[19], and severe septic conditions[15]. In 2011, the ISGLS defined PHLF as a postoperative reduction in the ability of the liver to maintain its synthetic, excretory, and detoxifying functions, which is characterized by an increased international normalized ratio and concomitant hyperbilirubinemia on or after postoperative day 5[13]. Treatments for PHLF must be selected carefully based on the etiology of the condition. Since it was proposed, most reports have employed the ISGLS definitions of PHLF (Table 2). In addition to the latter definitions, our grading system also includes information about the management strategies that are typically employed to treat each PHLF grade (Table 2).

BILE LEAKAGE

Bile leakage (BL) is a major complication of liver resection. The incidence of BL is reported to be 4.0% to 17%[20], and a previous meta-analysis did not find any difference in the incidence of BL between open and laparoscopic cases[21]. BL is defined as an increased bilirubin concentration in the drain or intra-abdominal fluid; *i.e.*, a bilirubin concentration at least 3 times greater than the simultaneously measured serum bilirubin concentration[22]. Once BL develops, it can sometimes lead to complications and can become difficult to manage without interventional radiology (IVR). One of our representative Grade C cases is shown in Figure 1. BL is usually managed with extensive IVR, and reoperations are rarely required. The ISGLS has also developed a grading system for BL[22]. Although the different grades of PHLF are well defined based on clinical symptoms and the management strategies employed, the definitions of each BL grade are too subjective. Therefore, our grading system includes clinical examples (Table 3).

ACUTE RENAL FAILURE

Acute renal failure (ARF) is associated with various postoperative complications. Renal failure is closely associated with PHLF and can lead to hepatorenal syndrome (HRS). The International Ascites Club (IASC) defined HRS using the following criteria[23-25]: (1) cirrhosis and ascites are present; (2) the patient's serum creatinine level is greater than 1.5 mg/dL (or 133 mmol/L); (3) no sustained improvement in the serum creatinine level (to a level of 1.5 mg/dL or less) is seen at least 48 h after diuretic withdrawal and volume expansion with albumin (recommended dose: 1 g/kg body weight per day up to a maximum of 100 g of albumin/d); (4) shock is absent; (5) the patient is not currently taking nor have they recently been taking nephrotoxic drugs; (6) parenchymal kidney disease, as indicated by proteinuria of greater than 500 mg/d, microhematuria (> 50 red blood cells/high power field), and/or abnormal renal ultrasonography, is absent (Verna EC1, Wagener G, Renal interactions in liver dysfunction and failure).

On the other hand, post-liver resection ARF is still poorly defined. Therefore, we have proposed a grading system for post-liver resection ARF (Table 4). The management of ARF mainly involves dehydration and the use of diuretics[26]. Most cases of Grade A and Grade B ARF are reversible and manageable via the latter approach. We defined cases in which the patient could not pass urine without continuous diuretic use as Grade B. On the other hand, Grade C cases were defined as those in which the patient required hemodialysis.

