

# Superantigen-SEA gene modified tumor vaccine for hepatocellular carcinoma: An *in vitro* study

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## Abstract

**AIM:** To construct an eukaryotic superantigen gene expression vector containing the recombinant gene of SEA and CD80 molecule transmembrane region (CD80TM), and to express staphylococcus enterotoxin A (SEA) on the membrane of hepatocellular carcinoma (HCC) cell to form a superantigen gene modified tumor vaccine for HCC.

**METHODS:** SEA and linker-CD80TM gene were amplified through PCR from plasmid containing cDNA of SEA and CD80. Gene fragments were then subcloned into the multiple cloning sites of retroviral vector pLXSN. Recombinant plasmid was transferred into HepG2 cells mediated with lipofectamine, positive clones were selected in culture medium containing G418. RT-PCR and indirect immunofluorescence studies confirmed that SEA was expressed specifically on HCC cell membrane. INF- $\gamma$ -ELISPOT study demonstrated that SEA protein was expressed on the membrane of HCC cells. Cytotoxicity of HepG2-SEA primed CTLs (SEA-T) was analyzed by  $^{51}\text{Cr}$  release assay. T cells cultured with rhIL-2 (IL-2-T) were used as control.

**RESULTS:** Restriction digestion and sequence analyses confirmed the correctness of length, position and orientation of inserted fusion genes. SEA was expressed on the surface of HepG2 cells, HepG2-SEA had strong stimulating effect on production of HepG2 specific CTL ( $P < 0.001$ ). SEA-T had enhanced cytotoxicity to HepG2 cells ( $P < 0.05$ ).

**CONCLUSION:** Tumor cell membrane expressed superantigen can be used to reinforce the immune effect of tumor cell vaccine for HCC, which provides a new method of the enhanced active immunotherapy for HCC.

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## INTRODUCTION

Autologous and allogeneic tumor cells have been used as tumor

vaccines in clinic for a long time<sup>[1,2]</sup>. The tumor cell-based vaccines possess the entire relevant tumor antigens recognized by the immune system and can be produced even without knowing the gene sequence of specific antigens. To identify the tumor specific antigen is time consuming, only few tumor specific antigens have been identified until now. But tumor cell-based immunization could not always elicit anti-tumor responses in sufficient magnitude to cause regression of established tumors due to scarcity of stimulatory surface molecules such as MHC-I, MHC-II or CD80<sup>[3]</sup>.

Superantigens are a family of bacterial and viral proteins that bind to MHC class II molecules as unprocessed proteins and activate a large number of T cells bearing T cell receptor variable region beta chain (TCR V $\beta$ )<sup>[4,5]</sup>. These T cells proliferate, and secrete cytokines (e.g. IFN- $\gamma$ , TNF- $\alpha$ , IL-2, and IL-12), induce strong cytolytic activity, and mediate tumor regression<sup>[6-12]</sup>. SEA is an important member of superantigen family. Anchoring SEA onto MHC-II negative tumor cells through monoclonal antibody has been demonstrated to direct T cell-mediated cytotoxicity against these tumors<sup>[13,14]</sup>. Other studies suggested that artificially anchoring a recombinant superantigen-transmembrane region chimera (SAG-TM) onto a tumor cell surface could substitute for the effect of MHC-II presentation<sup>[15]</sup>.

In the present study we sought to construct an eukaryotic expression vector containing recombinant gene of SEA and transmembrane region sequence of CD80 molecule (CD80TM). Recombinant gene was transfected into HCC cells, chimera protein (SEA-CD80TM) was expressed on the membrane of living cells. SEA gene modified cells were irradiated and used as a superantigen enhanced tumor cell-based vaccine for HCC, which was capable of inducing antitumor immunity *in vitro*.

## MATERIALS AND METHODS

### Reagents

EX Taq DNA polymerase, T4 DNA ligation kit Ver.2.0, and restriction endonuclease were obtained from TakaRa Biotechnology (Dalian). DNA isolation and purification kit was purchased from Shanghai ShunHua Biotechnology. Lipofectamine™ 2000 were from Invitrogen. IPTG, X-gal, DNA maker and FITC labeled sheep anti mouse IgG were from Sino-American Biotechnology. Dulbecco's modified Eagle media, Trizol and G418 were from GibcoBRL. Access RT-PCR system was from Promega. Human INF- $\gamma$ ELISPOT assay kit was from Diaclone, Recombinant human interleukin-2 (rhIL-2) and BD TriTEST™ antibody CD4 FITC/ CD8 PE/ CD3 PerCP were from BD Biosciences. Mouse anti-SEA monoclonal antibody was provided by the Department of Immunology, Fourth Military Medical University, P. R. China.

