Table 2. Lymphatic drainage pathways in the patients injected with ICG into the anterior surface of the uncinate process of the pancreas

No.	Anterior pancreaticoduodenal arcade	Behind the SMV	Left side of the SMA (J1, J2, J3 regions)	Longitudinally upward between the SMV and SMA	Origin of the middle colonic artery toward the transverse colon	PA region	Hepatoduodenal ligament
1	○	○	○	○	○	○	×
2	○	○	○	○	○	○	×
3	○	○	○	×	○	○	×
4	○	○	×	○	○	○	×
5	○	○	○	×	○	○	×
6	○	○	×	×	○	○	×
7	○	○	×	×	○	○	×
8	○	○	×	○	○	×	×
9	○	○	×	×	×	○	×
10	○	○	×	×	○	×	×

Table 3. Lymphatic drainage pathways in the patients injected with ICG into the posterior surface of the uncinate process of the pancreas

No.	Posterior pancreaticoduodenal arcade	Behind the SMV	Left side of the SMA (J1, J2, J3 regions)	Longitudinally upward between the SMV and SMA	Origin of the middle colonic artery toward the transverse colon	PA region	Hepatoduodenal ligament
11	○	○	×	○	○	○	×
12	○	○	×	×	○	○	×
13	○	○	×	○	×	○	○
14	○	○	×	○	○	○	×
15	○	○	×	○	×	○	○
16	○	○	×	○	×	○	×
17	○	○	×	×	×	○	○
18	○	×	×	×	×	○	×
19	○	×	×	×	×	○	×
20	○	×	×	×	×	×	×

uncinate process of the pancreas were observed with PDE. The lymphatic pathways were observed (1) along the anterior or posterior pancreaticoduodenal arcade, (2) running obliquely down behind the SMV, (3) passing behind the SMV and SMA and reaching the left side of the SMA (J1, J2, or J3 regions), (4) passing behind the SMV and running longitudinally upward between the SMV and SMA, (5) passing the origin of the middle colonic artery toward the transverse colon, (6) reaching the PA region, and (7) reaching the hepatoduodenal ligament.

Table 2 shows the lymphatic drainage pathways in the patients with injection of ICG into the anterior surface of the uncinate process of the pancreas (n = 10). We could identify the lymphatic flow along the anterior pancreaticoduodenal arcade in all 10 patients (100%) within 5 min after ICG injection. The lymphatic flow running obliquely down and shining fluorescent spots behind the SMV were found in all 10 patients (100%) with an ICG injection into the anterior surface, and then the lymphatic flow reaching on the left side of the SMA (J1, J2, or J3 region) was observed in 4 patients (40%). We observed the lymphatic flow running longitudinally upward between the SMV and SMA from the anterior surface in 4 patients (40%). Shining at the origin of the middle colonic artery was observed in 9 patients (90%) with the anterior injection. The lymphatic flow reaching the PA region was found in 8 patients (80%) who receive an ICG injection into the anterior surface, whereas the lymphatic flow reaching the hepatoduodenal ligament was not found in any patients with the anterior injection.

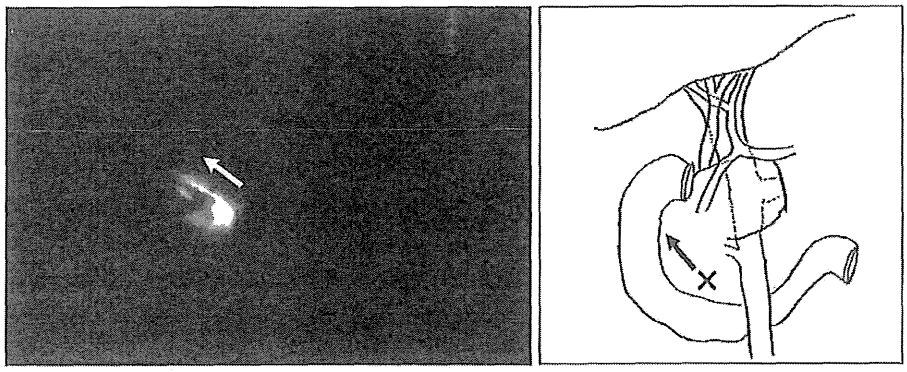

Fig. 1. Lymphatic flow from the anterior surface of the uncinate process of the pancreas toward the duodenal wall and then spread along the anterior pancreaticoduodenal arcade. × = Injection site, the arrow indicates the lymphatic flow visualized by the ICG fluorescence imaging system.

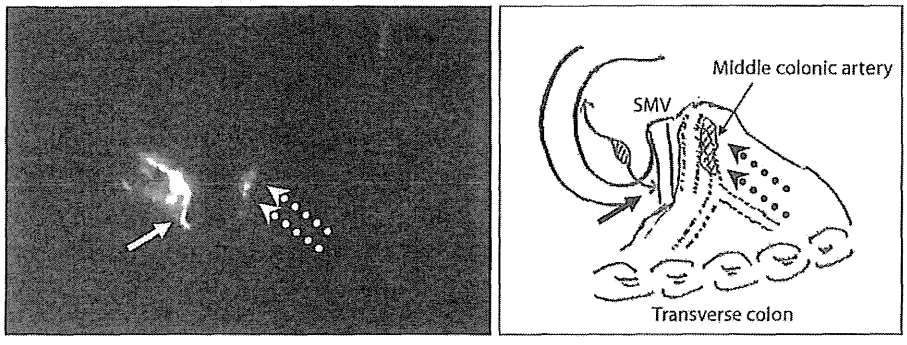

Fig. 2. Lymphatic flow running obliquely down behind the SMV was observed (a solid arrow), and at the same time, a shining spot at the origin of the middle colonic artery was observed (dotted arrows) in the patient who received an anterior injection.

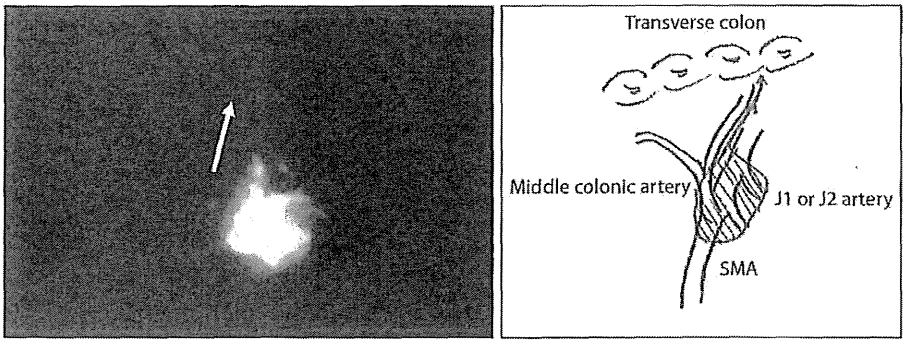

Fig. 3. Shining spots on the left side area of the SMA (J1 or J2) were observed in the patient who received an anterior injection. The lymphatic flow moving to the transverse colon through the middle colonic artery was observed.

Table 3 shows the lymphatic drainage pathways in the patients with an ICG injection into the posterior surface of the uncinate process (n = 10). We observed the lymphatic flow along the posterior pancreaticoduodenal arcade in all 10 patients (100%) immediately after ICG injection into the posterior surface. The lymphatic flow running obliquely down behind the SMV from the posterior surface of the uncinate process was found in 7 patients (70%), but reaching on the left side of the SMA was not observed in any patients with an ICG injection into the posterior surface. The lymphatic flow running longitudinally upward between the SMV and SMA from the posterior surface was observed in 5 patients (50%), and the lymphatic flow passing the origin of the middle colonic artery toward the transverse colon was observed in 3 patients (30%) with the posterior injection. The lymphatic flow reaching the PA region was found in 9 patients (90%) who received an ICG injection into the posterior surface, and the lymphatic flows reaching the hepatoduodenal ligament in 3 patients (30%).

Figure 1 shows the lymphatic flow toward the duodenal wall and then along the anterior pancreaticoduodenal arcade in case 1, with the injection of ICG at the anterior surface of the pancreas head. Figure 2 shows the lymphatic flow running obliquely down behind the SMV, and at the same time, the shining spot at the origin of the middle colonic artery, when ICG was injected in the anterior surface (case 1). Figure 3 shows the shining spots on the left side of the SMA (J1 and J2 regions), and the lymphatic flow passing the origin of the middle colic ar-

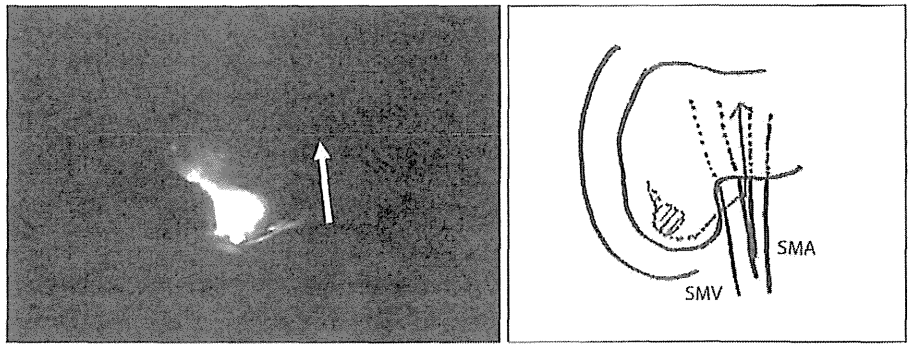

Fig. 4. Lymphatic flow running longitudinally upward between the SMV and SMA was observed in the patient who received a posterior injection.