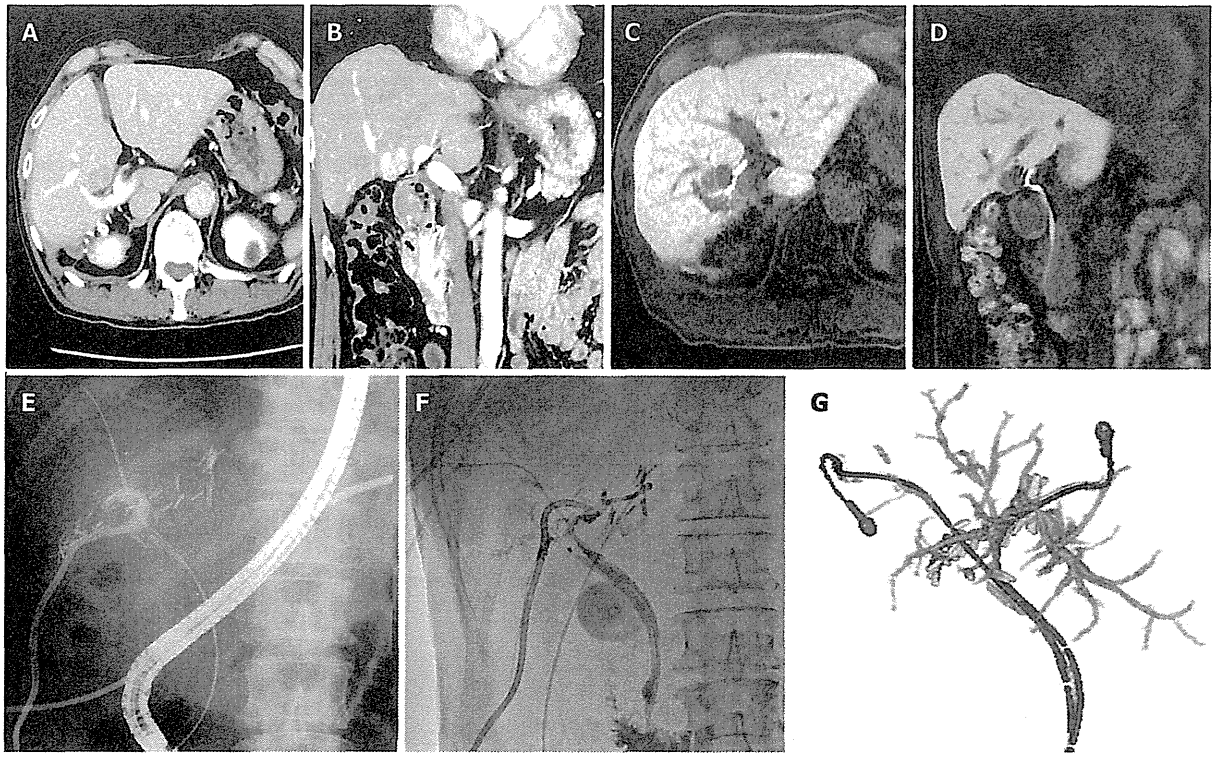

Figure 1 Representative grade C case in bile leakage. A 67-year-old man had hepatocellular carcinoma (diameter: 2 cm; A: Axial view; B: Coronal view) in segment S5 of his liver (located at the bifurcation of the bile duct in the hilar plate) (C: Axial view; D: Coronal view). The tumor was resected via enucleation; E: Bile leakage was detected and so endoscopic retrograde cholangiodrainage was performed together with percutaneous drainage of the resected pouch; F: Subsequently, stenosis of the left hepatic duct due to bile duct ischemia occurred. Percutaneous transhepatic cholangiodrainage was performed via the B3 duct; G: Three-dimensional reconstruction based on CT images obtained before the patient was discharged from hospital. CT: Computed tomography.

Table 1 Comparison between the modified grading system and the Clavien-Dindo classification

Modified grades	Clavien-Dindo classification	
Grade A	Grade I	Any deviation from the normal postoperative course that did not require special treatment
	Grade II	Cases requiring pharmacological treatment
Grade B	Grade IIIa	Cases requiring surgical or radiological interventions without general anesthesia
Grade C	Grade IIIb	Cases requiring surgical or radiological interventions performed under general anesthesia
	Grade IVa	Life-threatening complications involving single organ dysfunction
Grade D	Grade IVb	Life-threatening complications involving multiple organ dysfunction
	Grade V	Cases that resulted in death

Table 2 Grading system and representative management strategies for post-hepatectomy liver failure

Grades	Definition	Management strategies
Grade A	No change in the patient's clinical management strategy required or manageable with medication	Diuretics, selective digestive decontamination, lactulose, glucagon-insulin therapy, stronger neo-minophagen C
Grade B	Manageable without invasive treatment	FFP transfusion, hyperbaric oxygen therapy
Grade C	Invasive treatment required	Plasma exchange, artificial liver support, surgery (including liver transplantation)

Artificial liver support is including high-flow hemodialysis with FFP transfusion. FFP: Fresh frozen plasma.

ASCITES

Ascites is a common complication in patients who exhibit liver dysfunction or cirrhosis after liver resection[27]. One of the possible pathogenic mechanisms of the ascites seen after liver resection is portal flow resistance at the sinusoidal level due to a reduction in the volume of the portal vascular bed[28]. Hepatic outflow block can also cause increased portal flow resistance[29]. The acute phase after liver resection tends to involve edema in the interstitial organ space, which leads to increased portal flow resistance. The management of ascites after liver resection