### Plasmids

PBluescript II KS (+) plasmids containing cDNA of SEA and CD80 were constructed by Li *et al*<sup>[16]</sup>. Retroviral vector pLXSN was preserved in our laboratory.

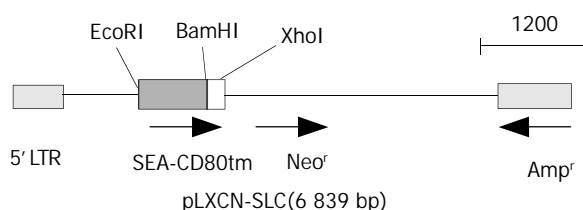
### Cell culture

Human HCC cell lines HepG2 and SMMC-7721 were cultured

in DMEM containing 10% heat-inactivated fetal calf serum, 100 units/ml penicillin, 100 µg/ml streptomycin, 0.292 mg/ml glutamine. The cell lines were incubated in a humidified 5% CO<sub>2</sub> incubator at 37 °C.

#### Polymerase chain reaction (PCR) and vector construction

The primers used for cloning SEA (774 bp) and CD80TM (147 bp) were as follows. SEA, forward: GCGAATTCGCATGAAAAACAGCATTTAC, reverse: CCGGATCCACTTGTATATAAATATATATCAAT. CD80TM, forward1: GGTGGCTCTGGCGGTGGCGGATCGGATAACCTGCTCCCATCC, forward2: CCGGATCCGGTGGAGGCGGTTTCAGGCGGAGGTGGCTCTGGCGGT, reverse: CCGCTCGAGTTATACAGGGCGTACACT. Linker (GGGSGGGSGGGSGGGS) was introduced into the upstream of CD80TM using two forward primers. PCR was performed in a 50 µl reaction system consisting of 1 µM each primer, 200 µM each dNTP, 5 µL 10×polymerase reaction buffer, and 1.25U EX Taq DNA polymerase with 1 µL SEA or CD80 gene vector. Samples were heated to 94 °C for 5 min followed by 30 cycles at 94 °C for 30 s, at 50 °C for 50 s, and at 68 °C for 1 min. The final extension was done at 72 °C for 7 min. Then 10 µl of each PCR product was electrophoresed on 1 % agarose gel containing 0.5 µg/mL EB, and PCR products were then purified from agarose gel according to the protocol of DNA purification kit. DNA fragments of SEA (S) and linker-CD80TM (LC) were subcloned into pBluescript II ks(+). The positive clones were selected from the transfected DH5α by the method described by Sambrook *et al*<sup>[17]</sup>. The constructed plasmids (designated as ks-S, ks-LC) were identified by restriction enzyme analysis. DNA sequences were verified in DNA sequencing core facility in Bioasia Biotechnology (Shanghai, China). Gene fragments were cut with *EcoRI*-*Bam*HI, *Bam*HI-*Xho*I, and inserted into the *EcoRI*-*Xho*I site of pLXSN to construct SAg gene expression vector pLXSN-SLC. The recombinant gene was verified by digestion with restriction endonuclease (Figure 1).



**Figure 1** Gene map of expression vector.

#### In vitro transfection of pLXSN-SLC

pLXSN-SLC was transfected into HepG2 cells using Lipofectamine™ 2000 reagent according to the manufacturer's instructions. Briefly, 2×10<sup>5</sup> HepG2 cells were seeded into 12-well culture plates and cultured for 18 hours until 90% to 95% confluence of the cells, transfection was performed with 1 µg of DNA per dish. Forty eight h after transfection, cells were passaged at 1:4 dilution into a fresh medium. The next day, G418 400 µg/mL was added into culture medium for the screening of stably transfected cells. The cells were then grown in G418 culture medium for 4 weeks and cloned by limiting dilution. Mock cell line transfected with empty pLXSN vector was selected in G418, and used as control (HepG2-pLXSN).