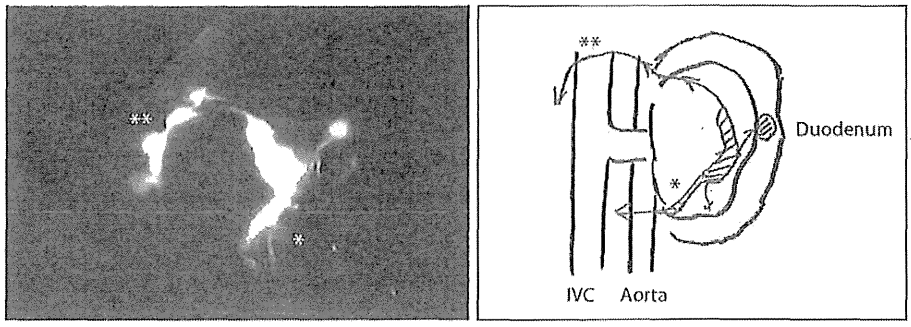

Fig. 5. Shining spots in the PA area were observed. There were two routes toward the PA area; one was the flow directly from the posterior pancreatic head (*), and the other was the flow passing the origin of the SMA toward the anterior inferior vena cava (**).

Table 4. Mean time to arrival at the shining spots from the uncinate process of the pancreas

Injection	Anterior or posterior pancreaticoduodenal arcade	Behind the SMV	Left side of the SMA (J1, J2, J3 regions)	Longitudinally upward between the SMV and SMA	Origin of the middle colonic artery toward the transverse colon	PA region	Hepatoduodenal ligament
Anterior surface of the uncinate process	2.0 min (0.5–4.5)	10.7 min (5–30)	41.8 min (7–80)	21.3 min (10–30)	8.4 min (1–30)	8.2 min (1–30)	–
Posterior surface of the uncinate process	1.6 min (0.5–4)	50.3 min (5–80)	–	38.2 min (5–78)	35.0 min (25–50)	7.2 min (1–50)	38 min (4–60)

Values in parentheses show the range of time to arrive after ICG injection.

tery toward the transverse colon in case 1, with the anterior injection. Figure 4 shows the lymphatic flow running longitudinally upward between the SMV and SMA when ICG was injected into the posterior surface (case 14). Figure 5 shows the lymphatic pathways reaching the PA regions from the anterior surface of the uncinate process (case 1). There were two routes that were observed to shine in the PA area; one was the lymphatic flow directly to the PA area from the posterior surface of the pancreas, and the other was the lymphatic flow passing the origin of the SMA toward the PA area, and then passing the anterior inferior vena cava and reaching the right side of the inferior vena cava.

The mean time to arrive at the each of the shining spots after ICG injection is shown in table 4. The mean arrival time of the drainage pathway reaching around the SMA was longer than that reaching the PA region following both the anterior and posterior injections (21.3 min, 38.2 vs. 8.1 min, 7.2 min).

Histopathological Examination of Tissues That Received ICG

We performed sampling of the PA tissue received in 11 of the 13 patients with pancreatic cancer who were found to have lymphatic flow to the PA tissue. None of the sampled tissue specimens contained malignant deposits on final histopathological examination. In only 1 patient with pancreatic cancer in whom we had observed shining in the left side of the SMA, we dissected en bloc the left side region of the SMA, and no malignant cells around the SMA were found in pathological examination.

Discussion

Several studies have tried to identify the lymphatic pathways in pancreatic cancer patients to determine the optimal lymphadectomy procedure, however the pattern of lymphatic drainage and draining areas remained poorly defined [22–35]. Deki and Sato [22] reported the lymphatic pathways of the pancreas by dissecting the lymphatic vessels using a cadaver. This report described that the lymphatic vessels running obliquely down and passing behind the SMA and SMV and linking with lymph nodes located between these vessels and the posterior layer of the mesentery were observed in the anterior pancreatic head. It has also been reported using autopsy specimens that the lymphatic pathway from the posterior pancreatic head drains toward the right or posterior side of the SMA and finally to the PA lymph nodes [23]. Although these studies did provide useful information, as they used only autopsy specimens, they could not identify the physiological lymphatic flow from the pancreas in a real-time fashion. In another study, sentinel lymph node mapping of pancreatic head cancer was studied by methylene blue dye injection, however the study failed and concluded that it was not possible to identify lymphatic drainage from the pancreatic head using their technology [34].

In this study, we injected ICG into the anterior or posterior surface of the uncinate process of the pancreas, after determining the optimal concentration of ICG by examining various concentrations [28], and then evaluated the time to arrive at the lymphatic drainage areas. The reasons that we chose the uncinate process as injected location of ICG are (1) cancer in the ventral pancreatic head occurs more often than that in the dorsal pancreatic head [20], and (2) it has been reported that lymph node metastasis around the SMA is most often in the cancer located in the uncinate process [18, 19].

We could identify the visual lymphatic drainage pathways from the head of the pancreas by ICG fluorescence imaging during surgery. Indeed, we found the lymphatic root passing behind the SMV and SMA around the left side of the SMA from the anterior pancreatic head, as the previous anatomic reports description, however we also simultaneously found several other roots including the PA region. With regard to the relationship between lymphatic drainage and the clinical significance of lymph node metastasis, of the 16 patients demonstrating lymphatic drainage to the PA region, 6 patients had no lymph node metastasis and 10 patients had lymph node metastasis in only the regional lymph node. As a result, we did not find any relationship between lymphatic drainage and the clinical outcome.

This study showed that (1) the lymphatic drainage to the PA region from the pancreatic head was more often than that to the left side of the SMA (J1, J2, and J3 regions), (2) the patients with lymphatic drainage into the left side of the SMA have a simultaneous pathway into the PA region, and (3) the time reaching around the SMA was longer than that reaching the PA region. Based on these results, a lymphadenectomy of the left side of the SMA, including the J1, J2, and J3 regions, might have a similar oncological impact as a lymphadenectomy around the PA region, that is, the circumferential clearance of the SMA may not be of benefit as PA lymph node dissection is for patients with pancreatic cancer, although prospective randomized controlled studies would be needed to prove our conclusion.

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Research Article

Identification of HLA-A24-Restricted Novel T Cell Epitope Peptides Derived from P-Cadherin and Kinesin Family Member 20A

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We here identified human leukocyte antigen-(HLA-)A*2402-restricted epitope peptides from Cadherin 3, type 1, P-cadherin (CDH3) and kinesin family member 20A (KIF20A) that were found to be specifically expressed in cancer cells through genome-wide expression profile analysis. CDH3-10-807 peptide and KIF20A-10-66 peptide successfully induced specific CTL clones, and these selectively responded to COS7 cells expressing both HLA-A*2402 and respective protein while did not respond to parental cells or COS7 cells expressing either HLA-A*2402 or respective protein. Furthermore, CTL clones responded to cancer cells that endogenously express HLA-A*2402 and respective protein, suggesting that CDH3-10-807 peptide and KIF20A-10-66 peptide are naturally presented on HLA-A*2402 molecule of human cancer cells. Our results demonstrated that CDH3-10-807 peptide and KIF20A-10-66 peptide are novel HLA-A24-restricted tumor-associated antigens and would be applicable for CTL-inducing cancer therapies.

1. Introduction

After identification of the melanoma antigen gene (MAGE) family as a tumor-associated antigen (TAA), a number of TAAs have been revealed by means of various approaches including SEREX and cDNA library screening [1–6]. Some TAAs, such as MAGE, gp100, and MUC1, have been applied to treat various cancers in clinical trials [7–9], and vaccine-based therapy is now considered as a promising approach to fight against various cancers [10–13].

We have identified dozens of genes specifically expressed in cancer cells by genome-wide expression profile analysis for cDNA microarray consisting of more than 30,000 cDNAs and expressed sequence tags (ESTs) [14]. Among them, two genes, Cadherin 3, type 1, P-cadherin (CDH3) and kinesin family member 20A (KIF20A), were found to be upregulated in pancreatic cancers [15, 16]. CDH3 is one of the classic cadherin family that plays a critical role in cell-cell adhesion and epithelial morphogenesis [17]. We reported that over-expression of CDH3 promoted the motility of cancer cells and blocking of CDH3 by anti-CDH3 antibody inhibited the migration of CDH3-expressing cells [15]. KIF20A is a member of the kinesin family, which is characterized to be a motor protein in cancer cells [18], and northern analysis indicated no expression of KIF20A among examined 23 normal tissues except testis. Furthermore, knock down of KIF20A expression with small interfering RNA suppressed the proliferation of pancreatic ductal adenocarcinoma cells [16].

Thus, both CDH3 and KIF20A would play oncogenic functions in pancreatic cancer cells and are attractive target molecules for cancer therapies including immunotherapy.

We here identified CDH3- and KIF20A-derived novel HLA-A*2402-restricted epitope peptides that can induce peptide-specific cytotoxic T lymphocyte (CTL), suggesting that these epitope peptide would be applicable to peptide-based cancer vaccine therapies for HLA-A*2402 positive pancreatic cancer patients.

2. Materials and Methods

2.1. Peptides. CDH3 and KIF20A-derived 9-mer and 10-mer peptides that have high binding affinity (binding score > 10) to HLA-A*2402 were predicted by the binding prediction software "BIMAS" (http://www-bimas.cit.nih.gov/molbio/hla_bind/) and were synthesized by Sigma-Aldrich Japan KK (Ishikari, Japan) according to a standard solid-phase synthesis method and purified by reversed-phase high-performance liquid chromatography (HPLC). HIV-A24 epitope peptide (RYLRDQQLL) [19] was also synthesized as a negative control. The purity (>90%) and the identity of the peptides were confirmed by analytical HPLC and mass spectrometry analysis, respectively. Peptides were dissolved in dimethylsulfoxide at 20 mg/mL and stored at −80°C.

