$CD4^+$ cell depletion, IFN-γ ELISPOT assay was performed utilizing HLA-A*2402-positive TISI cells (IHWG Cell and Gene Bank, Seattle, WA) stimulated by either vaccinated peptide or HIV-Env peptide (as control). Reaction in a MultiScreen-IP 96-plate (Millipore, Bedford, MA) was measured by an automated ELISPOT reader, Immuno-SPOT S4 (Cellular Technology Ltd, Cleveland, OH) with ImmunoSpot Professional Software Version 5.0 (Cellular Technology Ltd). All ELISPOT assays were performed in triplicate. The number of peptide-specific spots was calculated by subtracting the number of the spots of control cells from that of the cells stimulated by vaccinated peptide. The peptide-specific T cell response was classified into four grades (–, +, ++, and +++), according to the algorithm flow chart described in our previous report (+++ : the content rate of CTL is more than 0.2% , ++ : 0.02 - 0.2%, + : 0.01 - 0.02%, –: less than 0.01%) [25]. Sensitivity of ELISPOT assay was estimated as approximate average level utilizing proficiency panels conducted by Cancer Immunotherapy Consortium (CIC) in 2009 and 2011 [26].

Flow cytometry

Expression of peptide specific T cell receptor (TCR) was examined by FACS-CantoII (Becton Dickinson, San Jose, CA) using KIF20A-66/HLA-A*2402 dextramer-PE (KI F20A-dextramer) according to the manufacturer's instruction (Immudex, Copenhagen, Denmark). HIV-A24 epitope peptide (RYLRDQQLL)/MHC-dextramer (HIV-dextramer) was used as negative control. Briefly, cells were incubated with peptide-HLA-A*2402 dextramer-PE for 10 minutes at room temperature, then treated with FITC-conjugated anti-human CD8 monoclonal antibody (mAb), APC-conjugated anti-human CD3 mAb, PE-Cy7-conjugated anti-human CD4 mAb, and 7-AAD (BD Biosciences, San Jose, CA) at 4°C for 20 minutes. Analysis gate was set on the staining profiles using HIV-dextramer, and positive cell percentage ($dextramer^+$ cells/$CD3^+$ $CD4^-$ $CD8^+$ cells) was calculated by subtracting the percentage of HIV-$dextramer^+$ from that of KIF20A-$dextramer^+$.

Statistical analysis

StatView version 5.0 (SAS Institute Japan Ltd., Japan) was used for statistical analysis. TTP and OS curves were estimated using the Kaplan-Meier methodology and analyzed with a log-rank test. Mann–Whitney U test and Chi-square test were used to compare patient characteristics.

Results

The peptide vaccine treatment

A total of 31 patients with chemotherapy-refractory pancreatic cancer were enrolled in this trial. 16 patients had unresectable tumor and 15 had recurrent one after surgery. Tables 1 and 2 indicate clinicopathological information of the 31 patients, as well as the patients in control group, who received best supportive care in our and other hospitals (Table 1). The peptide in the amount of either 1.0 mg or 3.0 mg per body was examined in this phase I/II study. These dosages were well tolerated in the 31 patients with advanced pancreatic cancer. There is no severe adverse event (SAE) related to the peptide vaccine in the 1.0 mg/body-injected group, except the immunological response at injection sites. As well, no SAE was observed in the first 6 patients in the 3.0 mg/body-injected group during the first cycle in the treatment. Hence, we determined that 3.0 mg per body is an appropriate dose for phase II part in this study.

Immunological injection site reactions (ISRs) of all the 31 patients were evaluated. Clinical responses of 29 patients out of 31, who received at least one treatment cycle (4 injections), were evaluated by immuno-monitoring. ISRs, including adverse reactions on the skin in grades 1–3, was observed in 23 patients out of 29. It should be noted that there were two patients who were incompatible with further vaccination treatment due to the exclusion criteria, such as autoimmune hepatitis and interstitial pneumonia. The patient, who experienced grade 3 autoimmune hepatitis after 11 months of vaccination, was recovered after drug withdrawal. Another patient with the interstitial pneumonia was well recovered by hospital treatment without any steroid therapy. In these cases, we could not rule out the possibility whether these adverse events were related to vaccine treatments or not.

Clinical outcomes of eligible patients

Among the 29 patients examined in this trial, 21 patients yielded the status of "stable disease" (SD), while 8 resulted in "progressive disease" (PD) after one cycle of the treatment (injections of the peptide vaccine for 4 times) (Table 2). The rate of disease control at the time of one cycle was calculated to be 72%. 8 patients showed objective tumor response at target lesions (Figure 1). On the other hand, according to RECIST criteria, the other patients were not classified as partial response (PR), since the ratio of tumor shrinkage was insufficient. One patient (case 9) achieved "complete response" (CR) after SD over the long term (Table 2, Figures 1a, 2, and 3). The rate of objective response to the total was calculated to be 25.8%.

Case 9 describes a 33-year-old female ended up with CR after 25 months including a long period of SD (Figure 1a). This patient underwent pancreatoduodenectomy in November 2008 and was diagnosed with giant cell pancreatic cancer. Adjuvant chemotherapy utilizing gemcitabine was discontinued at the one course

Table 1 Clinical status and profile of the patients

	KIF20A peptide vaccine treatment	Best supportive care	
	Chiba (n = 31) *	Chiba (n = 9) *	Multi-center (n = 81) **
Age (average, (range))	61.3 (33–80)	64 (53–82)	64.5 (41–85)
Sex (Male: Female)	17:14	5:4	49:32
Performance status (0:1:2:3)	11:8:12:0	1:3:3:2	13:28:36:0 ***
Status of primary lesion (Resected: Unresected)	15:16	1:8	23:58
Median survival time (days)	142.0 ± 23.7	83.0 ± 33.5	62.0 ± 6.5
Mean survival time (days)	171.8 ± 23.8	93.3 ± 14.8	91.1 ± 11.6

*, Clinical data obtained at our institution, Chiba Tokushukai Hospital.
**, Clinical data of Multi-center (n = 81) include those obtained from Chiba and other three hospitals.
***, 4 cases were excluded, since Performance Status was not determined.

of drug administration, due to severe adverse reactions including hematopoietic toxicity. In February 2009, a progressive solitary liver metastasis was diagnosed (Figure 1a). There was no clinical sign of inflammation at the time of April 13th, 2009. White blood cell count ($2.8 \times 10^3/\mu$-l) and CRP level (0.02 mg/dl) were within normal limits. Vaccination started on April 23rd, 2009, and the tumor kept stable condition during the administration. After 8 months, shrinkage of the tumor size was observed. Vaccination was discontinued after 11 months, because the level of liver enzyme was increased and thus autoimmune hepatitis was suspected. Nonetheless, the tumor continued to shrink and became undetectable by CT 25 months after the start of administration. At the time of the submission of this manuscript, there is no sign of relapse or metastasis, and the general condition of the patient has been kept well with the performance status (PS) of zero.

Case 14 reports a 60-year-old male who showed objective response (Figure 1b). After pancreatoduodenectomy, gemcitabine treatment started in October 2008 and liver metastasis was found 3 months later. Followed by TS-1 chemotherapy, we found that metastatic lesions in the liver progressed after the condition of SD during 3 cycles of TS-1 treatment. After 1 cycle of the peptide vaccine, one target lesion of liver metastases located at S8 was shrunken. This lesion kept shrinking until September 2009, and became hardly detectable by CT scan. Similarly, a metastatic lesion in the lymph node was significantly shrunken until September 2009. However, the other target lesion (S4) in the liver showed no response to the vaccine treatment and the tumor progression was promoted after 2 cycles. Finally, the patient died at 220 days after the start of the vaccination.

In case 24, a 74-year-old male also showed objective response (Figure 1c). After distal pancreatectomy in August 2007, adjuvant chemotherapy utilizing gemcitabine was performed for 6 months and then switched to TS-1 because of the side effect. Bone metastasis was found in the xiphoid process by CT scan in April 2009. Radiation therapy was performed to the xiphoid process in May 2009, but the tumor did not respond well. The patient was enrolled into the peptide vaccine trial in July 2009 after one month of cooling off period. Bone metastasis started to shrink after one cycle of the peptide vaccine treatment. The precordial pain was rapidly diminished and well controlled without opioid treatment. After the 5th shot of the peptide, Grade 3 interstitial pneumonia was observed and the treatment was discontinued. The patient was hospitalized in one week of treatment without any steroid therapy and then well recovered. Even without the vaccination, pain was well controlled and tumor markers kept decreasing for the next two months. After the re-progression of the disease, gemcitabine was administered and no clinical effect was observed. Since the patient desired to receive the peptide vaccine again, we obtained an approval of the re-entry of this case from the Ethical committee. The vaccine treatment was restarted with careful monitoring, while neither adverse events nor clinical effect was observed in this second round of drug administration. His overall survival period from the first day of administration was 495 days.

The median overall survival time of 31 patients was 142 days, and the progression free survival period was 56 days (Figures 4a and 4b). In comparison with the control group without the vaccine treatment, who are the patients visited Chiba Tokushukai Hospital in the period between January 2007 and January 2009 (MST: 83 days), overall survival of the patients with the KIF20A-peptide vaccination was statistically significant ($p = 0.0468$, MST: 142 vs. 83 days) (Figure 4c). Moreover, MST of the patients who received BSC was 63 days. Compared to the control group in multi-center, Overall Survival of the vaccinated patients was significantly improved ($p = 0.0020$, MST: 142 vs. 63 days) (Figure 4c). Taken together, we concluded that the cancer vaccination utilizing KIF20A-derived peptide was significantly effective as immunotherapy against advanced pancreatic cancer.