#### Detection of SEA expression in transfected cells

Expression of SEA in screened monoclones was detected by RT-PCR. Total RNA was extracted from the tumor cells (HepG2 cells and SEA gene transfected or mock transfected HepG2 cells) using Trizol reagent. RNA (1.5 µg) was used to

synthesize cDNA in 20 µl reaction mixtures following standard protocol of Access RT-PCR system. PCR was carried out with 5 µl of RT products in 50 µl PCR reaction buffer containing *Tfi* polymerase, 1 mM MgCl<sub>2</sub>, annealing temperature was 50 °C. SEA cDNA was used as positive control in PCR. Indirect immunofluorescence was performed to locate the expressed SEA. Briefly, 2×10<sup>5</sup> SEA transfected HepG2 cells (HepG2-SEA) were plated into 12-well culture plates. At the bottom of each well a sterilized coverslip was placed in advance. HepG2 cells were plated as control. When the plated cells were 95-100% confluence, the coverslips were washed twice in cold PBS (0.01M) and fixed with 4% paraformaldehyde for 30 min at room temperature. Cells were stained following the standard protocol of indirect immunofluorescence. First antibody was 1:100 diluted mouse anti-SEA monoclonal antibody in PBS, irrelevant sheep anti-mouse IgG was used as control. Second antibody was 1:500 diluted FITC labeled sheep anti-mouse IgG in 0.1% evan blue. Coverslips were then mounted directly onto a glass slide with a tiny drop of 50% glycerol in PBS (9.1 mM Na<sub>2</sub>HPO<sub>4</sub>/1.7 mM NaH<sub>2</sub>PO<sub>4</sub>/50 mM NaCl, pH 7.4). Fluorescent images were captured at 490 nm using a Nikon Eclipse E1000 microscope attached to a MicroMax camera (Princeton Instruments, Trenton, NJ).

#### Preparation of T cell lines

Human peripheral blood mononuclear cells (PBMC) were isolated from healthy volunteers by routine density centrifugation, and stimulated with HepG2-SEA to establish SEA-reactive T-cell line (SEA-T). The T cell line was stimulated repeatedly by exposure to irradiated (4 000 rad) HepG2-SEA and rhIL-2 (200 U/mL) in complete medium for 2 weeks, and then stored in frozen. These cells were thawed 1 week before use. T-cell lines stimulated by HepG2-pLXSN (pLXSN-T), HepG2 (HepG2-T) or just maintained with rhIL-2 (IL-2-T) were also prepared and used as control. Cell phenotypes of T cell lines were analyzed using BD TriTEST™ antibody by FACS.

#### Elispot assay

PVDF 96-well plates were incubated with 100 µl of 70% ethanol for 10 min at room temperature, and washed three times in 100 µl of PBS. Capture antibody was added and incubated at 4 °C overnight. After washed in 100 µl of PBS, each well was added 100 µl of 2% skimmed dry milk in PBS, and incubated for 2 hours at room temperature. Wells were emptied and tapped on absorbent paper, 5×10<sup>4</sup> effector cells (including SEA-T, pLXSN-T, HepG2-T, IL-2-T) and 1×10<sup>4</sup> target cells (irradiated HepG2) were seeded to each well and incubated for 20 h in RPMI-1640 medium without IL-2 and serum. Plates were then washed and incubated with detection antibody in PBS-1% BSA for 1 hour and 30 min at 37 °C, and with streptavidin-alkaline phosphatase for 1 h at 37 °C. After washed, substrate (5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium) was added and incubated for 5-20 min. After the final washing, dark-violet spots on plate membranes were counted under microscope.

#### Cytotoxicity of HepG2-SEA stimulated T cell line

Cytotoxicity was measured in a standard 4-hour <sup>51</sup>Cr-release assay and expressed in formula: (%)specific lysis=100×(experimental - background cpm/maximal background cpm). Target cells (HepG2, SMMC-7721) were labeled for 2 hours with Na<sub>2</sub><sup>51</sup>CrO<sub>4</sub> (<sup>51</sup>Cr, New England Nuclear, Boston, MA), 250 µCi/1×10<sup>6</sup> cells. The cells were then washed twice and 5×10<sup>3</sup> cells per well were seeded in triplicate in v-bottomed microtiter plates. Effector cells (SEA-T, HepG2-T and IL-2-T) were added at an effector-to-target (E:T) cell ratio of 40:1, 25:1, 10:1, 5:1 and 3:1, respectively. The plates were incubated

at 37 °C and 5% CO<sub>2</sub> for 4 h, supernatants were collected, and the released <sup>51</sup>Cr was measured with a gamma counter (LKB-Wallac 1282; Stockholm, Sweden). Spontaneous release was estimated by incubation of target cells in medium alone, and maximum release by resuspending the wells with 0.1% Tween 20. Spontaneous release was typically less than 30% of maximum release.

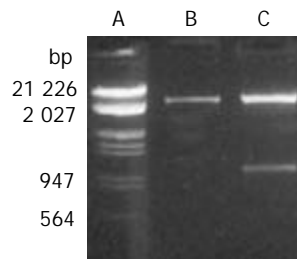
### Statistical analysis

All experiments were performed in triplicate, data were presented as the mean ± SD and analyzed using Student's *t* test.