2.2. Cell Lines. CDH3- and KIF20A- negative Human B-lymphoblastoid cell line TISI (HLA-A*2402) was purchased from the IHWG Cell and Gene Bank (Seattle, WA). Monkey kidney cell line COS7, human B-lymphoblastoid cell line Jiyoye (HLA-A32), human B-lymphoblastoid cell line EB-3 (HLA-A3/Aw32), and CDH3-expressing human lung cancer cell line H358 (HLA-A3) were purchased from American Type Culture Collection (Manassas, VA). CDH3-expressing human pancreatic cancer cell line PK-45P (HLA-A24/A33) and KIF20A-expressing human pancreatic cancer cell line PK-59 (HLA-A31/A33) were provided by Cell Resource Center for Biomedical Research, Tohoku University (Sendai, Japan). KIF20A-expressing human stomach cancer cell line MKN-45 (HLA-A24) and MiaPaCa-2 cells (HLA-A24) were purchased from Health Science Research Resources Bank (Osaka, Japan). TISI, Jiyoye, EB-3, H358, PK-45P, PK-59, and MKN-45 were maintained in RPMI1640 media (Invitrogen, Carlsbad, CA), COS7 were maintained in DMEM media (Invitrogen), and MiaPaCa-2 cells were maintained in EMEM media (Invitrogen). Each medium was supplemented with 10% fetal bovine serum (GEMINI Bio-Products, West Sacramento, CA) and 1% antibiotic solution (Sigma-Aldrich, ST. Louis, MO). The expression of CDH3 and KIF20A protein was confirmed by Western blotting using anti-CDH3 antibody (BD Transduction Labs., BD Biosciences, San Jose, CA) or anti-KIF20A antibody (Bethyl Laboratories, Montgomery, TX).

2.3. In Vitro Induction of Peptide-Specific CTL. To examine the ability to induce peptide-specific CTL, purified $CD8^+$ T cells were cocultured with autologous monocyte-derived mature dendritic cells (DCs) pulsed with peptide. Both $CD8^+$ T cells and DCs were prepared from peripheral blood mononuclear cells (PBMCs) of same HLA-A*2402-positive healthy volunteers. Briefly, PBMCs were isolated by Ficoll-Paque solution (GE Healthcare, Uppsala, Sweden), then cells were cultured in AIM-V medium (Invitrogen) containing 2% heat-inactivated autologous serum (AS). After the over night incubation, nonadherent cells were washed out, then 1000 U/mL of granulocyte-macrophage colony-stimulating factor (GM-CSF; R&D Systems, Minneapolis, MN) and 1000 U/mL of interleukin (IL)-4 (R&D Systems) were added in the culture to induce monocyte-derived DCs. To mature DCs, 0.1 KE/mL of OK-432 (Chugai Pharmaceutical Co., Tokyo, Japan) was added in the culture on day 5. Seven days later, DCs were pulsed with 20 μg/mL of synthesized peptide in AIM-V medium containing 3 μg/mL of β2-microglobulin (Sigma-Aldrich) at 37°C for 3 h [20] and incubated in the media containing 30 μg/mL of Mitomycin C (MMC) (Kyowa Hakko Kirin Co. Ltd., Tokyo, Japan) for 30 min. Following washing out residual peptide and MMC, cells were used as antigen-presenting cells to induce peptide-specific CTL. Generated monocyte-derived mature DCs expressed CD80, CD83, CD86, and HLA class II on their cell surface (data not shown). Autologous $CD8^+$ T cells were prepared from PBMCs derived from the same HLA-A*2402-positive donor by positive selection with Dynal CD8 positive isolation kit (Invitrogen) according to the manufacturer's instructions. 1.5×10^4 of peptide-pulsed DCs and 3×10^5 of $CD8^+$ T cells were cocultured in 0.5 mL of AIM-V medium supplemented with 10 ng/mL of IL-7 (R&D Systems) and 2% AS on 48-well plates (Corning Inc., Corning, CA). IL-2 (CHIRON, Emeryville, CA) was added to the culture at 20 IU/mL 3 days after coculture, and peptide-pulsed DCs were additionally supplied into the culture on days 7 and 14. Eight wells were prepared for CTL induction by every peptide in a single experiment. On day 21, interferon- (IFN-) γ production was examined by IFN-γ enzyme-linked immunospot (ELISPOT) assay under the stimulation with peptide-pulsed TISI cells.

2.4. IFN-γ Enzyme-Linked Immunospot (ELISPOT) Assay. T cell response to epitope peptide was measured by ELISPOT assay using IFN-γ ELISPOT kit and AEC substrate set (BD Pharmingen, San Diego, CA) according to the manufacturer's instruction. Briefly, TISI cells were pulsed with 20 μg/mL of respective peptide at 37°C for 20 h, and the residual peptide that did not bind to TISI cells was washed out to prepare peptide-pulsed TISI cells as the stimulator cells. 200 μL of cell culture suspension were distributed to two wells (100 μL each) on Multiscreen-IP 96-well plate (Millipore, Bedford, MA) following removing 500 μL of supernatant from each well from culture of "*in vitro* induction of peptide-specific CTL." Cells were co-incubated with peptide-pulsed TISI cells (1×10^4 cells/well) at 37°C for 20 h. The plates were analyzed by the automated ELISPOT reader, ImmunoSPOT S4 (Cellular Technology Ltd, Cleveland, OH) and ImmunoSpot Professional Software Version 5.0 (Cellular Technology Ltd). TISI cells pulsed with HIV-A24 epitope peptide (RYLRQQLLGI) were used as control. When the spot number in the peptide-stimulating

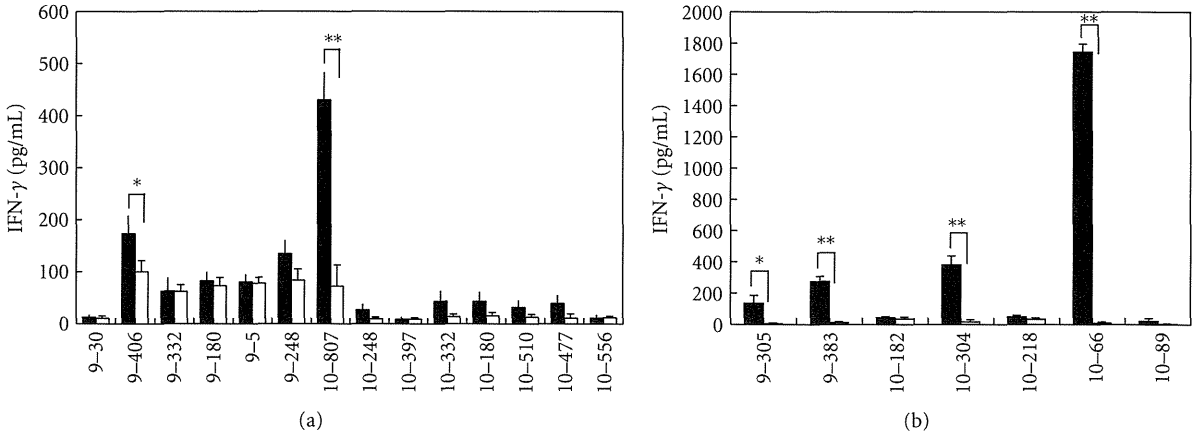

FIGURE 1: IFN-γ production from CTLs responding to CDH3- or KIF20A-derived peptides. IFN-γ production by CTLs induced with CDH3-derived peptides (a) or KIF20A-derived peptides (b) responding to respective peptide-pulsed HLA-A*2402 positive TISI cells. CTLs were expanded and harvested following "*in vitro* induction of peptide-specific CTL," and IFN-γ production was examined by IFN-γ ELISA. "Closed bar" indicates the mean IFN-γ production responding to TISI cells pulsed with indicated peptide, and "open bar" indicates the mean IFN-γ production responding to TISI cells pulsed with HIV-A24 peptide (negative control). All experiments were performed triplicate. Similar results were obtained in three to five independent experiments. $^*P < 0.05$, $^{**}P < 0.01$.