Table 2 Patient characteristics and clinical responses

No.	Age	Sex	Target lesion	Dose of peptide (mg)	Number of injection	Clinical response*	Objective Response	Response lesion	Injection site reaction(Grade)	CTL response	
										Pre-vaccination	Post-vaccination**
1	75	M	Local LNs	1	4	PD			0	N.A.	+
2	57	F	Local	1	11	PD			1	++	++
3	72	M	Liver	1	3	-			0	N.T.	N.T.
4	60	M	Lung, local LNs	1	19	SD	Yes	Lung metastasis	2	+	-
5	72	F	Primary , liver	1	12	PD			1	+	+++
6	65	F	Liver	1	4	PD			0	+	+
7	61	F	Local , liver	3	14	SD			2	+	+++
8	57	F	Primary, liver	3	10	SD			2	++	+++
9	33	F	Paraaortic LNs	3	29	SD	Yes(CR)	Liver metastasis	3	N.A.	+++
10	76	M	Liver	3	12	PD			2	-	++
11	55	F	Primary, lung	3	17	SD			1	+	-
12	58	M	Primary	3	5	PD			0	-	-
13	58	F	Live, lung, LNs	3	10	SD			1	-	++
14	60	M	Liver, LNs	3	17	SD	Yes	Liver metastasis, LNs	2	+++	+++
15	80	F	Liver, LNs, lung	3	5	PD			0	-	+
16	58	M	Primary, liver, lung	3	13	PD			1	-	++
17	49	M	Parimary	3	17	SD			2	+	+++
18	62	M	Primary, liver, LNs	3	7	SD			1	-	+++
19	61	M	Primarym, liver, lung, LNs	3	11	SD			2	-	+
20	58	M	LNs, lung	3	25	SD			2	+	+++
21	47	M	Primary, liver	3	13	SD			1	-	+
22	71	F	Liver, local LNs	3	7	SD	Yes	Liver metastasis	2	N.A.	++
23	50	M	Local, LNs	3	6	SD			0	N.A.	-
24	74	M	Bone	3	21	SD	Yes	Bone metastasis	2	N.A.	+++
25	69	F	Primary	3	2	-			0	N.T.	N.T.
26	80	M	Liver, lung	3	18	SD			1	+	+++
27	44	M	Liver, lung, local LNs	3	24	SD	Yes	Lung and liver metastasis	1	+	-
28	61	F	Peritoneal, local LNs	3	9	SD	Yes	Peritoneal metastasis	0	-	-
29	46	M	Liver	3	10	SD			2	-	+++
30	64	F	Liver	3	9	SD			2	-	+++
31	68	F	Liver	3	9	SD	Yes	Liver metastasis	2	+	+++

No.	PFS(day)	OS (day)	Pre-vaccination			Post-vaccination		
			WBC(/mm^3)	Lymphocyte (%)	Lymphocyte (/mm^3)	WBC(/mm^3)	Lymphocyte (%)	Lymphocyte (/mm^3)
1	36	36	7300	7	511	5300	10.5	557
2	26	108	7400	13	962	7900	8	632
3	31	31	7800	11	858	16200	10.5	1701
4	223	283	5100	21	1071	5200	10.5	546
5	24	128	2400	25.5	612	4200	10.8	454
6	26	40	4500	16.5	743	8000	4.1	328
7	55	155	4000	25	1000	6400	18.3	1171
8	56	145	4500	33	1485	14100	16	2256
9	>1219	>1219	2500	44.5	1113	3600	33	1188
10	27	142	2300	29.5	679	5800	11.5	667
11	112	225	2600	9	234	2200	11.5	253
12	32	32	4500	30	1350	2400	10.7	257
13	57	97	7100	15.5	1101	9100	10.5	956
14	169	220	2300	27	621	4100	19.5	800
15	24	44	8500	9.5	808	13300	4.8	638
16	28	182	4800	27	1296	6400	19.3	1235
17	169	309	6200	26.5	1643	7900	17.5	1383
18	93	93	4200	28	1176	6600	18.6	1228
19	57	105	10200	20.5	2091	28700	9	2583
20	169	332	10100	34	3434	7600	19.5	1482
21	56	249	6000	27.5	1650	9600	8	768
22	89	89	7000	11.5	805	5200	20	1040
23	148	148	7900	20	1580	11200	19	2128
24	415	495	3800	16	608	5600	17.8	997
25	11	11	7600	21.5	1634	7400	20.5	1517
26	112	207	6600	24	1584	7500	21.5	1613
27	115	317	2900	23.5	682	4000	25.5	1020
28	69	69	9000	26.5	2385	11200	7.5	840
29	52	388	4000	26	1040	5600	24.6	1378
30	56	69	4800	26.5	1272	8900	7.1	632
31	56	82	6800	33	2244	8300	19.5	1619

*Clinical response was evaluated one month after vaccination. PD, Progressive disease; SD, Stable disease; CR, Complete response; OR, Objective response.
**Best CTL response after vaccination. CTL responses were evaluated and classified based on the algorithm as described in Methods.
N.T. (Not Tested); CTL response was not tested in the samples in which PD was observed within one course of the treatment.
N.A. (Not Analyzed); CTL response was not analyzed because of the poor viability during the *in vitro* stimulation.

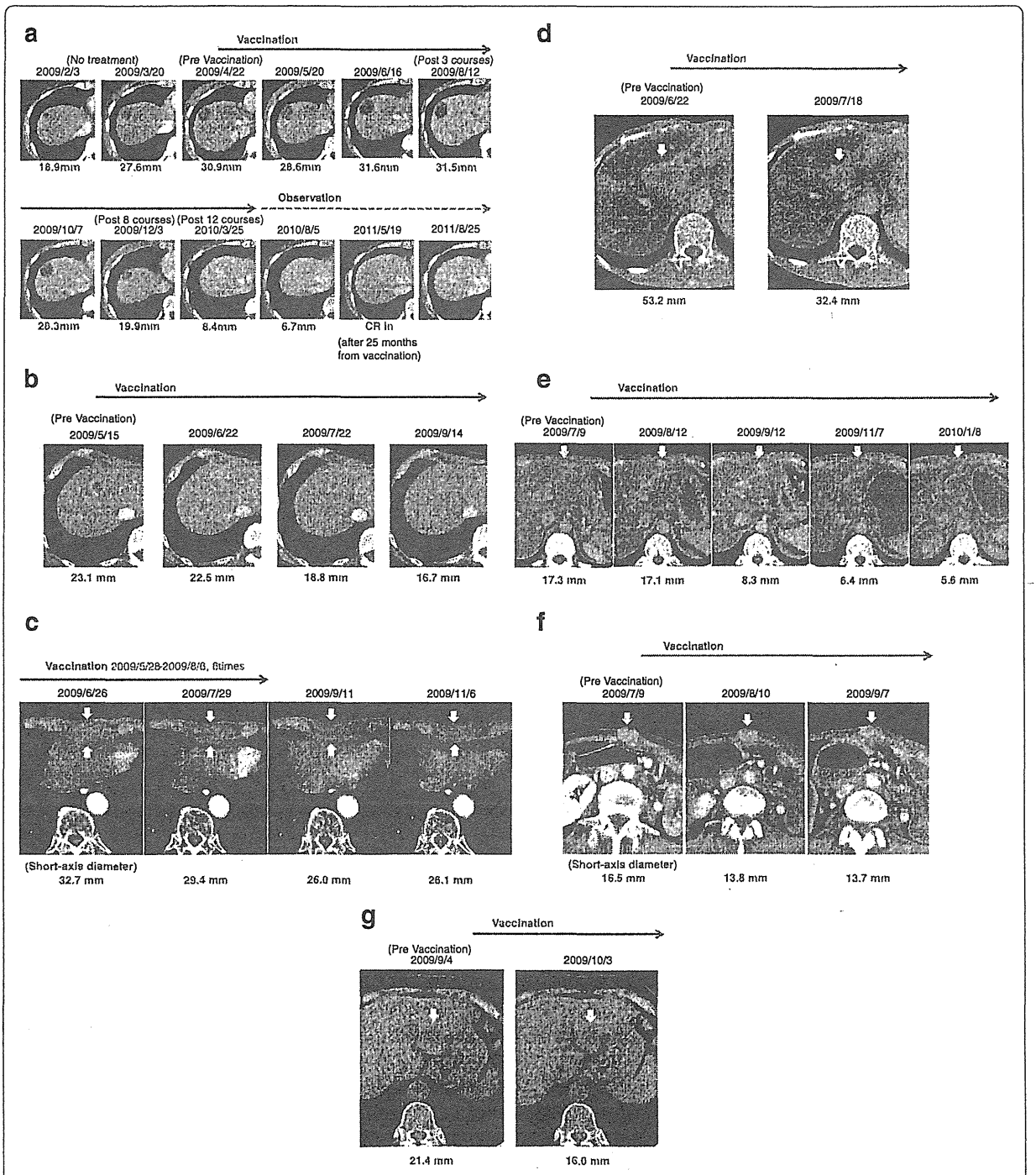

Figure 1 CT images of the target lesions showing objective tumor response. Representative CT images of the target lesions were shown, such as liver metastasis in case 9 **(a)**, liver metastasis in case 14 **(b)**, bone metastasis in xiphoid process in case 24 **(c)**, liver metastasis in case 22 **(d)**, liver metastasis in case 27 **(e)**, peritoneal metastasis in case 28 **(f)**, and liver metastasis in case 31 **(g)**. Arrow head indicates the target lesion.

CTL response and injection site reactions

We expected that the number of CTL responded to KIF20A peptide may be associated with the efficacy of the vaccine treatment. Therefore, CTL response was measured by ELISPOT assay in 29 patients who received the vaccination at least one cycle (Table 2). Among them, CTL responses in 24 patients were comparable in pre- and post-vaccination. In 16 patients out of 23 (70%), the

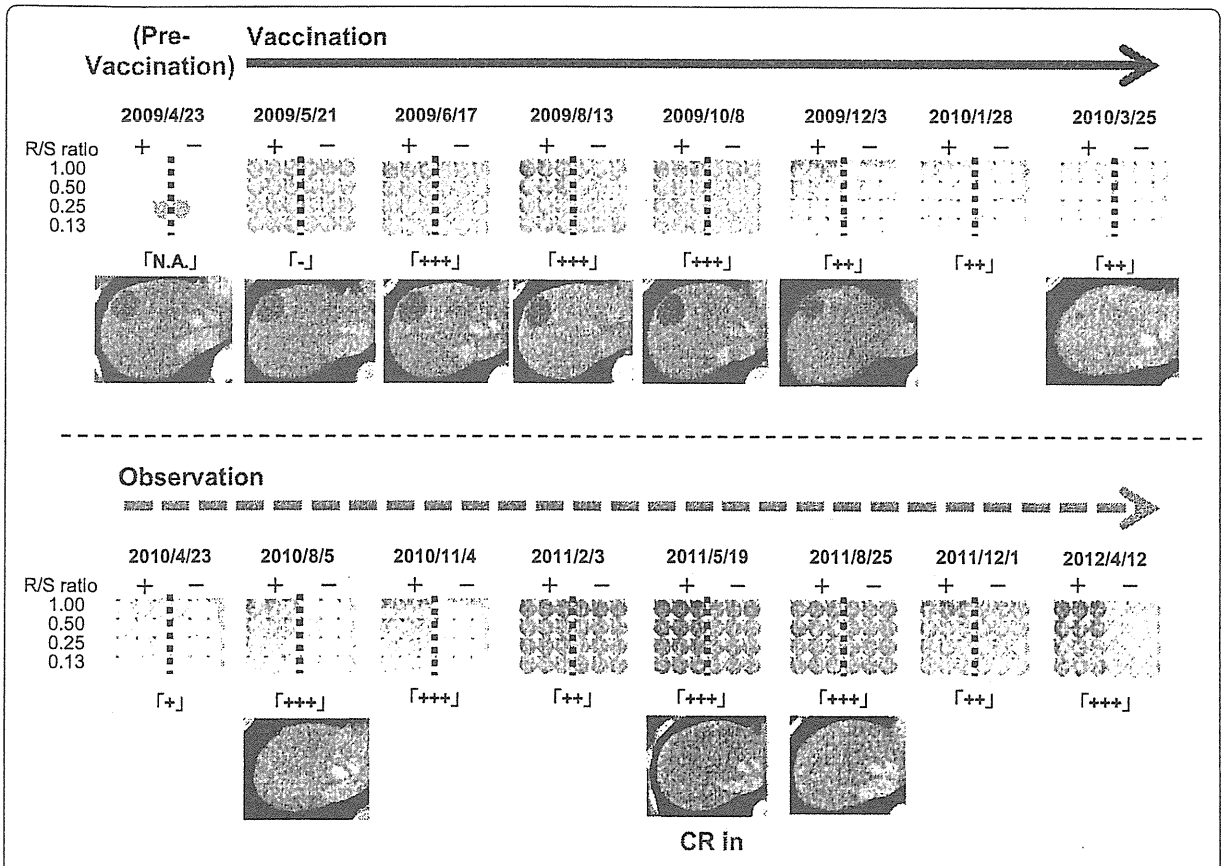

Figure 2 Peptide specific CTL response in case 9. Strong CTL responses specific to KIF20A-66 peptide were obtained at the time of 2 months after vaccination. The responses were kept strong positive during 2 years of the observation period. The number of the spots specific to peptide was calculated by subtracting the spot number in control wells from that in peptide-pulsed TISI cells.