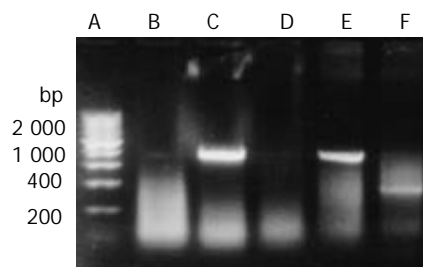
## RESULTS

### Insertion of SEA and linker-CD80TM into MCS of pLXSN

PCR products were subcloned into a pBluescript II ks (+) vector. DNA sequencing confirmed the correct sequence of both fragments. SEA gene was cloned into the *EcoR* I/*Xho* I site of pLXSN with linker-CD80TM. Recombinant gene SEA-linker-CD80TM was 1.0 kb with *EcoR* I and *Xho* I restriction site on each side, the size of pLXSN was 5.9 kb. Expressing vector pLXSN-SLC was digested by *EcoR* I/*Xho* I, 1.0 kb and 5.9 kb DNA fragments were appeared as expected (Figure 2).



**Figure 2** Enzyme digestion analysis of expression vector. A. Lambda DNA (*EcoR*I, *Hind*III); B. pLXSN (*EcoR*I, *xho*I); C. pLXSN-SLC (*EcoR*I, *xho*I).



**Figure 3** RT-PCR detected the transcription of SEA gene. A. 200 bp DNA marker; B. HepG2; C. HepG2 transfected with pLXSN-SLC; D. HepG2 transfected with pLXSN; E. Positive control; F. β-actin (RNA of C).

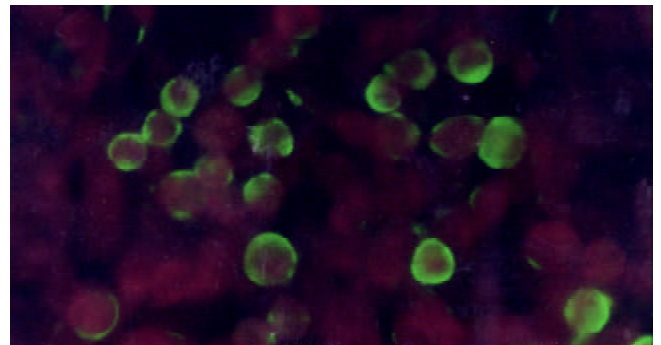
### SEA expressed on the membrane of HepG2

HepG2 was transfected with plasmid pLXSN-SLC and empty pLXSN vector, respectively. The expression of SEA was detected in HepG2 cells transfected with pLXSN-SLC only by RT-PCR (Figure 3). Indirect immunofluorescence test confirmed that most of the expressed SEA appeared to be located on the HepG2 cells membrane (Figure 4). SEA expression was detected in 5-10 percent of cells screened with G418. Irradiated HepG2-SEA cells were used as T cell stimulator in following experiments.

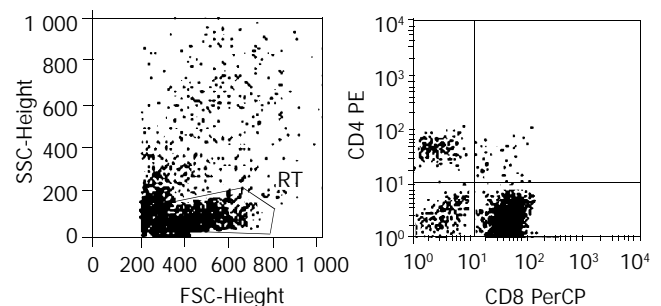
### Increased HepG2 reactive T cells with HepG2-SEA