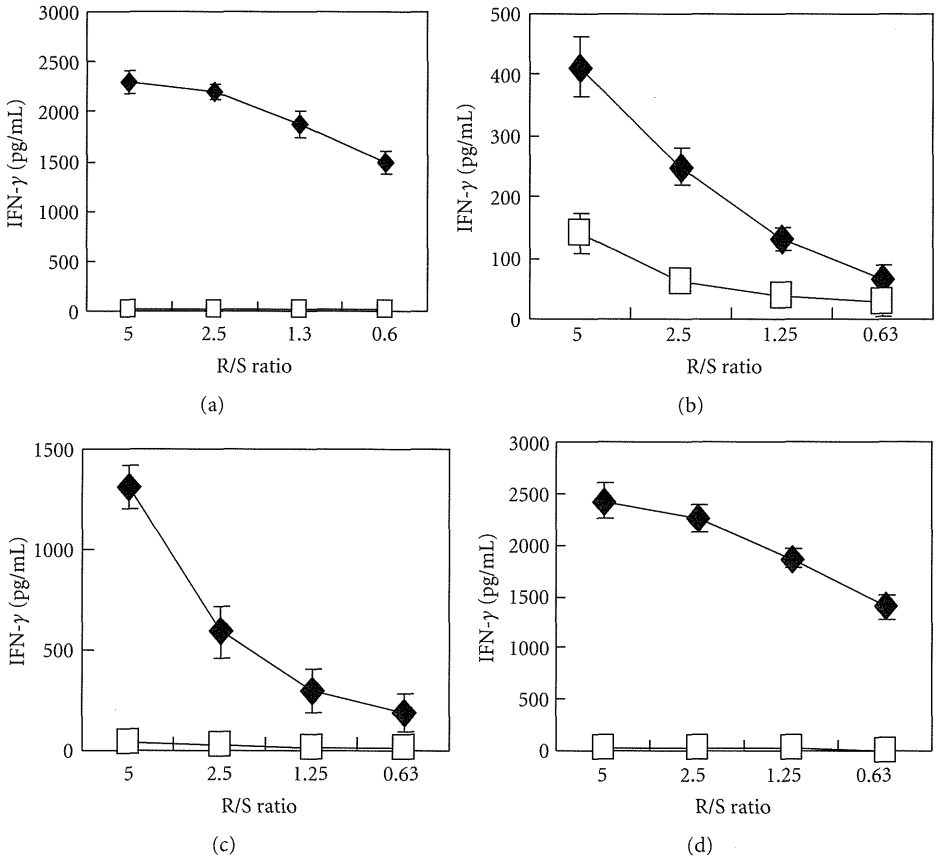

FIGURE 2: Peptide-specific IFN-γ production by CTL clones. IFN-γ production by CDH3-10-807 peptide-specific CTL clone (a), KIF20A-9-305 peptide-specific CTL clone (b), KIF20A-10-304 peptide-specific CTL clone (c), and KIF20A-10-66 peptide-specific CTL clone (d), when stimulated with TISI cell pulsed with corresponding peptide (closed diamond) or HIV-A24 peptide (open square). CTL clones produced significant amount of IFN-γ responding to corresponding peptide. IFN-γ ELISA was performed triplicate. R/S ratio, responder cell (CTL clone)/stimulator cell (TISI cell) ratio.

Table 1: Candidates of epitope peptide derived from CDH3 and KIF20A.

CDH3				KIF20A			
Start position	Amino acid sequence (mer)	Binding score	CTL induction	Start position	Amino acid sequence (mer)	Binding score	CTL induction
513	IYEVMVLAM (9)	37.5	–	308	IYNELLYDL (9)	432	–
667	LFLLLVLLL (9)	36	–	621	MYEEKLNIL (9)	432	–
30	VFREAEVTL (9)	24	+	67	VYLRVRPLL (9)	420	–
406	LYVEVTNEA (9)	16.6	+	499	KFSAIASQL (9)	56	–
332	KYEAHVPEN (9)	16.5	+	304	SFFEIYNEL (9)	44.352	–
180	KYELFGHAV (9)	15	+	187	IFNSLQGQL (9)	36	–
85	RSLKERNPL (9)	14.4	–	305	FFEIYNELL (9)	30	+
5	RGPLASLLL (9)	12	+	23	MFESTAADL (9)	30	–
652	KGGFILPVL (9)	11.2	–	256	SFDSGIAGL (9)	20	–
248	TYNGVVAYS (9)	10.5	+	298	RFSIWISFF (9)	20	–
65	LFSTDNDDF (9)	10	–	383	IFSIRILHL (9)	20	+
807	DYLNEWGSRF (10)	150	+	647	KIEELEALL (9)	17.28	–
248	TYNGVVAYSI (10)	105	+	625	KLNILKESL (9)	14.4	–
667	LFLLLVLLLL (10)	42	–	695	KLQQCKAEL (9)	13.2	–
397	DFEAKNQHTL (10)	30	+	726	FTIDVDKKL (9)	11.088	–
332	KYEAHVPENA (10)	21	+	688	QLQEVKAKL (9)	11.088	–
180	KYELFGHAVS (10)	15	+	308	IYNELLYDLL (10)	432	–
510	RNNIYEVMVL (10)	12	+	182	RSLALIFNSL (10)	24.192	+
5	RGPLASLLLL (10)	12	–	304	SFFEIYNELL (10)	24	+
477	RILRDPAGWL (10)	12	+	742	RLLRTELQKL (10)	15.84	–
556	CNQSPVRQVL (10)	10.1	+	739	KNIRLLRTEL (10)	15.84	–
				218	RQEEMKKLSL (10)	14.4	+
				70	RVRPLLPSEL (10)	12.672	–
				871	RILRSRRSPL (10)	12	–
				89	RIENVETLVL (10)	12	+
				364	KNQSFASTHL (10)	12	–
				66	KVYLRVRPLL (10)	11.2	+
				60	DSMEKVKVYL (10)	10.08	–

Start position indicated the number of amino acids from the N terminal of CDH3 and KIF20A.
Binding score was obtained using BIMAS program.
CTL induction was indicated as positive (+) or negative (–). Similar results were obtained 3–7 independent experiments using PBMC of 3–7 healthy volunteers.

well was more than 50 spots/well compared with that in the control well, we estimated that peptide-specific CTL were induced (positive) and subsequently expanded CTL from the positive well. Sensitivity of our ELISPOT assay was estimated as approximately average level by ELISPOT panel of Cancer Immunotherapy Consortium [CIC (http://www.cancerresearch.org/consortium/assay-panels/)].

2.5. CTL Expansion. Peptide-specific CTL obtained from CTL positive well of "*in vitro* induction of peptide-specific CTL" were expanded by the modified protocol based on the previously described methods [21–24]. Briefly, 5×10^5 of CTLs were cocultured with 5×10^6 of MMC-treated (30 μg/mL at 37°C for 30 min) EB-3 and Jiyoye cells in 25 mL of AIM-V containing 5% AS and 40 ng/mL of anti-CD3 mAb. The cultures were supplemented with IL-2 (final concentration: 120 IU/mL) 24 h later and fed with AIM-V medium containing 5% AS and IL-2 (30 IU/mL) on day 5, 8, and 11. On day 14, expanded T cells were harvested to examine specific response to epitope peptide by IFN-γ enzyme-linked immunosorbent assay (ELISA).

2.6. Establishment of Peptide-Specific CTL Clone. Peptide-specific CTL clones were established by limiting dilution method from the expanded CTLs specifically responding to epitope peptide. Briefly, T cells were diluted to 0.3, 1, and 3 cells/well in 96-well round-bottomed plates and cultured with 1×10^4 cells/well of MMC-treated EB-3 and Jiyoye cells in 150 μL of AIM-V containing 5% AS, 125 IU/mL of IL-2, and 30 ng/mL of anti-CD3 mAb. The culture was supplemented with IL-2 to the final concentration of 125 IU/mL on day 10. On day 14, IFN-γ production from peptide-specific CTL clones was examined by IFN-γ ELISA. Some peptide-specific CTL clones were expanded as described above.

2.7. IFN-γ ELISA. In some experiments, established CTLs were co-incubated with 1×10^4 cells of respective peptide-pulsed TISI cells or 5×10^4 cells of COS7 cells in 200 μL of AIM-V/5% AS media on 96-well round bottom plate (Corning Inc.). After 24 h incubation, cell free supernatants were harvested and IFN-γ production was examined by human

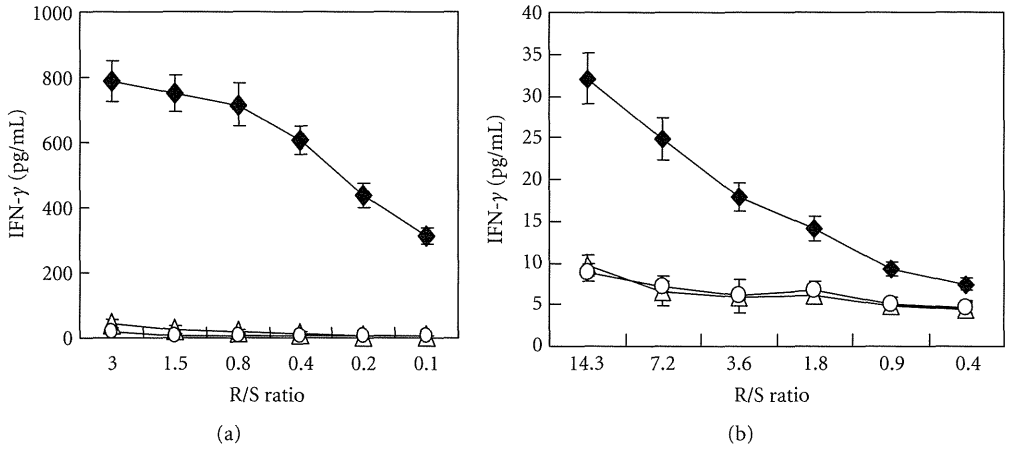

FIGURE 3: IFN-γ production by CTL clones responding to COS7 cells that expressing HLA-A*2402 and respective oncogene. (a) IFN-γ production by CDH3-10-807 peptide-specific CTL clone when exposed with COS7 cells expressing both HLA-A*2402 and CDH3 (closed diamond), HLA-A*2402 (open triangle), or CDH3 (open circle). (b) IFN-γ production by KIF20A-10-66 peptide-specific CTL clone when exposed with COS7 cells expressing HLA-A*2402 and KIF20A (closed diamond), HLA-A*2402 (open triangle), or KIF20A (open circle). Both CTL clones significantly produced IFN-γ responding to COS7 cells expressing HLA-A*2402 and corresponding gene. Similar results were obtained in three independent experiments. Independently induced other CTL clones also produced significant amount of IFN-γ when exposed with COS7 cell expressing both HLA-A*2402 and respective gene (data not shown). R/S ratio, responder cell (CTL clone)/stimulator cell (COS7 cell) ratio.

IFN-γ-specific ELISA kit (BD Pharmingen) according to the manufacturer's instructions.