intensity of CTL response was increased (Table 2), determined by the algorithm flow chart [25]. Of note, strong CTL response specific to KIF20A-66 was observed two months after the start of the vaccination in the patient of case 9, who achieved CR. This response kept strong for one year, and it was detectable even 2 years after the drug was discontinued (Figure 2). A flow cytometry assay demonstrated that the number of KIF20A-66 specific TCR in CD8-positive T cells was consistent with the grades classified according to our algorithm flow chart [25] (Figure 3a), compared to the negative control stain utilizing HIV-dextramer (Figure 3b). Also, injection site reactions were observed in 23 patients. MST of the patients with positive skin reaction was 182 days, while that of the patients with negative reaction was 42 days (Figure 5). These results demonstrate that CTL response and ISRs could be employed as biological markers to rapidly diagnose the efficacy of the peptide vaccination. Consistent with these results, when the 29 patients were classified into two groups in regard to the content ratio of lymphocyte (more than 16% (n = 23) vs. less than 16% (n = 6)), the group with higher number of lymphocyte yielded better prognosis with statistical significance (p = 0.0296). This result suggests that the number of lymphocyte is positively associated with the survival of the patients.

Discussion

Currently, there is no therapeutic strategy effective for the patients, whose pancreatic cancer is refractory to gemcitabine and TS-1. Combination therapy utilizing a couple of cytotoxic agents with gemcitabine has been investigated, but it has been failed to prove their clinical benefit so far [6-15]. We conducted an expression screening of proteins that were highly up-regulated in tumor cells, and not in normal cells, as a candidate of the target to develop novel anti-cancer drugs [20]. We successfully identified a member of kinesin super family protein 20A (KIF20A). Subsequently, we established an epitope peptide that were likely to be presented as an antigen in a HLA-A*2402- or HLA-A*0201-restricted manner [23,24,27]. In this report, we demonstrated that the KIF20A-derived peptide could improve the prognosis of the patients with advanced pancreatic cancer, suggesting that the KIF20A peptide vaccination is a promising approach as cancer immunotherapy.

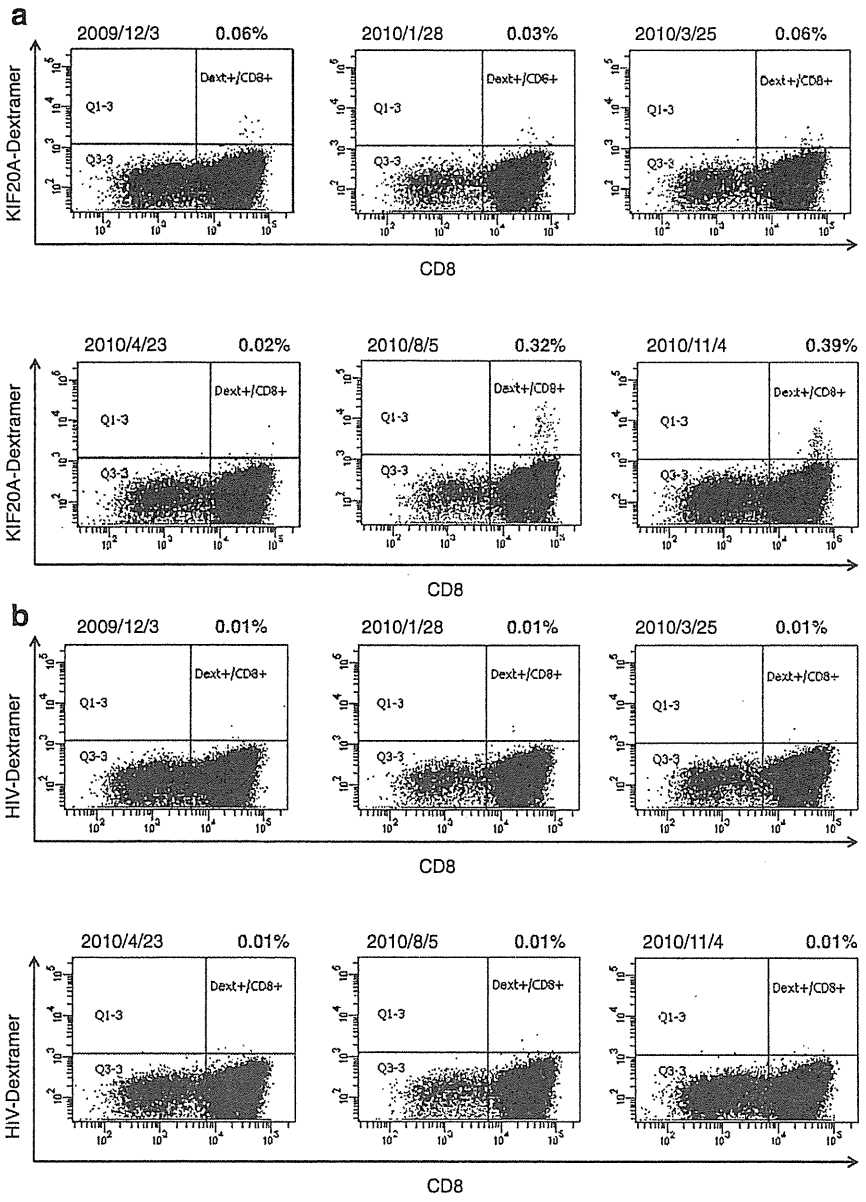

Figure 3 Flow cytometry analysis of KIF20A-66 specific TCR expression in $CD8^+$ cells in case 9. Cells were stained with either KIF20A-dextramer **(a)** or HIV-dextramer **(b)** after IVS as described in Methods section. The content rates of KIF20A-dextramer positive or HIV-dextramer positive cells (red dots) in $CD3^+$ $CD4^-$ $CD8^+$ cells are shown above panels in red.

In this clinical trial, we evaluated the safety and efficacy of KIF20A-66 peptide vaccine monotherapy for the patients with HLA-A*2402. This vaccine was well tolerated in the doses of 1.0 mg and 3.0 mg/body, although we do not exclude the possibility of two adverse events related to vaccination. The MST of 31 patients was 142 days in this phase I/II trial, indicating that vaccine treatment utilizing KIF20A-66 peptide provides survival benefit. Therefore, we concluded that the peptide vaccination improved overall survival period of the patients with advanced pancreatic cancer, who were resistant to chemotherapy. A placebo-controlled clinical trial should be required to further establish this peptide vaccine as a standard immunotherapy against pancreatic cancer.

We realized, during the course of peptide vaccination, that an induction of peptide-specific CTL and positive skin reaction were observed in the majority of the patients. We assure that these reactions could be employed as biomarkers of preferable clinical responses. Therefore, the number of CTL induced by peptide injection and the skin reaction at an injection site were analyzed. As we expected,

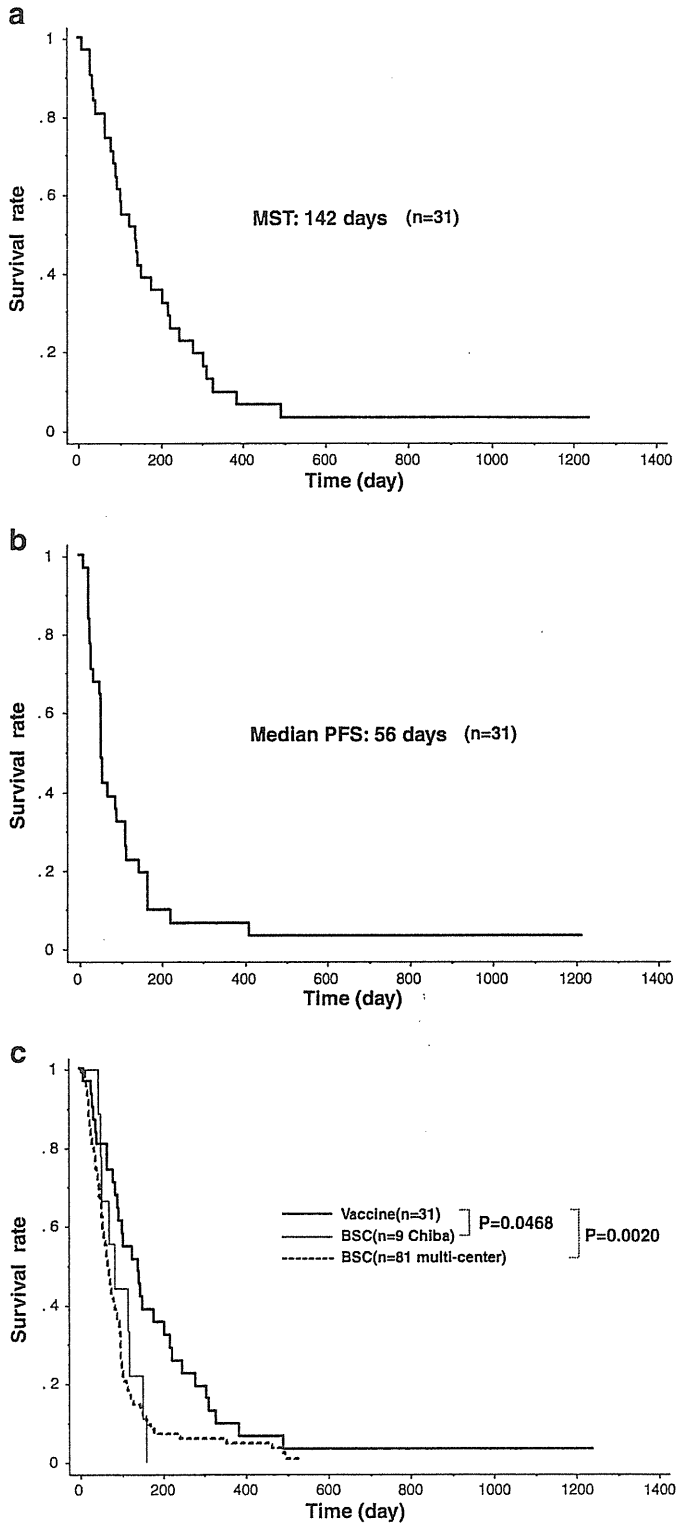

Figure 4 (See legend on next page.)