Interferon-γ (IFN-γ) ELISPOT assay was used to detect the

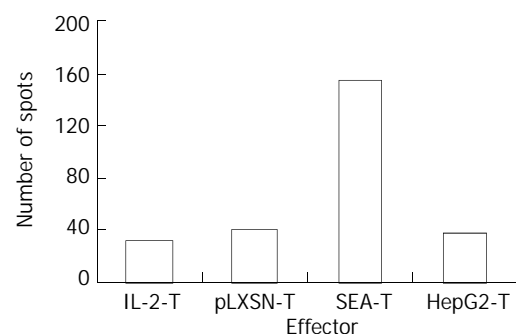
specificity of HepG2 CD8<sup>+</sup> T cells quantitatively. Cells tested included unstimulated PBMC (IL-2-T) and polyclonal T-lymphocyte lines stimulated by HepG2-SEA, HepG2-pLXSN or HepG2. After a short term of culture *in vitro*, the percentage of positive cells was more than 90% for CD3<sup>+</sup>, 80% to 90% for CD8<sup>+</sup>, and 5% to 10% for CD4<sup>+</sup> (Figure 5). The number of HepG2 reactive CTL in the SEA-T cell line was much higher than that of HepG2-T (155.67±7.22 vs 38.03±8.18, *t*=18.67, *P*<0.0001), pLXSN-T (39.50±6.26, *t*=21.06, *P*<0.0001) or IL-2-T (32.00±4.26, *t*=25.56, *P*<0.0001) (Figure 6). Both the size and density of spots in SEA-T group were greater than those of controls (data not shown). The difference of spot number between HepG2-T and IL-2-T cell lines was not significant (*t*=-1.13, *P*=0.321).



**Figure 4** SEA expressed on the membrane of HepG2 (indirect immunofluorescence). HepG2-SEA cells were stained according to the standard protocol of indirect immunofluorescence. First antibody is mouse anti-SEA IgG and second antibody is FITC labeled sheep anti-mouse IgG diluted in 0.1% evan blue. Positive signal is green while the background is red. SEA was located on the membrane of HepG2. Untransfected HepG2 cells did not express SEA (data not show).



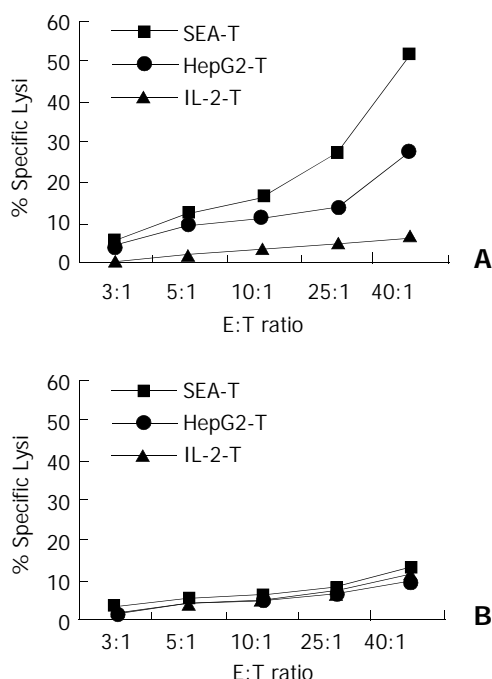
**Figure 5** Lymphocyte phenotype analysis. 1×10<sup>5</sup> SEA-T cells were stained with BD TriTEST™ antibody CD4 FITC/CD8 PE/CD3 PerCP, and assayed by FACS. CD3<sup>+</sup>90.75%, CD8<sup>+</sup>84.46%, CD4<sup>+</sup>6.29%.



**Figure 6** HepG2-SEA increased the number of hepG2 specific CTL in T lymphocyte line. Each bar represents average of spots in triplicate wells.

### Enhanced cytotoxicity elicited by membrane expressed SEA

Cytotoxicity of the SEA-T effector cell line against HepG2, and SMMC-7721 target cells was measured (Figure 7). HepG2-T and IL-2-T cells were used as control. In contrast, SEA-T was highly efficient in directing specific lysis of HepG2 cells when compared with HepG2-T ( $51.55 \pm 5.051\%$  vs  $33.16 \pm 4.54$ ,  $t=4.47$ ,  $P=0.011$ ). The two T cell lines had effective cytotoxicity to SMMC-7721 cells.



**Figure 7** Membrane expressed SEA enhanced specific lysis on autologous tumor cells. Cytotoxicity was measured in a standard 4-hour  $^{51}\text{Cr}$ -release assay. Target cells (HepG2, SMMC-7721) were seeded in triplicate in v-bottomed microtiter plates at a concentration of  $5 \times 10^3$  cells per well. Effector cells were added at an effector-to-target (E:T) cell ratio of 40:1, 25:1, 10:1, 5:1 and 3:1 respectively. A: SEA-T was highly efficient in directing specific lysis of HepG2 cells (squares), HepG2-T has moderate cytotoxicity on HepG2 (cross) cells ( $P<0.001$ ). B: None of T cell line has killing effect on SMMC-7721 cells.