2.8. Cytotoxicity Assay. Specific cytotoxic activity of induced CTL clones was tested by a 4 h ^{51}Cr release assay as previously described [25]. Data are represented as the mean ± SD of triplicate samples.

2.9. Transfection of HLA-A24 and/or Oncogene (CDH3 or KIF20A). HLA-A*2402 coding region was obtained from TISI cells. The cDNA encoding an open reading frame of HLA-A*2402 gene with FLAG tag or oncogene (CDH3 or KIF20A) coding region with the Myc tag sequence was amplified with PCR and cloned into pcDNA3.1 vector (Invitrogen). COS7 cells transiently expressing HLA-A*2402 and/or oncogene were prepared by the transfection of the vectors encoding respective genes using lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction. The expression of HLA-A*2402 and oncogene-derived protein was confirmed by Western blotting using anti-Myc (Upstate Biotechnology, Lake Placid, NY) or anti-FLAG antibody (Sigma-Aldrich). Two days after transfection, the transfected cells were harvested with versene (Invitrogen) and used to stimulate peptide-specific CTL clones. IFN-γ production by CTLs was examined by IFN-γ-specific ELISA.

2.10. Flow Cytometry. Expression of peptide-specific T cell receptor (TCR) was examined on FACS-CantoII (Becton Dickinson, San Jose, CA) using peptide-HLA-A*2402 dextramer-PE (Immudex, Copenhagen, Denmark) (CDH3-10-807/MHC-dextramer-PE and KIF20A-10-66/MHC-dextramer-PE) according to the manufacturer's instructions. HIV-A24 epitope peptide (RYLRDQQLL)/MHC-dextramer was used as negative control. Briefly, expanded CTL lines were incubated with peptide-HLA-A*2402 dextramer-PE for 10 minutes at room temperature, then treated with FITC-conjugated anti-human CD8 mAb, APC-conjugated anti-human CD3 mAb, PE-Cy7-conjugated anti-human CD4 mAb, and 7-AAD (BD Pharmingen) at 4°C for 20 minutes.

3. Results

*3.1. Induction of CTL Responding to CDH3- or KIF20A-Derived Peptide Restricted with HLA-A*2402.* Based on the analysis with the binding prediction software "BIMAS," we synthesized 21 CDH3-derived epitope-peptides and 28 KIF20A-derived epitope-peptides that were expected to have high affinity to HLA-A*2402 molecule and activate CTLs (Table 1).

HLA-A*2402-positive CD8$^+$ T cells were cocultured with autologous DCs pulsed with respective peptide, and then peptide-specific IFN-γ production was analyzed by ELISPOT. Fourteen peptides derived from CDH3 and 7 peptides derived from KIF20A were able to induce peptide-specific CTLs producing IFN-γ (Table 1). Amongst these peptides, we successfully obtained CTLs that specifically produced significant amount of IFN-γ after CTL expansion when CDH3-9-406, CDH3-10-807, KIF20A-9-305, KIF20A-9-383, KIF20A-10-304, and KIF20A-10-66 peptide were pulsed (Figures 1(a) and 1(b)).

3.2. Establishment of CDH3- or KIF20A-Derived Peptide-Specific CTL Clones. Subsequently, we attempted to establish CTL clones by a limiting dilution. CDH3-10-807-, KIF20A-9-305-, KIF20A-10-304-, or KIF20A-10-66-specific CTL clones were established and produced a potent amount of

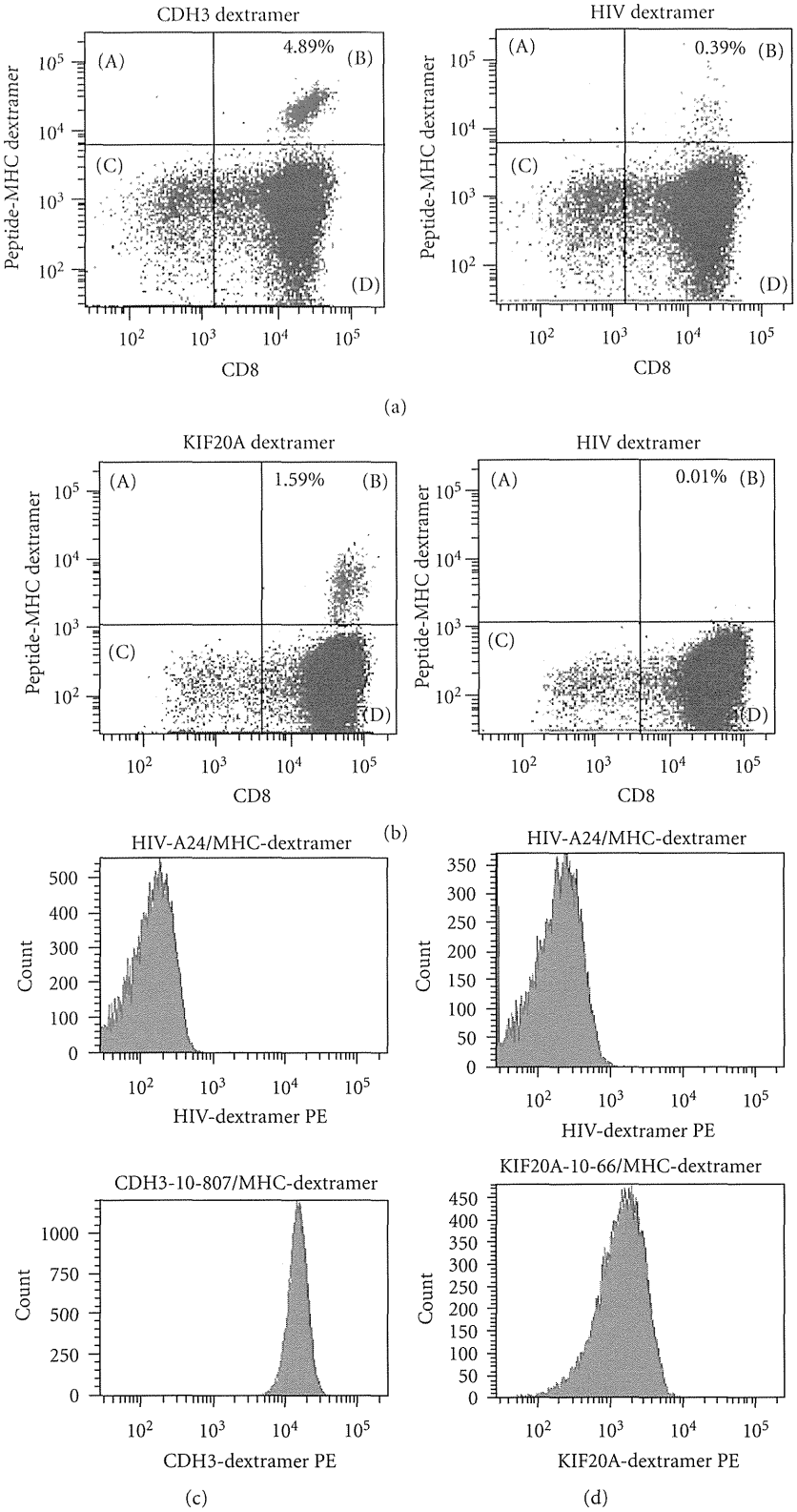

FIGURE 4: Peptide-specific TCR expression on CTL clones. (a) CDH3-10-807/HLA-A*2402-specific TCR expressing cells in expanded CTLs following "*in vitro* induction of peptide-specific CTL." (b) KIF20A-10-66/HLA-A*2402-specific TCR expressing cells in expanded CTLs following "*in vitro* induction of peptide-specific CTL." Results staining with anti-human CD8 mAb and CDH3-10-807/MHC-dextramer-PE or KIF20A-10-66/MHC-dextramer-PE are presented following gating on CD3-positive cells (left panels). Results staining with anti-human CD8 mAb and HIV-A24/MHC-dextramer-PE are presented as negative control following gating on CD3 positive cells (right panels). (c) CDH3-10-807/HLA-A*2402-specific TCR expression on CDH3-10-807-specific CTL clone. (d) KIF20A-10-66/HLA-A*2402-specific TCR expression on KIF20A-10-66-specific CTL clone. Staining with HIV-A24/MHC-dextramer-PE was used as negative control. CTL clones were $CD3^+$ and $CD8^+$ as expected (data not shown). Similar results were obtained in independent all experiments to examine CTL induction.

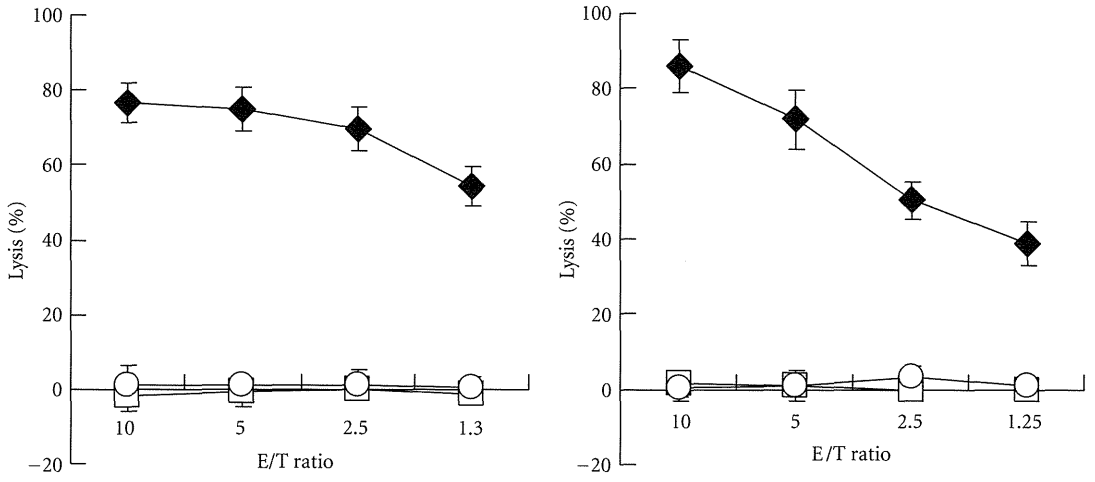

FIGURE 5: Cytotoxic activity of CTL clones against peptide-pulsed TISI cells. Cytotoxic activity of CDH3-10-807 peptide-specific CTL clone (a) and KIF20A-10-66 peptide-specific CTL clone (b) against HLA-A*2402-positive TISI cells pulsed with respective peptide (close diamond), HIV-A24 peptide (open square), or TISI cells without peptide pulse (open circle). E/T ratio, effector cell (CTL clone)/target cell (TISI cell) ratio. Similar results were obtained in three independent experiments using same CTL clone and in independent experiments using other CTL clones.