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Figure 4 Overall survival and progression free survival in phase I/II trial. Overall survival of the patients was shown in Kaplan-Meier plots (n = 31) **(a)**. MST of the patients with peptide vaccine was 142 days. PFS of the patients with peptide vaccine was 56 days **(b)**. In comparison with the control patients who were treated with best supportive care in Chiba Tokushukai Hospital (n = 9), overall survival of the patients with the KIF20A-peptide vaccination was fairly improved (p = 0.0468, MST: 142 vs. 83 days). In comparison with the BSC patients (n = 81), overall survival of the vaccinated patients in Chiba Tokushukai Hospital was significantly improved (p = 0.0020, MST: 142 vs. 63 days) **(c)**.

high level of CTL response specific to KIF20A-66 peptide resulted in CR in case 9. The liver metastasis continuously shrunk even after the peptide vaccination was discontinued (Figure 1a), and there was no sign of recurrence or metastasis at the time of 40 months after the vaccination started. Since biopsy of the tumor lesion was not performed during or after the vaccination, there is no information regarding the tumor infiltrating lymphocyte (TIL). This example indicates that positive correlation between tumor shrinkage and immunological reactions is of clinically interest (Figure 2). On the other hand, there is no CTL induction detected in Case No. 4, 27, and 28, while objective shrinkages were observed in these patients during the course of treatment. Since the number of CTL is usually low in peripheral blood, the CTL induction is measured after the stimulation utilizing respective peptide and IL-2 to yield higher detection limit. Despite this procedure, it is assumed that the intensity of CTL induction and the efficacy of vaccine treatment are not necessarily correlated according to a linear function, possibly due to the high expression levels of MHC Class I and/or targeted antigen KIF20A in tumor cells. Therefore, development of sensitive and reliable methods to detect CTL is required to evaluate the results of peptide vaccine treatment in the patients.

The US FDA published the guidance for the therapeutic cancer vaccine [28], describing that it is hard to expect clinical benefit of the vaccine treatment for the patients after multiple chemotherapy regimens due to very poor immune status. However, unlike many trials tested so far utilizing other peptide vaccines, this clinical study was quite successful. Our results clearly demonstrate that therapeutic cancer vaccination is still a promising approach for advanced pancreatic cancer after the failure of standard chemotherapy. In general, patients with relapsed or recurrent metastatic disease receive multiple treatments for their cancer. These therapies may be detrimental to the immune system, and adequate time is required for the cancer vaccine to elicit a detectable immune response. Given such therapeutic conditions affect the results of peptide vaccination, the use of adjuvant setting and the cohort study during an early treatment of the vaccine may be necessary to better understand a cause-and-result relationship of cancer immunotherapy. Furthermore, it is important to develop the peptides with the higher immunogenicity against active oncoproteins. Indeed, we have examined several peptides derived from a variety of cancer-testis antigens that have the oncogenic activity, including KIF20A, DEPDC1, MPHOSPH1, URLC10(LY6K),TTK, KOC1(IMP3),

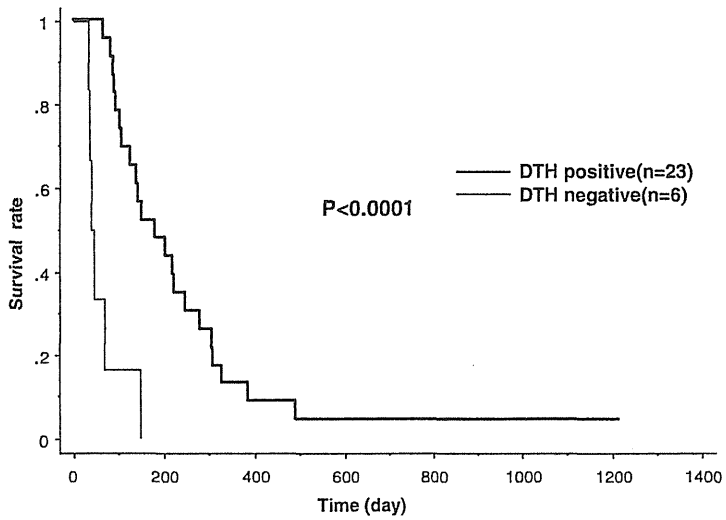

Figure 5 Correlation between OS and ISR. The local immune reactions at the site of injection were observed in 23 patients. MST of the patients who had injection site reaction was 182 days, while MST of the patients without such reaction (n = 6) was 42 days (p < 0.0001).

CDCA1, RNF43, and TOMM34 [16,17,20,22-25,27,29]. We propose that the trial of the cocktail vaccine of these high immunogenic peptides including KIF20A-66 will provide with better treatment and cure for cancer.

Abbreviations

HLA: Human leukocyte antigen; CR: Complete response; SD: Stable disease; PD: Progressive disease; MST: Median survival time; CTL: Cytotoxic T lymphocyte; 5-FU: 5-fluorouracil; ECOG: Eastern cooperative oncology group; RECIST: Response evaluation criteria in solid tumors; OS: Overall survival; PFS: Progression free survival; ISRs: Injection site reactions; IFA: Incomplete freund's adjuvant; ELISPOT: Enzyme-linked immunospot; PBMC: Peripheral blood mononuclear cell; IFN: Interferon; CIC: Cancer immunotherapy consortium; SAE: Severe adverse event; PR: Partial response; TIL: Tumor infiltrating lymphocyte.

Competing Interests

The authors declare that they have no financial competing interest.

Authors' contribution

SA designed, performed, and evaluated clinical study. KT participated as the main coordinator and investigator regarding the immunological data analysis and evaluation. KY, HM, and HY analyzed control studies in their hospitals. SA wrote the manuscript. All authors read and approved the final manuscript.

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References

1. Jemal A, Siegel R, Xu J, Ward E: **Cancer statistics, 2010.** *CA Cancer J Clin* 2010, 60:277–300.
2. Sener SF, Fremgen A, Menck HR, Winchester DP: **Pancreatic cancer: a report of treatment and survival trends for 100,313 patients diagnosed from 1985–1995, using the National Cancer Database.** *J Am Coll Surg* 1999, 189:1–7.
3. Bramhall SR, Allum WH, Jones AG, Allwood A, Cummins C, Neoptolemos JP: **Treatment and survival in 13,560 patients with pancreatic cancer, and incidence of the disease, in the West Midlands: an epidemiological study.** *Br J Surg* 1995, 82:111–115.
4. Rothenberg ML, Moore MJ, Cripps MC, Andersen JS, Portenoy RK, Burris HA 3rd, Green MR, Tarassoff PG, Brown TD, Casper ES, *et al*: **A phase II trial of gemcitabine in patients with 5-FU-refractory pancreas cancer.** *Ann Oncol* 1996, 7:347–353.
5. Burris HA 3rd, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, *et al*: **Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial.** *J Clin Oncol* 1997, 15:2403–2413.
6. Berlin JD, Catalano P, Thomas JP, Kugler JW, Haller DG, Benson AB 3rd: **Phase III study of gemcitabine in combination with fluorouracil versus gemcitabine alone in patients with advanced pancreatic carcinoma: Eastern Cooperative Oncology Group Trial E2297.** *J Clin Oncol* 2002, 20:3270–3275.
7. Rocha Lima CM, Green MR, Rotche R, Miller WH Jr, Jeffrey GM, Cisar LA, Morganti A, Orlando N, Gruia G, Miller LL: **Irinotecan plus gemcitabine results in no survival advantage compared with gemcitabine monotherapy in patients with locally advanced or metastatic pancreatic cancer despite increased tumor response rate.** *J Clin Oncol* 2004, 22:3776–3783.
8. Louvet C, Labianca R, Hammel P, Lledo G, Zampino MG, Andre T, Zaniboni A, Ducreux M, Aitini E, Taieb J, *et al*: **Gemcitabine in combination with oxaliplatin compared with gemcitabine alone in locally advanced or metastatic pancreatic cancer: results of a GERCOR and GISCAD phase III trial.** *J Clin Oncol* 2005, 23:3509–3516.
9. Abou-Alfa GK, Letourneau R, Harker G, Modiano M, Hurwitz H, Tchekmedyian NS, Feit K, Ackerman J, De Jager RL, Eckhardt SG, O'Reilly EM: **Randomized phase III study of exatecan and gemcitabine compared with gemcitabine alone in untreated advanced pancreatic cancer.** *J Clin Oncol* 2006, 24:4441–4447.
10. Van Cutsem E, van de Velde H, Karasek P, Oettle H, Vervenne WL, Szawlowski A, Schoffski P, Post S, Verslype C, Neumann H, *et al*: **Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer.** *J Clin Oncol* 2004, 22:1430–1438.
11. Bramhall SR, Schulz J, Nemunaitis J, Brown PD, Baillet M, Buckels JA: **A double-blind placebo-controlled, randomised study comparing gemcitabine and marimastat with gemcitabine and placebo as first line therapy in patients with advanced pancreatic cancer.** *Br J Cancer* 2002, 87:161–167.
12. Heinemann V, Quietzsch D, Gieseler F, Gonnermann M, Schonekas H, Rost A, Neuhaus H, Haag C, Clemens M, Heinrich B, *et al*: **Randomized phase III trial of gemcitabine plus cisplatin compared with gemcitabine alone in advanced pancreatic cancer.** *J Clin Oncol* 2006, 24:3946–3952.
13. Herrmann R, Bodoky G, Ruhstaller T, Glimelius B, Bajetta E, Schuller J, Saletti P, Bauer J, Figer A, Pestalozzi B, *et al*: **Gemcitabine plus capecitabine compared with gemcitabine alone in advanced pancreatic cancer: a randomized, multicenter, phase III trial of the Swiss Group for Clinical Cancer Research and the Central European Cooperative Oncology Group.** *J Clin Oncol* 2007, 25:2212–2217.
14. Boeck S, Hoehler T, Seipelt G, Mahlberg R, Wein A, Hochhaus A, Boeck HP, Schmid B, Kettner E, Stauch M, *et al*: **Capecitabine plus oxaliplatin (CapOx) versus capecitabine plus gemcitabine (CapGem) versus gemcitabine plus oxaliplatin (mGemOx): final results of a multicenter randomized phase II trial in advanced pancreatic cancer.** *Ann Oncol* 2008, 19:340–347.
15. Okusaka T, Funakoshi A, Furuse J, Boku N, Yamao K, Ohkawa S, Saito H: **A late phase II study of S-1 for metastatic pancreatic cancer.** *Cancer Chemother Pharmacol* 2008, 61:615–621.
16. Okuno K, Sugiura F, Hida JI, Tokoro T, Ishimaru E, Sukegawa Y, Ueda K: **Phase I clinical trial of a novel peptide vaccine in combination with UFT/LV for metastatic colorectal cancer.** *Exp Ther Med* 2011, 2:73–79.
17. Kono K, Mizukami Y, Daigo Y, Takano A, Masuda K, Yoshida K, Tsunoda T, Kawaguchi Y, Nakamura Y, Fujii H: **Vaccination with multiple peptides derived from novel cancer-testis antigens can induce specific T-cell responses and clinical responses in advanced esophageal cancer.** *Cancer Sci* 2009, 100:1502–1509.
18. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, *et al*: **Sipuleucel-T immunotherapy for castration-resistant prostate cancer.** *N Engl J Med* 2010, 363:411–422.
19. Schwartzentruber DJ, Lawson DH, Richards JM, Conry RM, Miller DM, Treisman J, Gailani F, Riley L, Conlon K, Pockaj B, *et al*: **gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma.** *N Engl J Med* 2011, 364:2119–2127.
20. Nakamura T, Furukawa Y, Nakagawa H, Tsunoda T, Ohigashi H, Murata K, Ishikawa O, Ohgaki K, Kashimura N, Miyamoto M, *et al*: **Genome-wide cDNA microarray analysis of gene expression profiles in pancreatic cancers using populations of tumor cells and normal ductal epithelial cells selected for purity by laser microdissection.** *Oncogene* 2004, 23:2385–2400.
21. Boon T: **Tumor antigens recognized by cytolytic T lymphocytes: present perspectives for specific immunotherapy.** *Int J Cancer* 1993, 54:177–180.
22. Suda T, Tsunoda T, Daigo Y, Nakamura Y, Tahara H: **Identification of human leukocyte antigen-A24-restricted epitope peptides derived from gene products upregulated in lung and esophageal cancers as novel targets for immunotherapy.** *Cancer Sci* 2007, 98:1803–1808.
23. Taniuchi K, Nakagawa H, Nakamura T, Eguchi H, Ohigashi H, Ishikawa O, Katagiri T, Nakamura Y: **Down-regulation of RAB6KIFL/KIF20A, a kinesin involved with membrane trafficking of discs large homologue 5, can attenuate growth of pancreatic cancer cell.** *Cancer Res* 2005, 65:105–112.
24. Osawa R, Tsunoda T, Yoshimura S, Watanabe T, Miyazawa M, Tani M, Takeda K, Nakagawa H, Nakamura Y, Yamaue H: **Identification of HLA-A24-restricted**