### DISCUSSION

T cells have been found to possess various potent antitumor effects through releasing cytotoxic effector molecules such as perforin or growth-suppressive cytokines IFN- $\gamma$  and TNF- $\alpha$ <sup>[18-21]</sup>. However, the frequency of tumor-specific T cells could be generally too low and insufficient to interfere with progressive tumor growth. The goal of this experiment was to examine the possibility of developing a new experimental vaccine from irradiated HepG2 cells that were transfected to express superantigen-SEA on membrane (designed as HepG2-SEA) to augment cellular and humoral anti-tumor immune responses. Superantigen is the most effective T cell activator. Upon stimulation by superantigens, naïve T cells could respond and then become quickly anergized and/or deleted, while mature T cells could not become anergized<sup>[22-24]</sup>. Characteristics of superantigens can thus be exploited to enhance specific antigen responses. Because superantigens can cause anergy and/or deletion of potentially competing naïve T cells bearing the same V $\beta$  element(s), thus there is less “competition” for cytokines and the desired specific immune response can be amplified.

HCC cells do not induce an effective immunological rejection process because of their weak antigenicity, the abnormal expression of MHC molecules as well as the

deletion or deficiency of costimulatory molecules. In the present study, the expressed superantigen-SEA on the membrane of HepG2 cells was confirmed by RT-PCR and indirect immunofluorescence assays. When HepG2-SEA cells were used as a vaccine, two stimulating signals would present to T cells simultaneously, one was SEA, the other was tumor-associated antigens expressed on the surface of HepG2 cells, which facilitated the occurrence of specific anti-tumor immune reaction.

IFN- $\gamma$  ELISPOT (enzyme-linked immunospot) assay was used for the quantitative detection of HepG2-specific CD8 $^{+}$  T cells. This technique could detect T cells that secrete a given cytokine (e.g., IFN- $\gamma$ ) in response to an antigenic stimulation<sup>[25]</sup>. The size and density of spots represented the quantity of IFN- $\gamma$  secreted by single CTL<sup>[18]</sup>. The results showed that the number of HepG2 cell specific CTLs in repeatedly stimulated T cell line (SEA-T) with new vaccines was significantly increased than that of HepG2 cell-based vaccines ( $P<0.001$ ). Cytotoxic assay demonstrated that SEA-T was highly efficient in directing specific lysis of HepG2 cells other than SMMC-7721 cells. When compared with HepG2-T, the difference induced by new vaccines had statistical significance ( $P=0.011$ ). So we conclude that tumor cell membrane expressed SEA can stimulate the proliferation of tumor specific CTL and enhance the specific lysis on autologous tumor cells.

This superantigen modified tumor cell-based vaccine has an advantage over ordinary tumor cell-based vaccines in three aspects. First, this vaccine could make up for the disadvantage of low-level expression of class II MHC molecule on the surface of most tumor cells<sup>[26]</sup>. It has been found that T cell activation by SEA superantigen could occur in the absence or presence of MHC class II<sup>[14,27-29]</sup>, and artificially anchoring a superantigen onto a cell surface through transmembrane protein could substitute for MHC-II presentation<sup>[15]</sup>. Second, although most immunotherapeutic strategies have focused on activating tumor-specific CD8 $^{+}$  T cells, optimal antitumor activity could be achieved if both CD4 $^{+}$  and CD8 $^{+}$  tumor specific T cells were induced<sup>[30,31]</sup>. Likewise, if tumor immunity plays a role in limiting the recurrence of primary tumor or the future onset of metastatic diseases, then CD4 $^{+}$  and CD8 $^{+}$  immunological memory should be optimized. Both CD4 $^{+}$  and CD8 $^{+}$  T cells can be activated by bacterial superantigens. Third, superantigens could preferentially direct cytotoxicity against MHC-II-positive cells<sup>[32-34]</sup>, *in vivo* administration of intact superantigens in sufficiently therapeutic amount could produce unwanted cytotoxicity to normal cells. Anchoring of superantigen and other TM fusion proteins on the surface of tumor cells would offer a novel strategy for the use of superantigens as immunostimulatory molecules. This method can confine the effect of SEA within the tumor, thus decreasing the side effects of treatment.

As presented above, irradiated HepG2-SEA cells could be used as an effective tumor cell-based vaccine *in vitro*. The superantigen enhanced vaccine is superior to tumor cell alone on the prime of tumor cell specific T cells, which provides a new strategy for the addition of superantigen as immunostimulatory molecules in tumor therapy. *In vivo* studies are currently under way to evaluate the effectiveness of these superantigen based vaccines for cancer therapy.