IFN-γ specifically responding to the stimulator cells pulsed with the respective peptide, but not HIV-A24 peptide (Figures 2(a)–2(d)).

*3.3. Recognition of Cells Endogenously Expressing Both HLA-A*2402 and Respective Protein by Peptide-Specific CTL Clones.* We then examined that the established peptide-specific CTL clones can recognize cells that express HLA-A*2402 and the target proteins. COS7 cells were transfected with plasmid designed to express HLA-A*2402 molecule and/or that to express the full length protein of CDH3 or KIF20A. We confirmed expression of these proteins by western blotting (data not shown). CDH3-10-807 peptide responding CTL clone substantially produced IFN-γ when exposed to COS7 cells that expressing both HLA-A*2402 and CDH3, but not COS7 cells that expressing either HLA-A*2402 or CDH3 (Figure 3(a)). Similarly, KIF20A-10-66 peptide responding CTL clone produced significant amount of IFN-γ when exposed to COS7 cells that expressing both HLA-A*2402 and KIF20A, but not COS7 cells that expressing either HLA-A*2402 or KIF20A (Figure 3(b)). Both CTL clones also produced IFN-γ responding to COS7 cells, which transfected with pIRES-vector containing both HLA-A*2402 and respective oncogene (data not shown). On the other hand, CTL clones responding to KIF20A-9-305 peptide or KIF20A-10-304 peptide did not produce IFN-γ when exposed to COS7 cells expressing both HLA-A*2402 and KIF20A (data not shown). Only CDH3-10-807 peptide and KIF20A-10-66 peptide, but not other candidate peptides, were able to induce CTL responding to COS7 cells expressing HLA-A*2402 and CDH3 or KIF20A, albeit we have tried several times using PBMC derived from different healthy donors (data not shown).

3.4. Peptide-Specific T Cell Receptor Expression. Expression of CDH3-10-807/HLA-A*2402- or KIF20A-10-66/HLA-A*2402-specific T cell receptor (TCR) was examined using CDH3-10-807/MHC-dextramer-PE or KIF20A-10-66/MHC-dextramer-PE. Significant population of $CD3^+$ $CD8^+$ cells, but not $CD3^+$ $CD8^-$ cells, expressed CDH3-10-807/HLA-A*2402- or KIF20A-10-66/HLA-A*2402- specific TCR after expansion of cells obtained by "*in vitro* induction of peptide-specific CTL" (Figures 4(a) and 4(b)). As expected, CTL clones established by CDH3-10-807 peptide- or KIF20A-10-66 peptide-pulsed cells were CD8 positive and expressed respective peptide/HLA-A*2402-specific TCR (Figures 4(c) and 4(d)).

3.5. Cytotoxic Activity of CTLs. We also examined cytotoxic activity of CTL clones. CDH3-10-807 or KIF20A-10-66 peptide-specific CTL clone demonstrated cytotoxic activity against HLA-A*2402-positive TISI cells when respective peptide was pulsed, but not when HIV-A24 peptide was pulsed or peptide was not pulsed (Figures 5(a) and 5(b)). These results suggested that CDH3-10-807 or KIF20A-10-66 peptide-specific CTL clone specifically exerted cytotoxic activity responding to respective epitope peptide binding to HLA-A*2402 on cells.

We, then, finally examined the cytotoxic activity against cancer cells, which endogenously expressed CDH3 or KIF20A gene. Expression of CDH3 protein was confirmed in HLA-A*2402-positive PK-45P cells and HLA-A*2402-negative H358 cells, but HLA-A*2402-positive MiaPaca-2 cells did not express CDH3 (Figure 6(a)). CDH3-10-807 peptide-specific CTL clone exerted significant cytotoxic activity against CDH3-expressing HLA-A*2402-positive PK-45P cells, but not H358 or MiaPaca-2 cells (Figure 6(b)).

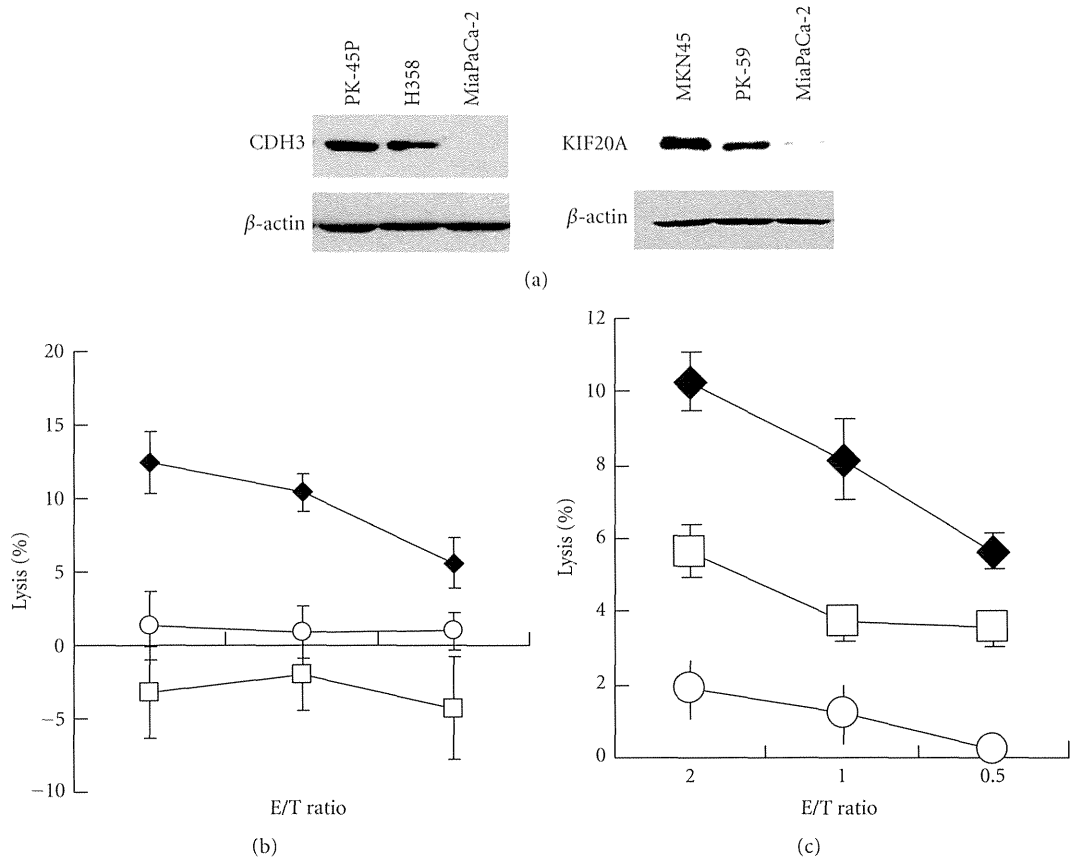

FIGURE 6: Cytotoxic activity against tumor cells expressing respective gene. (a) The expression of CDH3 and KIF20A protein in tumor cells used in cytotoxic assay. (b) Cytotoxic activity of CDH3-10-807 peptide-specific CTL clone against CDH3-expressing HLA-A*2402-positive PK-45P cells (closed diamond), CDH3-expressing HLA-A*2402-negative H358 cells (open square), or MiaPaCa-2 cells (open circle). Cytotoxicity against PK-45P cells was significantly higher than those against other cells. (c) Cytotoxic activity of KIF20A-10-66 peptide-specific CTL clone against KIF20A-expressing HLA-A*2402-positive MKN-45 cells (closed diamond), KIF20A-expressing HLA-A*2402-negative PK-59 cells (open square), or MiaPaCa-2 cells (open circle). Cytotoxicity against MKN-45 cells or PK-59 cells are significantly higher than that against MiaPaCa-2 cells, although cytotoxic activity against PK-59 cells was significantly lower compared with that against MKN-45 cells. E/T ratio, effector cell (CTL clone)/target cell (tumor cell) ratio. Similar results were obtained in three independent experiments using same CTL clone and in independent experiments using other CTL clones.

Expression of KIF20A protein was confirmed in HLA-A*2402-positive MKN-45 cells and HLA-A*2402-negative PK-59 cells, but HLA-A*2402-positive MiaPaca-2 cells did not express KIF20A (Figure 6(a)). KIF20A-10-66 peptide-specific CTL clone exerted significant cytotoxic activity against KIF20A-expressing HLA-A*2402-positive MKN-45 cells, but not MiaPaca-2 cells (Figure 6(c)). KIF20A-10-66 peptide-specific CTL clone demonstrated cytotoxic activity against KIF20A-expressing HLA-A*2402-negative PK-59 cells; however, this cytotoxicity was always less when compared with that against KIF20A-expressing HLA-A*2402-positive MKN-45 cells (Figure 6(c) and data not shown).