novel T Cell epitope peptides derived from P-cadherin and kinesin family member 20A. *J Biomed Biotechnol* 2012, 2012:848042.
25. Kono K, Iinuma H, Akutsu Y, Tanaka H, Hayashi N, Uchikado Y, Noguchi T, Fujii H, Okinaka K, Fukushima R, *et al*: **Multicenter, phase II clinical trial of cancer vaccination for advanced esophageal cancer with three peptides derived from novel cancer-testis antigens.** *J Transl Med* 2012, 10:141.
26. Janetzki S, Panageas KS, Ben-Porat L, Boyer J, Britten CM, Clay TM, Kalos M, Maecker HT, Romero P, Yuan J, *et al*: **Results and harmonization guidelines from two large-scale international Elispot proficiency panels conducted by the Cancer Vaccine Consortium (CVC/SVI).** *Cancer Immunol Immunother* 2008, 57:303–315.
27. Imai K, Hirata S, Irie A, Senju S, Ikuta Y, Yokomine K, Harao M, Inoue M, Tomita Y, Tsunoda T, *et al*: **Identification of HLA-A2-restricted CTL epitopes of a novel tumour-associated antigen, KIF20A, overexpressed in pancreatic cancer.** *Br J Cancer* 2011, 104:300–307.
28. Guidance for Industry: *Clinical Considerations for Therapeutic Cancer Vaccines.* U.S. Department of Health and Human Services; 2011. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/UCM278673.pdf.
29. Yasuda S, Tsuchiya I, Okada K, Tanaka A, Suzuki T, Sadahiro S, Takeda K, Yamamoto S, Nakui M: **Significant clinical response of advanced colon cancer to peptide vaccine therapy: a case report.** *Tokai J Exp Clin Med* 2012, 37:57–61.

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RESEARCH ARTICLE Open Access

Association between variations in the fat mass and obesity-associated gene and pancreatic cancer risk: a case–control study in Japan

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Abstract

Background: It is clear that genetic variations in the fat mass and obesity-associated (FTO) gene affect body mass index and the risk of obesity. Given the mounting evidence showing a positive association between obesity and pancreatic cancer, this study aimed to investigate the relation between variants in the FTO gene, obesity and pancreatic cancer risk.

Methods: We conducted a hospital-based case–control study in Japan to investigate whether genetic variations in the FTO gene were associated with pancreatic cancer risk. We genotyped rs9939609 in the FTO gene of 360 cases and 400 control subjects. An unconditional logistic model was used to estimate the odds ratio (OR) and 95% confidence interval (CI) for the association between rs9939609 and pancreatic cancer risk.

Results: The minor allele frequency of rs9939609 was 0.18 among control subjects. BMI was not associated with pancreatic cancer risk. Compared with individuals with the common homozygous TT genotype, those with the heterozygous TA genotype and the minor homozygous AA genotype had a 48% (OR=1.48; 95%CI: 1.07–2.04), and 66% increased risk (OR=1.66; 95%CI: 0.70–3.90), respectively, of pancreatic cancer after adjustment for sex, age, body mass index, cigarette smoking and history of diabetes. The per-allele OR was 1.41 (95%CI: 1.07–1.85). There were no significant interactions between TA/AA genotypes and body mass index.

Conclusions: Our findings indicate that rs9939609 in the FTO gene is associated with pancreatic cancer risk in Japanese subjects, possibly through a mechanism that is independent of obesity. Further investigation and replication of our results is required in other independent samples.

Keywords: The fat mass and obesity-associated gene, Pancreatic cancer, rs9939609, Case–control study

Background

In 2010, approximately 28,000 Japanese subjects died from pancreatic cancer, making it the fifth leading cause of cancer deaths in Japan [1]. Despite extensive research efforts, the etiology of pancreatic cancer remains poorly understood. Cigarette smoking and long-standing type II diabetes are two well-established risk factors, based on consistent findings from epidemiologic studies [2,3]. In addition, being overweight and obese have been implicated in the development of pancreatic cancer [4], with statistically significant, positive associations observed in large cohort studies conducted in Western countries [5-7], and corroborated in at least four meta-analyses [8-11] and three pooled analyses [12-14]. The positive association between body mass index (BMI) and pancreatic cancer, however, has not been clearly observed in Asian populations. To date, four cohort studies have examined the association between BMI and pancreatic cancer in Asians, but the results have been inconsistent and inconclusive [15-18].

Recently, genome-wide association (GWA) studies have identified at least 30 loci that affect BMI and the

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risk of obesity [19]. Among these loci, the fat mass and obesity-associated (FTO) gene, which was first identified in a GWA study of diabetes in 2007 [20], has the strongest influence on BMI and obesity. Rs9939609, located in the first intron of the FTO gene, was found to be associated with both BMI and type II diabetes in subsequent GWA studies in diverse populations [21-23]. The association of rs9939609 with various traits, including hip circumference, energy intake and total mortality has also been studied [24-26]. In addition, rs9939609 genotypes have been linked with the risk of prostate, breast and endometrial cancers [27-29]. The association between genetic variations in the FTO gene and the risk of pancreatic cancer, however, is not clear. Of the three studies that examined this association, only one case–control study, conducted at the MD Anderson Cancer Center in the United States, reported that the minor A allele of FTO, rs9939609, was associated with an increased risk of pancreatic cancer among overweight subjects [30]. Another two studies examined rs8050136 of the FTO gene, with one study reporting a positive association [31], and the other no association [32].

Given the mounting evidence showing a positive association between obesity and pancreatic cancer, we hypothesized that variants in the FTO gene may be associated with pancreatic cancer risk through effects on obesity or other mechanisms. In a search of the literature for obesity-related genetic variants, we found that FTO rs9939609 was the most widely studied single nucleotide polymorphism (SNP), and has been found to exert strong effects on BMI, as well as diabetes. Furthermore, it showed strong linkage disequilibrium with other SNPs in the FTO gene, such as rs8050135 and rs17817449 [22]. We therefore investigated the association between FTO rs9939609 and pancreatic cancer risk in a case–control study in Japan.

Methods

Study subjects

Our study is an ongoing hospital-based case–control study focusing on the role of genetic polymorphisms and gene-environment interaction in pancreatic cancer. For the present analysis, eligible cases were patients aged older than 20 years, who were newly diagnosed with pancreatic cancer in five hospitals located in central, north and Tokyo metropolitan areas from April 1, 2010 through May 15, 2012. The diagnosis of pancreatic cancer was based on imaging modalities or pathologic reports. The response rate among cases was 85% (441/516) as of July 1, 2012. Almost all of the cases were approached within a week after the diagnosis of pancreatic cancer, and very few cases died before they were invited to participate in our study. During the same period, we recruited control subjects with no diagnosis of cancer from inpatients and outpatients from the participating hospitals where the cases were enrolled, as well as relatives of inpatients, and individuals undergoing a medical checkup in one of the participating hospitals. Control subjects were eligible if they were more than 20 years old and had no prior cancer diagnoses. Recruitment of controls was accomplished by approaching eligible participants in the hospitals who satisfied the study requirements, and the response rate was 98% (525/534). Control subjects had a variety of diseases, such as anemia, gastric ulcer, and irritable bowel syndrome. Control subjects were matched with case patients according to sex and age (within 10-year categories). As a result, data from 360 case patients and 400 control subjects were included in the present analysis.

All subjects provided written, informed consent. This study was approved by the ethical board of Aichi Medical University (Nagakute, Japan), the Institutional Review Board (IRB) of Cancer Institute Hospital (Tokyo, Japan), the IRB of Kanagawa Cancer Center Hospital (Kanagawa, Japan), the IRB of Tokyo Metropolitan Komagome Hospital (Tokyo, Japan), and the IRB of Sapporo Medical University (Sapporo, Japan).

Data collection

Study subjects were asked to fill out a self-administered questionnaire including information on demographic characteristics, medical history, and lifestyle factors, such as cigarette smoking, alcohol consumption and dietary intake. For body weight, data on usual weight over the year prior to study entry as well as weight at age 20 were reported by the study participants. For current or former smokers, we collected detailed data on smoking exposure, including smoking status (never, former, or current smokers), average number of cigarettes smoked per day, age at starting and quitting, and duration of smoking. For subjects with type II diabetes, we recorded the age at diagnosis. In addition to the questionnaire survey, all consenting participants provided a 7-mL venous blood sample. Genomic DNA was extracted from peripheral lymphocytes at SRL Hachioji Laboratory and then stored at -30°C at the Department of Public Health, Aichi Medical University.

Genotyping assays

Genotyping was performed using the Taqman SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA) at the laboratory of Aichi Cancer Center Research Institute, Nagoya, Japan. Laboratory staff were blinded to case or control status. Four quality control samples were included in each assay, and the successful genotyping rate was 100%.

Statistical analysis

Case–control differences in selected demographic characteristics and risk factors were evaluated using t tests (for continuous variables) and Chi-square tests (for categorical variables). A chi-square test was used to test genotype frequencies in control subjects for Hardy-Weinberg equilibrium (HWE) by comparing observed genotype frequencies with those expected under HWE. A co-dominant genomic model was assumed for SNP effects. Unconditional logistic regression methods were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between rs9939609 genotypes and pancreatic cancer risk. Homozygous carriers of the common FTO rs9939609 T allele served as the reference group. All analyses were adjusted for age (continuous), sex (male or female), BMI (<20, 20–22.4, 22.5-24.9, ≥25.0), history of diabetes (yes or no), and cigarette smoking (current, former, never smokers). ORs were also estimated for the variant allele on the basis of a log-additive model. The interaction of genotype-BMI and genotype-history of diabetes with respect to pancreatic cancer risk was assessed using the likelihood ratio test. Because recent-onset diabetes may result from pancreatic cancer, we performed an analysis excluding cases who had onset of diabetes within 2 years prior to the diagnosis of pancreatic cancer.