### REFERENCES

- 1 Teshima T, Mach N, Hill GR, Pan L, Gillesen S, Dranoff G, Ferrara JL. Tumor cell vaccine elicits potent antitumor immunity after allogeneic T-cell-depleted bone marrow transplantation. *Cancer Res* 2001; **61**: 162-171
- 2 Nelson WG, Simons JW, Mikhak B, Chang JF, DeMarzo AM, Carducci MA, Kim M, Weber CE, Baccala AA, Goeman MA, Clift

- SM, Ando DG, Levitsky HI, Cohen LK, Sanda MG, Mulligan RC, Partin AW, Carter HB, Piantadosi S, Marshall FF. Cancer cells engineered to secrete granulocyte-macrophage colony-stimulating factor using *ex vivo* gene transfer as vaccines for the treatment of genitourinary malignancies. *Cancer Chemother Pharmacol* 2000; **46**(Suppl): S67-S72
- 3 **Antonia SJ**, Seigne J, Diaz J, Muro-Cacho C, Extermann M, Farmelo MJ, Friberg M, Alsarraj M, Mahany JJ, Pow-Sang J, Cantor A, Janssen W. Phase I trial of a B7-1 (CD80) gene modified autologous tumor cell vaccine in combination with systemic interleukin-2 in patients with metastatic renal cell carcinoma. *J Urol* 2002; **167**: 1995-2000
  - 4 **Marrack P**, Kappler J. The staphylococcal enterotoxins and their relatives. *Science* 1990; **248**: 705-711
  - 5 **Fraser JD**. High-affinity binding of staphylococcal enterotoxins A and B to HLA-DR. *Nature* 1989; **339**: 221-223
  - 6 **Kotzin BL**, Leung DY, Kappler J, Marrack P. Superantigens and their potential role in human disease. *Adv Immunol* 1993; **54**: 99-166
  - 7 **Leung DY**, Gately M, Trumble A, Ferguson-Darnell B, Schlievert PM, Picker LJ. Bacterial superantigens induce T cell expression of the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen, via stimulation of interleukin 12 production. *J Exp Med* 1995; **181**: 747-753
  - 8 **Sriskandan S**, Evans TJ, Cohen J. Bacterial superantigen-induced human lymphocyte responses are nitric oxide dependent and mediated by IL-12 and IFN- $\gamma$ . *J Immunol* 1996; **156**: 2430-2435
  - 9 **Lando PA**, Hedlund G, Dohlsten M, Kalland T. Bacterial superantigens as anti-tumour agents: induction of tumour cytotoxicity in human lymphocytes by staphylococcal enterotoxin A. *Cancer Immunol Immunother* 1991; **33**: 231-237
  - 10 **Ochi A**, Migita K, Xu J, Siminovitch K. *In vivo* tumor immunotherapy by a bacterial superantigen. *J Immunol* 1993; **151**: 3180-3186
  - 11 **Dow SW**, Elmslie RE, Willson AP, Roche L, Gorman C, Potter TA. *In vivo* tumor transfection with superantigen plus cytokine genes induces tumor regression and prolongs survival in dogs with malignant melanoma. *J Clin Invest* 1998; **101**: 2406-2414
  - 12 **Shu S**, Krinock RA, Matsumura T, Sussman JJ, Fox BA, Chang AE, Terman DS. Stimulation of tumor-draining lymph node cells with superantigenic staphylococcal toxins leads to the generation of tumor-specific effector T cells. *J Immunol* 1994; **152**: 1277-1288
  - 13 **Wang Q**, Yu H, Zhang L, Ju D, Pan J, Xia D, Yao H, Zhang W, Wang J, Cao X. Adenovirus-mediated intratumoral lymphotactin gene transfer potentiates the antibody-targeted superantigen therapy of cancer. *J Mol Med* 2002; **80**: 585-594
  - 14 **Ihle J**, Holzer U, Krull F, Dohlsten M, Kalland T, Niethammer D, Dannecker GE. Antibody-targeted superantigens induce lysis of major histocompatibility complex class II-negative T-cell leukemia lines. *Cancer Res* 1995; **55**: 623-628
  - 15 **Wahlsten JL**, Mills CD, Ramakrishnan S. Antitumor response elicited by a superantigen-transmembrane sequence fusion protein anchored onto tumor cells. *J Immunol* 1998; **161**: 6761-6767
  - 16 **Li Z**, Sui Y, Jiang Y, Lei Z, Shang J, Zheng Y. Reconstruction of SEA-B7.1 double signals on human hepatocellular carcinoma cells and analysis of its immunological effect. *Biochem Biophys Res Commun* 2001; **288**: 454-461