No homologous sequence to CDH3-10-807 peptide or KIF20A-10-66 peptide was demonstrated by the homology research using the BLAST algorithm http://blast.ncbi.nlm.nih.gov/Blast.cgi (data not shown), suggesting that these peptide would be the unique epitope peptide presented on HLA-A*2402 of CDH3 or KIF20A-expressing cells.

Taken together, presented results suggested that CDH3-10-807 peptide-specific or KIF20A-10-66 peptide-specific CTLs exert potent IFN-γ production and cytotoxic activity specifically responding to HLA-A*2402-positive cancer cells expressing CDH3 or KIF20A, respectively.

4. Discussion

Pancreatic cancer is one of the most malignant cancers, since 5-year survival rate is only 5% and the therapeutic modalities are very limited [26, 27]. Both CDH3 and KIF20A were upregulated in the majority of pancreatic cancers and have oncogenic functions [15, 16]. Thus, CDH3 and KIF20A would be promising target molecules to develop novel therapeutic strategies for pancreatic cancer. Hence, we identified HLA-A*0201-restricted peptides derived from CDH3 and KIF20A [28, 29].

In present study, we successfully identified HLA-A*2402-restricted novel epitope peptides derived from both CDH3 and KIF20A and demonstrated that these peptides could induce specific CTLs producing potent amount of IFN-γ and

exert cytotoxic activity. Established CDH3-10-807-specific CTL clones or KIF20A-10-66-specific CTL clones responded to CDH3- or KIF20A-introduced COS7 cells as well as CDH3 or KIF20A endogenously expressing cancer cells (PK-45P or MKN-45) in HLA-A24-restricted manner. These results indicated that induction of CDH3-10-807-specific CTLs or KIF20A-10-66-specific CTLs would exert antitumor effect against pancreatic cancers in HLA-A24-positive patients.

Predicted binding score of KIF20A-10-66 peptide to HLA-A*2402 was relatively low when compared with that of CDH3-10-807 peptide. We previously reported epitope peptides derived from RNF43 and IMP-3, and those peptides also have low affinity to HLA molecule [22, 24]. Interestingly, both peptides have been already applied for clinical trials as peptide-based immunotherapy and CTL were obtained in many cancer patients [11, 12]. These results suggested that some peptides possibly induce CTLs albeit binding score was low by BIMAS prediction and KIF20A-10-66 peptide, as well as CDH3-10-807 peptide, possibly induces CTL in cancer patients.

Recent improvement and development of cancer therapies, including combined treatments of standard therapies (chemotherapy, radiotherapy, and surgical resection), substantially improved the survival of advanced cancer patients [27]. However, unfavorable adverse events are still often observed. On the other hand, immune therapies inducing cancer-cell-specific CTLs are now developed to improve the efficacy against cancers and the quality of life of patients. Ongoing several clinical trials using epitope peptides derived from TAA have been proving the evidence that CTL-inducing therapies are much less harmful to the patients [10–12]. However, efficacy of some vaccine therapy trials is still limited mainly due to the development of escaping variant cancer cells that lost targeted TAA expression during the treatment [30]. Therefore, it is generally thought that the therapeutic efficacy would be improved when the origin of vaccinated peptide is functionary essential molecule for cancer cell survival, proliferation, and/or motility. We have been screening epitope peptides derived from cancer-specific genes and reported several epitope peptides, which can elicit specific CTL responses [21–24]. Some of these peptides have been already applied for translational researches of multipeptide vaccine to treat esophageal cancer and colorectal cancer [11, 12]. Moreover, multiple-antigen vaccine therapy was suggested to more effectively hinder escape mechanisms in the guidance from Food and Drug Administration (Guidance for Industry: Clinical Considerations for Therapeutic Cancer Vaccines). Thus, we believe that identification of CTL-inducible epitope peptides derived from several molecules that play critical roles in various types of cancer is important to develop multipeptide cocktail, and that resulted in the improvement of efficacy of CTL-inducing cancer therapies.

Presented results demonstrated that CDH3-10-807 peptide and KIF20A-10-66 peptide pulsed DCs induced specific CTL to possibly exert antitumor effect. The immunogenicity of CDH3-10-807 peptide and KIF20A-10-66 peptide should be examined in patients bearing these genes-expressing cancers, and we are now going to conduct clinical trials.

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特集 消化器がんにおけるがんワクチン療法

Gastrointestinal Research

消化器がんにおけるがんワクチン療法 —臨床試験と治験の現状—

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Summary

2010年4月に米国食品医薬品局（FDA）により前立腺がんに対しsipuleucel-T（Provenge®）が承認された．国際的には現在，承認に向けての多数のがん免疫療法の臨床試験が進行中であり，その開発競争がますます加熱している．このなかには膵がん，大腸がんといった消化器がんも含まれている．一方，国内でも現在，多くの医師主導型臨床試験がおこなわれており，そのなかで有望な結果が得られ，企業治験へ進んでいるものもある．われわれも膵がんと食道がんを対象としてペプチドワクチン療法の臨床試験をおこなっているが，とくに膵がんを対象とした医師主導型臨床試験では第Ⅰ相試験の結果が評価され，企業主導の臨床治験をおこなうまで至っている．FDAは2009年9月にがん治療用ワクチンに関する臨床的考察として企業向けのガイダンス（ドラフト版）を公表し，がんワクチンはその作用機序を考慮すると抗がん剤とは異なった考え方で開発を進める必要があることをこと明示している．わが国では厚生労働省や医薬品医療機器総合機構（PMDA）からそのような動きはみられていないが，日本バイオセラピィ学会が2011年11月に「がん治療用ペプチドワクチンガイダンス（案）」を作成し，今後，広くパブリックコメントを募集し，本格的なガイダンス作成に取り組んでいくことになっている．

Key words

がんワクチン療法　ペプチドワクチン療法　消化器がん　臨床試験　治験　米国食品医薬品局（FDA）ガイダンス

はじめに

がんワクチン療法は2004年のRosenbergの悲観的報告[1]に代表される低迷期を乗り越え，近年，再び注目を集めている．国際的には2010年4月に米国食品医薬品局（Food and Drug Administration：FDA）によりsipuleucel-T（Provenge®；米国Dendreon社）が承認され[2]，がんワクチン療法の開発競争がますます加熱している．国内でも現在，多数の施設で多くの医師主導型臨床試験がおこなわれており，そのなかのいくつかでは有望な結果が報告されて企業主導の治験へと進んでいる．われわれも消化器固形がんのなかでも難治がんとされる膵がんと食道がんを対象としてペプチドワクチン療法の臨床試験をおこなっているが，とくに膵がんを対象とした医師主導型臨床試験[3]

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表 1. 開発中の消化器がんに対するおもな治療的ワクチン

製品名	スポンサー	開発品の内容	対象がん腫	開発段階
GV-1001	Pharmexa A/S GemVax & KAEL	hTERT ペプチド	膵がん	第Ⅲ相試験 終了
Telo Vac GV1001	Royal Liverpool 大学	hTERT ペプチド	膵がん	第Ⅲ相試験 実施中
Hyper Acute	NewLink Genetics	α-Gal 遺伝子導入膵がん細胞株	膵がん	第Ⅲ相試験 実施中
OncoVAX	Vaccinogen	患者腫瘍細胞ワクチン	大腸がん	前期第Ⅲ相試験終了

では第Ⅰ相試験の結果が適正に評価され，その結果を踏まえて企業主導の臨床治験をおこなうまで至っている．

本稿では，消化器がんを中心にがんワクチン療法の開発における臨床試験・治験の現状とわれわれのおこなった試験の概要につき述べる．

1 国際的ながんワクチン療法開発の現状

1991 年に Boon らにより細胞傷害性 T 細胞（cytotoxic T lymphocyte：CTL）に認識されるがん関連抗原が同定されて以来[4]，さまざまながん腫において腫瘍抗原が同定され，基礎研究を経て現在まで多くの臨床試験がおこなわれてきた．そして規制当局によりすでにスイス，ロシア，キューバ，オランダなどでがんワクチン療法が承認されており，2010 年 4 月には米国 FDA により前立腺がんに対する樹状細胞（dendritic cell：DC）ワクチン製剤である sipuleucel-T が承認された[2]．現在も承認に向けての多数のがん免疫療法の臨床試験が進行中であり[5]，ますます開発競争が激しくなっている．このなかの多くは前立腺がん，悪性黒色腫，肺がん，腎がんに対するものであるが，膵がん，大腸がんといった消化器がん（表 1）も含まれている．

2 FDA がん治療用ワクチンのガイダンスからみるがんワクチン開発の留意点

前述したように 2010 年 4 月に米国 FDA により前立腺がんに対して sipuleucel-T が承認されたが，それに先立ち FDA は 2009 年 9 月にがん治療用ワクチンに関する臨床的考察として企業向けのガイダンス（ドラフト版）[6]を公表し（http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm182443.htm），広く意見を求めた．

その背景に，従来の抗がん剤とまったく違う考え方で臨床試験デザインを考慮する必要があり，その理由としてはワクチンの投与が臨床効果に結びつくためには相当な時間を要するためであると述べられている．その内容をみると，医師主導臨床試験を立案するにおいても参考になるいくつかの注目すべきポイントがあるので，その一部を紹介し，われわれなりの解説を加えた．

1）対象患者について

〔進行した患者の場合は投与開始から病状悪化までの期間が短く，抗腫瘍免疫応答が得られるまでの時間が確保できない可能性があるのに対し，残存病変のない患者や微小ながんをもつ患者では

がんワクチンによる免疫活性化のための適切な時間を確保することができる.〕

以前より多くのがんワクチンに携わる研究者は，切除不能進行がん・再発がんを対象とするよりむしろ術後のアジュバントなどがよい適応となると考えてきた．しかし，その場合，微妙な差を検出できるだけの莫大な症例数と費用，時間が必要であるため，研究者はやむを得ず進行再発患者を対象として試験をおこなっているという現実がある.