All P-values were two-sided, with P<0.05 indicating statistical significance. All statistical analyses were performed using SAS 9.2 (SAS Institute, Inc., Cary, NC, USA).

Results

The distribution of genotypes among control subjects did not deviate from the Hardy-Weinberg equilibrium (P=0.94). The minor allele frequency (MAF) was 0.18 among control subjects. Table 1 summarizes the characteristics of cases and controls. Both groups had a similar distribution of sex and 10-year age groups. The mean age was 65.1±8.1 years for cases, and 58.5±9.1 years for controls. Cases were more likely to be current smokers and have a history of diabetes compared with controls. Current smokers had an approximately 2.9-fold increased risk of pancreatic cancer compared with nonsmokers, after adjustment for age, sex, BMI, and history of diabetes (OR=2.86; 95%CI: 1.79-4.57). Individuals who had a BMI of 30 or more had a 1.21-fold increased risk, but the association was not statistically significant. Similar results were obtained in an additional analysis in which BMI at age 20 was used (data not shown). Risk of pancreatic cancer was significantly increased among subjects reporting a history of diabetes (OR=2.94; 95%CI: 1.90-4.57). The significant, positive association remained after excluding pancreatic cancer cases with recent-onset diabetes (OR=1.92; 95%CI: 1.20–3.08). Among control subjects, the mean BMI was 22.7±3.1 for the TT genotype, 23.2±3.3 for the TA genotype, and 21.1±2.9 for the AA genotype.

Table 1 Association between variations in the fat mass and obesity-associated gene and pancreatic cancer risk: a case–control study in Japan

Characteristics	Case patients (N=360)	Control subjects (N=400)	OR (95% CI)
Age group			Matching factor
<50	12 (3.3)	19 (4.8)	
50-59	44 (12.2)	79 (19.8)	
60-69	141 (39.2)	170 (42.5)	
70-79	138 (38.3)	115 (28.8)	
≥80	25 (6.9)	17 (4.3)	
Sex			Matching factor
Female	215 (59.7)	226 (56.5)	
Male	145 (40.3)	174 (43.5)	
Body mass index (kg/m^2)			
<25	278 (77.2)	312 (78.0)	1.00
25.0-29.9	64 (17.8)	75 (18.7)	0.96 (0.65-1.43)
≥30	16 (4.4)	12 (3.0)	1.21 (0.53-2.77)
Unknown	2 (0.6)	1(0.3)	-
Smoking status			
Non-smokers	145 (40.2)	202 (50.5)	1.00
Former smokers	119 (33.1)	140 (35.0)	1.23 (0.82-1.85)
Current Smokers	96 (26.7)	58 (14.5)	2.86 (1.79-4.57)
History of diabetes			
No	269 (74.7)	362 (90.5)	1.00
Yes	87 (24.2)	35 (8.7)	2.94 (1.90-4.57)
Unknown	4 (1.1)	3 (0.8)	-

OR: odds ratio; CI: confidence interval.
OR was adjusted for sex, age, smoking status and history of diabetes.

Table 2 shows the association between variants in the FTO gene (rs9939609) and pancreatic cancer risk. Compared with individuals with the TT genotype, the multivariate adjusted OR for developing pancreatic cancer was 1.48 (95%CI: 1.07–2.04) among those with the TA

Table 2 Association between the FTO rs9939609 and pancreatic cancer risk

FTO rs9939609	Cases	Control subjects	Age- and sex-adjusted OR	Multivariable-adjusted OR
TT	213	271	1.00	1.00
TA	133	116	1.49 (1.09-2.03)	1.48 (1.07-2.04)
AA	14	13	1.49 (0.67-3.29)	1.66 (0.70-3.90)

OR: odds ratio ; CI: confidence interval.
Multivariable adjusted OR: adjusted for age, sex, body mass index, cigarette smoking and history of diabetes.

genotype, and 1.66 (95%CI: 0.70–3.90) among those with the AA genotype. Under the dominant model, the OR was 1.49 (95%CI: 1.09–2.05) among carriers of the TA/AA genotype. Under the log-additive model, each additional copy of minor allele A was associated with a 1.4-fold increased risk of pancreatic cancer (OR=1.41, 95% CI: 1.07–1.85).

We found no significant interaction between FTO rs9939609 and BMI (Table 3). Individuals with both a TA/AA genotype and a history of diabetes had a 3.7-fold increased risk of pancreatic cancer compared with those with a TT genotype and no history of diabetes (Table 4), but a test for the interaction was not statistically significant.

Discussion

This was a hospital-based case–control study in Japan to investigate whether genetic variations in the FTO gene were associated with pancreatic cancer risk. The main findings of our study were: 1) individuals with the FTO rs9939609 TA genotype had a significant 1.5-fold increased risk of pancreatic cancer compared with those with the TT genotype; and 2) a combination of the FTO rs9939609 TA/AA genotype and a history of diabetes significantly increased the pancreatic cancer risk, with an OR of 3.70 (95%CI: 1.59–8.63).

We found that obesity, defined as a BMI of 30 or more, was associated with 1.2-fold increased risk of pancreatic cancer, but this association was not statistically significant. In contrast to evidence of a positive association between obesity and pancreatic cancer in Western countries, available data on the role of obesity in pancreatic cancer in Japanese are inconclusive. There have been no prospective studies that have observed a clear, dose–response relation between baseline BMI and pancreatic cancer risk in the Japanese population [15,16]. Given that less than 5% of the subjects were obese in this study, it might be difficult to observe significant associations. The small percentage of obese people may be the main reason for the inconclusive results on BMI and pancreatic cancer in Asians, including Japanese [15-18]. In addition, differences in body fat distribution, in genetic predisposition to obesity and in lifestyle factors between Caucasians and Asians may contribute to the inconsistent results on BMI and pancreatic cancer risk in Asian populations [33,34].

Table 3 Joint associations of the FTO rs9939609 and BMI with respect to pancreatic cancer risk

Genotype	BMI	Cases/control subjects	Age- and sex-adjusted OR	Multivariable-adjusted OR
TT	<25	166/220	1.00	1.00
TA/AA	<25	112/92	1.69 (1.20-2.40)	1.68 (1.18-2.41)
TT	≥25	45/51	1.29 (0.81-2.04)	1.20 (0.75-1.94)
TA/AA	≥25	35/36	1.35 (0.81-2.25)	1.21 (0.71-2.07)
				P for interaction=0.29

Multivariable OR: adjusted for age, sex, cigarette smoking and history of diabetes.

Table 4 Joint associations of the FTO rs 9939609 and history of diabetes with respect to pancreatic cancer risk

Genotype	History of diabetes	Cases/ control subjects	Age- and sex-adjusted OR	Multivariable-adjusted OR
TT	No	163/243	1.00	1.00
TA/AA	No	106/119	1.38 (0.99-1.93)	1.41 (1.00-1.98)
TT	Yes	34/26	1.76 (1.01-3.07)	1.70 (0.96-3.00)
TA/AA	Yes	24/8	4.03 (1.75-9.24)	3.70 (1.59-8.63)
				P for interaction=0.28

Cases were excluded if the onset of diabetes was within 2 years prior to the diagnosis of pancreatic cancer.
Multivariable OR: adjusted for age, sex, body mass index, and cigarette smoking.

Because of the positive association between obesity and pancreatic cancer in Caucasians and the plausible mechanisms, several research groups have hypothesized that variants in obesity-related genes might be associated with pancreatic cancer risk. The association between rs9939609 in the FTO gene was reported in one previous hospital–based case–control study conducted at the MD Anderson Cancer Center, Texas, USA [30]. Of the 15 obesity- and diabetes-associated genotypes in the FTO gene, rs9939609 was found to be positively associated with pancreatic cancer risk in persons who were overweight, whereas no increased risk was observed in persons who had a BMI of less than 25 kg/m^2 [30]. In contrast, our study showed a significant, positive association between rs9939609 TA/AA genotype and pancreatic cancer risk in individuals with a BMI of less than 25 kg/m^2. We consider that the difference in minor allele frequency (MAF) may be the main reason, given the fact that the MAF was 18% in our study, much lower than the 38% in the MD Anderson Cancer Center case–control study. The possible differences in selection of cases and controls, patterns of linkage disequilibrium and effects of gene-gene interactions may also account for the inconsistent findings. In addition to rs9939609, rs8050136 in the FTO gene was found to be associated with pancreatic cancer risk in individuals of European ancestry [31]; however, no association was noted in another case–control study [32].

In our study, FTO rs9939609 genotypes were associated with pancreatic cancer risk. However, the mean BMI did not differ among rs9939609 genotypes for control subjects, and no significant interaction was observed between rs9939609 TA/AA genotypes and BMI with

respect to pancreatic cancer risk. It is possible that the positive association observed between rs9939609 genotypes and pancreatic cancer risk may be driven by a mechanism other than adiposity. Diabetes, a well-established risk factor for pancreatic cancer, is a possible candidate. There is evidence suggesting that Asian people are more susceptible to insulin resistance at a lesser degree of obesity than Caucasians [33,34]. Besides its close association with adiposity, FTO has been shown to be associated with susceptibility to type II diabetes [21,22]. We found that individuals with a TA/AA genotype and a history of diabetes were at a 3.7-fold increased risk of pancreatic cancer. However, a test for the interaction was not statistically significant. Another possibility is that FTO is just a proxy of as yet unidentified causal variants, and it is those variants that exert their effects on rs9939609 and influence pancreatic cancer risk. Given that the function of the FTO gene is largely unknown, further studies are needed to comprehensively evaluate multiple SNPs in the FTO gene and elucidate the mechanisms by which FTO rs9939609 influences pancreatic cancer risk.

Our study has several limitations. First, it is well-known that two significant issues, namely selection bias and recall bias, plague case–control studies. Our results might have been biased if hospital controls did not represent the same population from which the cases were derived. However, the allele frequencies observed among control subjects in our study were similar to those reported in the studies of Asian populations [22]. In particular, the MAF of FTO rs9939609 was 18% in our control subjects, which is very close to that reported from a sample of 100 Japanese included in the HapMap project. Moreover, the risk estimates for current smokers and individuals with a history of diabetes were comparable to those estimated from cohort or population-based case–control studies [2,3], providing indirect evidence that selection bias might not be a serious concern in our study. Second, as for recall bias, while the analysis of the association between pancreatic cancer and BMI based on self-reported weight and height might be affected by recall bias, the association with the obesity-related genotype was not. Third, although our study included a relatively large sample size compared with previous studies conducted in Japan, the sample size may not have been large enough to detect significant gene-environment interactions in subgroups. Finally, it is possible that the results could represent a chance association and therefore replication in other independent samples is required. Despite these limitations, there are several advantages of the hospital-based design adopted in our study, including rapid case ascertainment, a high response rate from both cases and controls, and high quality genotyping.

Conclusion

Our findings indicate that rs9939609 in the FTO gene is associated with pancreatic cancer risk in Japanese subjects, possibly through a mechanism that is independent of obesity. Because of the limited statistical power, our results need replication in other independent samples. The fast-increasing prevalence of overweight/obesity and type II diabetes in Asians provides a good opportunity to further address this association and its underlying mechanisms.

Competing interests

The authors declare no conflict of interest.