  - 17 **Sambrook J**, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual, 2nd ed. New York: Cold Spring Harbor Laboratory Press 1989
  - 18 **Clay TM**, Hobeika AC, Mosca PJ, Lysterly HK, Morse MA. Assays for monitoring cellular immune responses to active immunotherapy of cancer. *Clin Cancer Res* 2001; **7**: 1127-1135
  - 19 **Street SE**, Cretny E, Smyth MJ. Perforin and interferon- $\gamma$  activities independently control tumor initiation, growth, and metastasis. *Blood* 2001; **97**: 192-197
  - 20 **Masson D**, Tschopp J. Isolation of a lytic, pore-forming protein (perforin) from cytolytic T-lymphocytes. *J Biol Chem* 1985; **260**: 9069
  - 21 **Merger M**, Viney JL, Borojevic R, Steele-Norwood D, Zhou P, Clark DA, Riddell R, Maric R, Podack ER, Croitoru K. Defining the roles of perforin, Fas/FasL, and tumour necrosis factor alpha in T cell induced mucosal damage in the mouse intestine. *Gut* 2002; **51**: 155-163
  - 22 **Stohl W**, Elliott JE, Lynch DH, Kiener PA. CD95 (Fas)-based, superantigen-dependent, CD4+ T cell-mediated down-regulation of human *in vitro* immunoglobulin responses. *J Immunol* 1998; **160**: 5231-5238
  - 23 **Torres BA**, Perrin GQ, Mujtaba MG, Subramaniam PS, Anderson AK, Johnson HM. Superantigen enhancement of specific immunity: antibody production and signaling pathways. *J Immunol* 2002; **169**: 2907-2914
  - 24 **Mahlknecht U**, Herter M, Hoffmann MK, Niethammer D. The toxic shock syndrome toxin-1 induces anergy in human T cells *in vivo*. *Hum Immunol* 1996; **45**: 42-45
  - 25 **Herr W**, Schneider J, Lohse AW, Meyer zum Buschenfelde KH, Wolfel T. Detection and quantification of blood-derived CD8+ T lymphocytes secreting tumor necrosis factor alpha in response to HLA-A2.1-binding melanoma and viral peptide antigens. *J Immunol Methods* 1996; **191**: 131-142
  - 26 **Baskar S**. Gene-modified tumor cells as cellular vaccine. *Cancer Immunol Immunother* 1996; **43**: 165-173
  - 27 **Gidlöf C**, Dohlsten M, Lando P, Kalland T, Sundstrom C, Totterman TH. A superantigen-antibody fusion protein for t-cell immunotherapy of human B-lineage malignancies. *Blood* 1997; **89**: 2089-2097
  - 28 **Lando PA**, Dohlsten M, Hedlund G, Akerblom E, Kalland T. T cell killing of human colon carcinomas by monoclonal-antibody-targeted superantigens. *Cancer Immunol Immunother* 1993; **36**: 223-228
  - 29 **Holzer U**, Bethge W, Krull F, Ihle J, Handgretinger R, Reisfeld RA, Dohlsten M, Kalland T, Niethammer D, Dannecker GE. Superantigen-staphylococcal-enterotoxin-A-dependent and antibody-targeted lysis of GD2-positive neuroblastoma cells. *Cancer Immunol Immunother* 1995; **41**: 129-136
  - 30 **Mortara L**, Gras-Masse H, Rommens C, Venet A, Guillet JG, Bourgault-Villada I. Type 1 CD4+ T-cell help is required for induction of antipeptide multispecific cytotoxic T lymphocytes by a lipopeptidic vaccine in rhesus macaques. *J Virol* 1999; **73**: 4447-4451
  - 31 **Yu Z**, Restifo NP. Cancer vaccines: progress reveals new complexities. *J Clin Invest* 2002; **110**: 289-294
  - 32 **Torres BA**, Kominsky S, Perrin GQ, Hobeika AC, Johnson HM. Superantigens: the good, the bad, and the ugly. *Exp Biol Med* 2001; **226**: 164-176
  - 33 **Herrmann T**, Maryanski JL, Romero P, Fleischer B, MacDonald HR. Activation of MHC class I-restricted CD8+ CTL by microbial T cell mitogens. Dependence upon MHC class II expression of the target cells and V beta usage of the responder T cells. *J Immunol* 1990; **144**: 1181-1186
  - 34 **Hansson J**, Ohlsson L, Persson R, Andersson G, Ilback NG, Litton MJ, Kalland T, Dohlsten M. Genetically engineered superantigens as tolerable antitumor agents. *Proc Natl Acad Sci U S A* 1997; **94**: 2489-2494