2）安全性と用量増加

〔用いるがんワクチンが数多くの臨床試験において広く使用されてきたクラスのものであれば安全性はすでに確立されている．このような状況では開始用量を設定するうえで前臨床試験は必要がない．開始用量と用量増加スケジュールは，過去にヒトへ投与された経験から導き出すことが可能である．多くのがんワクチン臨床試験では非常にまれな状況を除き最大耐量（maximum tolerated dose：MTD）は認められていない．用量-毒性曲線は平坦であり，考えられる最も高い用量は，毒性よりも，製造上または投与部位の解剖学的な問題により規定される.〕

とくにペプチドワクチンなどはすでに多くの種類のペプチドが人に投与されその安全性が報告されている．第Ⅰ相試験においては少なくとも皮下投与の場合，開始用量は過去の経験から設定可能であり，毒性をみるための用量増量は意味がないと考えてよいのであろう．ただ第Ⅱ相試験への推奨投与量の決定に関しては触れられていない.

3）免疫モニタリング

〔ワクチンが免疫応答を実際に引き起こすかを知るのみならず，後の開発や後期臨床試験デザインおよびその評価において免疫モニタリングはきわめて重要である．2種以上の複数の免疫学的アッセイ法を用いることが推奨される.〕

それぞれのがんワクチンに応じたアッセイ法を用いる必要がある．第Ⅱ相試験以降へ進める根拠ともなるため，rationale に合致する再現性の高い方法を用いることが望まれる.

4）効果発現の遅延とその評価

〔がんワクチンは投与後に免疫を誘導するための期間が必要であり，投与後早期には腫瘍が増殖し，その後引きつづいて抗腫瘍反応が起こる場合がある．このワクチンの遅延効果のため生存曲線は治療後早期には差を示さないが，治療効果がある場合は後期に曲線の分離が起こることが観察される．これにより Cox モデルを適用した場合には，比例的ハザード仮定にしたがわず，統計学的仮説を実証するには症例数の増加を余儀なくされる事態が生じる.〕

つまり抗腫瘍免疫応答が誘導されるまである程度時間を要し，その後，誘導された抗腫瘍免疫活性が治療効果を示すと考えられるため，早期と後期ではハザード比が変化すると考えられる．このような場合の統計解析には Harrington-Fleming 検定[7]が適切といわれている[8].

以上の内容の詳細はぜひ原文を参照されたい．なお 2011 年 10 月にドラフト版でなく最終版が発行されるに至っている（http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/）.

3 わが国における消化器がんに対する医師主導型臨床試験の現状

わが国でも 1980 年代より腫瘍免疫の基礎研究が盛んにおこなわれてきた．1991 年にがん関連抗原が同定[4]されて以降は，研究の中心ががんワクチン療法にシフトし，さまざまな基礎研究，臨床研究がおこなわれ，多くの研究論文も発表された．しかし，将来の創薬を念頭に置いて立案された臨床試験は非常に少ないといわざるを得ない．しかし，最近の数年間の進歩はめざましいものが

表 2. わが国における消化器がんに対するがんワクチン療法の臨床試験

対象がん腫	試験数	施設		投与ワクチン		試験デザイン	
膵がん	17	単施設	16	ペプチド	16	第Ⅰ相試験	6
		他施設	1	DC	1	第Ⅰ/Ⅱ相試験	5
						第Ⅱ相試験	5
						その他	1
食道がん	15	単施設	12	ペプチド	13	第Ⅰ相試験	9
		他施設	3	DC	1	第Ⅰ/Ⅱ相試験	2
				蛋白	1	第Ⅱ相試験	3
						その他	1
肝細胞がん	15	単施設	15	ペプチド	15	第Ⅰ相試験	4
						第Ⅰ/Ⅱ相試験	4
						第Ⅱ相試験	7
大腸がん	14	単施設	11	ペプチド	13	第Ⅰ相試験	3
		他施設	3	DC	1	第Ⅰ/Ⅱ相試験	7
						第Ⅱ相試験	4
胃がん	10	単施設	10	ペプチド	10	第Ⅰ相試験	2
						第Ⅰ/Ⅱ相試験	6
						第Ⅱ相試験	2
胆管がん	9	単施設	9	ペプチド	9	第Ⅰ相試験	5
						第Ⅰ/Ⅱ相試験	1
						第Ⅱ相試験	3
消化器がん	11	単施設	12	ペプチド	13	第Ⅰ相試験	9
		他施設	3	DC	1	第Ⅰ/Ⅱ相試験	2
				蛋白	1	第Ⅱ相試験	3
						その他	1

2011年10月の時点でclinicaltrials.govまたはUMINに登録されている消化器がんを対象とした国内臨床試験を列挙した．胃がん・大腸がんおよび膵がん・胆管がんの重複試験2件を含む．

ある．これは新規腫瘍抗原の同定，免疫モニタリング法の開発，免疫抑制メカニズムの解明，アジュバントの開発など基礎研究の進歩に加えて，（とくに臨床側の）研究者自身ががんワクチンの臨床的有用性を客観的に証明するために充分に計画された臨床試験の重要性を認識するようになったためと考えられる．

2011年10月の時点で，clinicaltrials. govまたは大学病院医療情報ネットワーク（University Hospital Medical Information Network：UMIN）に登録されている国内臨床試験のなかで，消化器がんを対象とするがんワクチン療法の臨床試験は95件あり（表2），その対象がん腫の内訳は膵がん17件，食道がん15件，肝細胞がん15件，大腸がん14件，胃がん10件，胆管がん9件，消化器がん11件（重複あり）である．そのほとんどはペプチドを用いた試験であるが，DCワクチンや蛋白を用いたものも一部に含まれる．標的分子はmelanoma antigen-encoding gene（MAGE)-A4, Wilms tumor（WT)[9]，NY-ESO-1[10]など代表的な腫瘍抗原からkinesin-like family member 20A（KIF20A)，up-regulated lung cancer 10

表 3. わが国におけるがんワクチン療法の臨床治験

企業	治験薬コード	対象疾患	進捗状況
塩野義製薬	S-488410	食道がん	第Ⅰ/Ⅱ相試験実施中
	S-288310	膀胱がん	第Ⅰ/Ⅱ相試験実施中
大塚製薬・扶桑薬品工業	OTS11101	固形がん	第Ⅰ相試験終了
	OCV-101	膵がん	第Ⅱ相試験登録終了
	OTS102	胆道がん	第Ⅱ相試験登録終了
		膵がん	第Ⅱ/Ⅲ相試験登録終了
	OCV-105	膵がん	第Ⅰ相試験登録終了
イミュノフロンティア	IMF-001	食道がん	第Ⅰ相試験実施中
メルクセローノ	Stimuvax（BLP25）	非小細胞肺がん	第Ⅱ相試験実施中
大日本住友・中外製薬	WT4869	骨髄異形成症候群	第Ⅰ/Ⅱ相試験実施中

（URLC10），TTK protein kinase（TTK），insulin-like growth factor 2 mRNA binding protein 3（KOC1），ring finger protein 43（RNF43）などの新規に同定されたがん精巣抗原，さらに血管内皮増殖因子受容体（vascular endothelial growth factor receptor：VEGFR）1，R2などさまざまであり[11]，さらにテーラーメイド型[12]のペプチド選択をおこなっている試験など多岐にわたる．また大半は単施設の試験であり，試験デザインは第Ⅰ相試験あるいは第Ⅱ相試験である．すべてが順調に登録されているわけではないが，予定登録患者数を合計すると3,542人にのぼる．

4 わが国における消化器がんに対する臨床治験の現状と問題点

前述の医師主導型臨床試験のなかのいくつかでは有望な結果が報告され[3)9)10)13)]，企業主導の治験へと進んでいる．2011年10月の段階で表3に示すような企業治験が進行中である．消化器がんでは現在，膵がん，食道がん，胆道がんを対象にした治験がおこなわれている．このなかでは後述するように進行膵がんに対するゲムシタビン塩酸塩（gemcitabine：GEM）とVEGFR2ペプチド（OTS102：大塚製薬・扶桑薬品工業）の治験が先行しており，2010年1月で第Ⅱ/Ⅲ相試験として153例の症例登録が終了し，現在，追跡調査中である（執筆時）．

5 和歌山県立医科大学第2外科におけるペプチドワクチン療法の臨床試験

われわれは，東京大学医科学研究所の中村祐輔教授が中心となって組織されたペプチドワクチン療法臨床研究ネットワークであるCaptivation Network[11]のメンバーとして，将来の創薬をめざしたペプチドワクチン療法の臨床研究をおこなっている．われわれは消化器固形がんのなかでも難治がんとされる食道がんと膵がんを対象として，2つの臨床試験をおこなった．

1つは，CpG-oligodeoxynucleotide（CpG-ODN）の強力な1型インターフェロン（IFN）の産生誘導能に注目し，human leukocyte antigen（HLA）-A＊2402陽性標準治療不応の進行食道扁平上皮がん患者を対象に新規がん精巣抗原であるURLC10およびTTK由来エピトープペプチド[14]