Authors' contribution

SK supervised the study, SK, YL, KY designed the study, YL drafted the manuscript and conducted the statistical analysis. JU and KM performed genotyping and SNP data analysis. HI, MU, NE, HN, MM participated in data collection. All authors read and approved the final manuscript.

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References

1. Statistics and Information Department, Minister's Secretariat: *Vital Statistics of Japan.* Tokyo: Minister of Health and Welfare; 2010.
2. Iodice S, Gandini S, Maisonneuve P, Lowenfels AB: **Tobacco and the risk of pancreatic cancer: a review and meta-analysis.** *Langenbecks Arch Surg* 2008, **393**:535–545.
3. Ben Q, Xu M, Ning X, Liu J, Hong S, Huang W, Zhang H, Li Z: **Diabetes mellitus and risk of pancreatic cancer: A meta-analysis of cohort studies.** *Eur J Cancer* 2011, **47**:1928–1937.
4. Calle EE, Kaaks R: **Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms.** *Nat Rev Cancer* 2004, **4**:579–591.

5. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ: **Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults.** *N Engl J Med* 2003, **348**:1625–1638.
6. Rapp K, Schroeder J, Klenk J, Stoehr S, Ulmer H, Concin H, Diem G, Oberaigner W, Weiland SK: **Obesity and incidence of cancer: a large cohort study of over 145,000 adults in Austria.** *Br J Cancer* 2005, **93**:1062–1067.
7. Michaud DS, Giovannucci E, Willett WC, Colditz GA, Stampfer MJ, Fuchs CS: **Physical activity, obesity, height, and the risk of pancreatic cancer.** *JAMA* 2001, **286**:921–929.
8. Larsson SC, Orsini N, Wolk A: **Body mass index and pancreatic cancer risk: A meta-analysis of prospective studies.** *Int J Cancer* 2007, **120**:1993–1998.
9. Aune D, Greenwood DC, Chan DS, Vieira R, Vieira AR, Navarro Rosenblatt DA, Cade JE, Burley VJ, Norat T: **Body mass index, abdominal fatness and pancreatic cancer risk: a systematic review and non-linear dose–response meta-analysis of prospective studies.** *Ann Oncol* 2012, **23**:843–852.
10. Berrington de Gonzalez A, Sweetland S, Spencer E: **A meta-analysis of obesity and the risk of pancreatic cancer.** *Br J Cancer* 2003, **89**:519–523.
11. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M: **Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies.** *Lancet* 2008, **371**:569–578.
12. Arslan AA, Helzlsouer KJ, Kooperberg C, Shu XO, Steplowski E, Bueno-de -Mesquita HB, Fuchs CS, Gross MD, Jacobs EJ, Lacroix AZ, Petersen GM, Stolzenberg-Solomon RZ, Zheng W, Albanes D, Amundadottir L, Bamlet WR, Barricarte A, Bingham SA, Boeing H, Boutron-Ruault MC, Buring JE, Chanock SJ, Clipp S, Gaziano JM, Giovannucci EL, Hankinson SE, Hartge P, Hoover RN, Hunter DJ, Hutchinson A, *et al*: **Anthropometric measures, body mass index, and pancreatic cancer: a pooled analysis from the Pancreatic Cancer Cohort Consortium (PanScan).** *Arch Intern Med* 2010, **170**:791–802.
13. Jiao L, Berrington de Gonzalez A, Hartge P, Pfeiffer RM, Park Y, Freedman DM, Gail MH, Alavanja MC, Albanes D, Beane Freeman LE, Chow WH, Huang WY, Hayes RB, Hoppin JA, Ji BT, Leitzmann MF, Linet MS, Meinhold CL, Schairer C, Schatzkin A, Virtamo J, Weinstein SJ, Zheng W, Stolzenberg-Solomon RZ: **Body mass index, effect modifiers, and risk of pancreatic cancer: a pooled study of seven prospective cohorts.** *Cancer Causes Control* 2010, **21**:1305–1314.
14. Genkinger JM, Spiegelman D, Anderson KE, Bernstein L, van den Brandt PA, Calle EE, English DR, Folsom AR, Freudenheim JL, Fuchs CS, Giles GG, Giovannucci E, Horn-Ross PL, Larsson SC, Leitzmann M, Männistö S, Marshall JR, Miller AB, Patel AV, Rohan TE, Stolzenberg-Solomon RZ, Verhage BA, Virtamo J, Willcox BJ, Wolk A, Ziegler RG, Smith-Warner SA: **A pooled analysis of 14 cohort studies of anthropometric factors and pancreatic cancer risk.** *Int J Cancer* 2011, **129**:1708–1717.
15. Luo J, Iwasaki M, Inoue M, Sasazuki S, Otani T, Ye W, Tsugane S, JPHC Study Group: **Body mass index, physical activity and the risk of pancreatic cancer in relation to smoking status and history of diabetes: a large-scale population-based cohort study in Japan--the JPHC stud.** *Cancer Causes Control* 2007, **18**:603–612.
16. Lin Y, Kikuchi S, Tamakoshi A, Yagyu K, Obata Y, Inaba Y, Kurosawa M, Kawamura T, Motohashi Y, Ishibashi T, JACC Study Group: **Obesity, physical activity and the risk of pancreatic cancer in a large Japanese cohort.** *Int J Cancer* 2007, **120**:2665–2671.
17. Jee SH, Yun JE, Park EJ, Cho ER, Park IS, Sull JW, Ohrr H, Samet JM: **Body mass index and cancer risk in Korean men and women.** *Int J Cancer* 2008, **123**:1892–1896.
18. Kuriyama S, Tsubono Y, Hozawa A, Shimazu T, Suzuki Y, Koizumi Y, Suzuki Y, Ohmori K, Nishino Y, Tsuji I: **Obesity and risk of cancer in Japan.** *Int J Cancer* 2005, **113**:148–157.
19. McCarthy MI: **Genomics, type 2 diabetes, and obesity.** *N Engl J Med* 2010, **363**:2339–2350.
20. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, *et al*: **A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity.** *Science* 2007, **316**:889–894.
21. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, *et al*: **A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants.** *Science* 2007, **316**:1341–1345.
22. Li H, Kilpeläinen TO, Liu C, Zhu J, Liu Y, Hu C, Yang Z, Zhang W, Bao W, Cha S, Wu Y, Yang T, Sekine A, Choi BY, Yajnik CS, Zhou D, Takeuchi F, Yamamoto K, Chan JC, Mani KR, Been LF, Imamura M, Nakashima E, Lee N, Fujisawa T, Karasawa S, Wen W, Joglekar CV, Lu W, Chang Y, *et al*: **Association of genetic variation in FTO with risk of obesity and type 2 diabetes with data from 96,551 East and South Asians.** *Diabetologia* 2012, **55**:981–995.
23. Wen W, Cho YS, Zheng W, Dorajoo R, Kato N, Qi L, Chen CH, Delahanty RJ, Okada Y, Tabara Y, Gu D, Zhu D, Haiman CA, Mo Z, Gao YT, Saw SM, Go MJ, Takeuchi F, Chang LC, Kokubo Y, Liang J, Hao M, Le Marchand L, Zhang Y, Hu Y, Wong TY, Long J, Han BG, Kubo M, Yamamoto K, *et al*: **Meta-analysis identifies common variants associated with body mass index in east Asians.** *Nat Genet* 2012, **44**:307–311.
24. Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, Najjar S, Nagaraja R, Orrú M, Usala G, Dei M, Lai S, Maschio A, Busonero F, Mulas A, Ehret GB, Fink AA, Weder AB, Cooper RS, Galan P, Chakravarti A, Schlessinger D, Cao A, Lakatta E, Abecasis GR: **Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits.** *PLoS Genet* 2007, **3**:e115.
25. Speakman JR, Rance KA, Johnstone AM: **Polymorphisms of the FTO gene are associated with variation in energy intake, but not energy expenditure.** *Obesity* 2008, **16**:1961–1965.
26. Zimmermann E, Kring SI, Berentzen TL, Holst C, Pers TH, Hansen T, Pedersen O, Sørensen TI, Jess T: **Fatness-associated FTO gene variant increases mortality independent of fatness–in cohorts of Danish men.** *PLoS One* 2009, **4**:e4428.
27. Lewis SJ, Murad A, Chen L, Davey Smith G, Donovan J, Palmer T, Hamdy F, Neal D, Lane JA, Davis M, Cox A: **Associations between an obesity related genetic variant (FTO rs9939609) and prostate cancer risk.** *PLoS One* 2010, **5**:e13485.
28. Kaklamani V, Yi N, Sadim M, Siziopikou K, Zhang K, Xu Y, Tofilon S, Agarwal S, Pasche B, Mantzoros C: **The role of the fat mass and obesity associated gene (FTO) in breast cancer risk.** *BMC Med Genet* 2011, **12**:52.
29. Delahanty RJ, Beeghly-Fadiel A, Xiang YB, Long J, Cai Q, Wen W, Xu WH, Cai H, He J, Gao YT, Zheng W, Shu XO: **Association of obesity-related genetic variants with endometrial cancer risk: a report from the Shanghai Endometrial Cancer Genetics Study.** *Am J Epidemiol* 2011, **174**:1115–1126.
30. Tang H, Dong X, Hassan M, Abbruzzese JL, Li D: **Body mass index and obesity- and diabetes-associated genotypes and risk for pancreatic cancer.** *Cancer Epidemiol Biomarkers Prev* 2011, **20**:779–792.
31. Pierce BL, Austin MA, Ahsan H: **Association study of type 2 diabetes genetic susceptibility variants and risk of pancreatic cancer: an analysis of PanScan-I data.** *Cancer Causes Control* 2011, **22**:877–883.
32. Prizment AE, Gross M, Rasmussen-Torvik L, Peacock JM, Anderson KE: **Genes related to diabetes may be associated with pancreatic cancer in a population-based case–control study in Minnesota.** *Pancreas* 2012, **41**:50–53.
33. Wulan SN, Westerterp KR, Plasqui G: **Ethnic differences in body composition and the associated metabolic profile: a comparative study between Asians and Caucasians.** *Maturitas* 2010, **65**:315–319.
34. Lee JW, Brancati FL, Yeh HC: **Trends in the prevalence of type 2 diabetes in Asians versus whites: results from the United States National Health Interview Survey, 1997–2008.** *Diabetes Care* 2011, **34**:353–357.

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BRIEF ARTICLE

Ultrasound-guided *vs* endoscopic ultrasound-guided fine-needle aspiration for pancreatic cancer diagnosis

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Abstract

AIM: To clarify the effectiveness and safety of endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) for the diagnosis of pancreatic cancer (PC).

METHODS: Patients who were diagnosed with unresectable, locally advanced or metastatic PC between February 2006 and September 2011 were selected for this retrospective study. FNA biopsy for pancreatic tumors had been performed percutaneously under extracorporeal ultrasound guidance until October 2009; then, beginning in November 2009, EUS-FNA has been performed. We reviewed the complete medical records of all patients who met the selection criteria for the following data: sex, age, location and size of the targeted tumor, histological and/or cytological findings, details of puncture procedures, time from day of puncture until day of definitive diagnosis, and details of severe adverse events.

RESULTS: Of the 121 patients who met the selection criteria, 46 had a percutaneous biopsy (Group A) and 75 had an EUS-FNA biopsy (Group B). Adequate cytological specimens were obtained in 42 Group A patients (91.3%) and all 75 Group B patients ($P = 0.0192$), and histological specimens were obtained in 41 Group A patients (89.1%) and 65 Group B patients (86.7%). Diagnosis of malignancy by cytology was positive in 33 Group A patients (78.6%) and 72 Group B patients (94.6%) ($P = 0.0079$). Malignancy by both cytology and pathology was found in 43 Group A (93.5%) and 73 Group B (97.3%) patients. The mean period from the puncture until the cytological diagnosis in Group B was 1.7 d, which was significantly shorter than that in Group A (4.1 d) ($P < 0.0001$). Severe adverse events were experienced in two Group A patients (4.3%) and in one Group B patient (1.3%).

CONCLUSION: EUS-FNA, as well as percutaneous needle aspiration, is an effective modality to obtain cytopathological confirmation in patients with advanced PC.

Key words: Endoscopic ultrasound-guided fine needle aspiration; Percutaneous needle aspiration; Pancreatic cancer

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INTRODUCTION

Pancreatic cancer (PC) is currently the fifth leading cause of cancer-related mortality in Japan. Although complete surgical removal of the tumor is the only chance of cure, almost all PC patients are initially diagnosed as having advanced unresectable disease despite recent improvements in diagnostic techniques. In recent decades, techniques were developed to obtain proof of cancer from the primary tumor in PC patients. Pancreatic juice cytology *via* endoscopic retrograde pancreatography was initially developed to meet this challenge; however, in practical settings the positive rate for cancer cells has remained low, indicating the presence of false-negative results[1,2]. Ultrasonography-guided fine-needle aspiration (US-FNA) biopsy or computed tomography (CT)-guided FNA biopsy appears to provide a more definitive diagnosis of PC[3,4]. US-FNA is convenient but its usefulness is limited for masses in the pancreatic tail. In contrast, CT-guided FNA is the biopsy procedure of choice to assess pancreatic lesions. However, this technique is time-consuming and is limited by a substantial false-negative rate of approximately 20%[5]. In addition, there have been concerns about percutaneous cancer seeding[6,7]. Recently, endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) has been developed as a more feasible method to obtain definitive specimens for cytological and/or histological examinations for diagnosis of PC[8-12]. Three years ago, we began to perform EUS-FNA although until that time US-FNA was the standard technique at our institute.

In the current study, we retrospectively examined the diagnostic ability of EUS-FNA for PC compared with US-FNA.

MATERIALS AND METHODS

Patients

The inclusion criteria were: (1) the patient underwent US-FNA between February 2006 and October 2009 or EUS-FNA between November 2009 and September 2011 at the Cancer Institute Hospital, Tokyo, Japan for suspected PC; and (2) the patient was subsequently diagnosed as having clinical stage III or IV PC. Unresectable PC, which was indicated by International Union Against Cancer clinical stage III (locally advanced disease: T4N0-1 and M0) or IV (metastatic disease: T1-4N0-1 and M1), was diagnosed by CT.

The exclusion criteria were: (1) a contraindication for EUS (esophageal stenosis, duodenal stenosis, ileus, or perforation of the digestive tract); and (2) a contraindication for EUS-FNA and US-FNA (severe cardiovascular disease or respiratory disease, poor performance status, difficulty in visualization of the target, bleeding tendency, or impossibility of ensuring the puncture route).

Patients who met the selection criteria were identified from the database in our division, which was updated daily.

US- and EUS-FNA procedures

A short admission, usually for one or two nights, was mandatory according to the protocol for FNA biopsy of a suspected pancreatic tumor in our division. FNA biopsy for pancreatic tumors had been performed percutaneously under extracorporeal ultrasound guidance (US-FNA) until October 2009; then, beginning in November 2009, FNA biopsies have been performed under EUS guidance (EUS-FNA). In general, FNA examinations were performed and managed by Ishii H until October 2009 and by Matsuyama M since November 2009. Written informed consent was obtained from each patient before the examination.

US-FNA was performed using SSA-550A (Toshiba, Tokyo, Japan) as the ultrasound device and SONOPSY C1 21G (Hakko, Osaka, Japan) as the ultrasound-guided biopsy needle. After systemic premedication and percutaneous local anesthesia, FNA was performed 1-3 times repeatedly until adequate material was obtained. Pathological examination of the obtained materials and cytological examination of the needle-washing water were done. There was no on-site cytotechnologist during the performance of US-FNA.

EUS-FNA was performed using EU-ME1 and UCT240-AL5 (Olympus, Tokyo, Japan) as the EUS system and the Echo-Tip ULTRA 22G (Wilson-Cook, Bloomington, IN, United States) as the ultrasound-guided biopsy needle. After systemic premedication and pharyngeal local anesthesia, FNA was performed endoscopically *via* the stomach or duodenum. Aspiration puncture was repeated until an on-site cytology screener confirmed that adequate materials had been obtained.

After the examination, patients stayed in the hospital overnight and were discharged the following morning if no problems were revealed by physical examination, complete blood count tests and biochemistry tests that included serum amylase level. Three to 7 d later, the patients came to the outpatient clinic for an explanation of the results of the biopsy and examination for late adverse events, and were then able to start chemotherapy.

The final diagnosis was based on pathology results or clinical follow-up of > 6 mo.

Statistical analysis

We reviewed the complete medical records of all patients who met the selection criteria for the following data: sex, age, location and size of the targeted tumor, histological and/or cytological findings of the obtained specimens, details of puncture procedures, time from day of puncture until the day of definitive diagnosis, and details of severe adverse events, if any. The tumor status (location and size) was determined by dynamic CT before puncture. Frequency analysis was performed with Fisher's exact test for 2×2 tables, χ^2 test for 3×2 tables, and Mann-Whitney test. All analysis were performed using the statistical software SPSS 11.0J for Windows. Statistical significance was defined as a two-sided P value ≤ 0.05.

Table 1 Characteristics of patients and comparison of results of percutaneous biopsy with those of endoscopic ultrasound-guided fine-needle aspiration

	Percutaneous biopsy Group A	EUS-FNA Group B	*P* value
Patients	46	75	
Site of puncture			
Pancreas	46	74	> 0.9999
Head/body/tail	12/32/2	34/31/9	0.0114
Sex (male/female)	25/21	39/36	> 0.8525
Age, yr			> 0.8466
≥ 65	28	48	
< 65	18	27	
Tumor diameter, mm (range)	44.8 (18-111)	25.5 (7-70)	
≥ 40	30	25	0.0007
< 40	16	50	
Passes (range)	2.26 (1-4)	2.85 (2-5)	< 0.0001
Adequate specimens obtained[1] *n* (%)			
Cytology	42 (91.3)	75 (100)	0.0192
Histology	41 (89.1)	65 (86.7)	0.7812
Positivity for cancer *n* (%)			
Cytology	33 (78.6)	72 (94.6)	0.0079
Histology	33 (80.5)	51 (78.4)	> 0.9999
Total *n* (%)	43 (93.5)	73 (97.3)	0.3672
Complications *n* (%)	2 (4.3)	1 (1.3)	> 0.5567
	Fever[1]	Peritonitis[1]	
	Bleeding[1]		
Time from puncture to definitive diagnosis			
Cytology, d (range)	4.05 (0-8)	1.65 (0-5)	< 0.0001
Histology, d (range)	3.95 (2-7)	3.18 (2-10)	0.7066

[1]An on-site pathologist was available for endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) but not for ultrasonography-guided-FNA.

RESULTS

US-FNA was performed in 48 patients from February 2006 until October 2009. Two cases (renal cell carcinoma and malignant lymphoma) were excluded from the analysis of US-FNA because the patients did not have primary PC. EUS-FNA was attempted in 125 cases and was successfully performed in 123 cases from November 2009 until September 2011. Among these, 48 patients did not meet the selection criteria (lymph node metastasis, 34 cases; other pancreatic tumor, 10 cases; other abdominal tumor, three cases, and mediastinum tumor, one case). EUS-FNA could not be performed in two patients because of difficulty of visualization due to total gastrectomy in one case, and impossibility of ensuring the puncture route in the other. Thus, 46 patients who underwent US-FNA (Group A) and 75 who underwent EUS-FNA (Group B) were eligible for analysis.

Table 1 shows the characteristics of the study subjects. The distribution of the target tumor in the pancreas differed significantly between the two groups, with the tumor location more frequent in the pancreatic head/tail than in the pancreatic body in Group B. The maximum diameter of the target tumor ranged from 18 to 111 mm (median, 44.8 mm) in Group A and from 7 to 70 mm (median, 25.5 mm) in Group B. A significantly larger number of target tumors were < 40 mm in Group B than in Group A ($P = 0.0007$).

Table 1 shows a comparison of the results of percutaneous biopsy with those of EUS-FNA. Adequate cytological and histological specimens were obtained in 42 (91.3%) and 41 (89.1%) Group A patients ($n = 46$), respectively, and in 75 (100%) and 65 (86.7%) Group B patients ($n = 75$).

Results of cytology indicated the presence of cancer cells in 33 Group A patients (78.6%) and in 72 Group B patients (94.6%). Histological studies showed cancer tissue in 33 (80.5%) and 51 (78.4%) patients in Group A and Group B, respectively. In total, a cancer diagnosis was made in 43 Group A (93.5%) and 73 Group B (97.3%) patients by cytology and/or histology. These 116 patients were diagnosed with pancreatic adenocarcinoma by cytology/histology as well as by imaging and their subsequent clinical course. The final diagnosis of PC in the remaining five patients for whom there was no cytological or histological proof was confirmed by the clinical course until April 2012. The positive cytology/histology rate did not differ between the two groups.

Total puncture procedures per patient varied from one to five, with a median of 3. The frequency of multiple punctures, that is, > 2, was significantly higher in Group B than in Group A. Time from the day of puncture until the day of the final cytological diagnosis varied from 0 to 8 d (median, 4.1 d) in Group A and from 0 to 5 d (median, 1.7 d) in Group B. The period was significantly shorter in Group B than in Group A. The time from the day of puncture until the day of the final histological diagnosis varied from 2 to 7 d (median, 4.0 d) in Group A and 2 to 10 d (median, 3.2 d) in Group B, with no significant difference between the two groups.

Severe adverse events occurred in two Group A patients (4.3%) and in one Group B patient (1.3%). In Group A, one patient developed a high fever, which required hospitalization but resolved with only symptomatic treatment. The other Group A patient experienced upper gastrointestinal bleeding, which was confirmed by endoscopy to be related to the needle biopsy. This patient was treated by blood transfusion and antiulcer medication and was hospitalized for 1 wk without surgical intervention. The adverse event in Group B was an abdominal abscess that required surgical drainage. The patient experienced continuous abdominal pain one night after EUS-FNA, and dynamic CT demonstrated an abscess in front of the pancreatic body tumor, which was clearly related to the EUS-FNA puncture. Fortunately, she recovered after surgery and antibiotic therapy and could receive chemotherapy thereafter. There was no cancer seeding event up to 6 mo from the time of puncture in any patient in either group.

DISCUSSION

The aim of the current study was to investigate the results of two different approaches to obtain pancreatic biopsy specimens, which are a percutaneous approach and EUS-FNA, because this issue has seldom been ad-