CD80 transfected human hepatocellular carcinoma cells activate cytotoxic T lymphocytes to target HCC cells with shared tumor antigens

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Abstract. Human HCC cell lines (BEL-7402, SMMC-7721 and QGY-7703) do not express CD80 molecules, although they express MHC class I molecules and ICAM-1. HCC's poor immunogenicity may therefore be due to lack of CD80 molecules. This study first investigated whether CD80 molecules could provide minimal co-stimulatory signal for establishing an efficient anti-tumor immunity in HCC and second, whether the transfection of CD80 into the BEL-7402 cell line could induce T cell activation for targeting other HCC cell lines expressing shared common antigens. The transfection of cDNA encoding CD80 into ICAM-1⁺ HCC BEL-7402 cells was confirmed by flow cytometrical analysis. The CD80-transfected cells could enhance the immunogenicity of BEL-7402 cells as detected by T cell proliferation assay, and also activated the T cells at a higher proliferation rate comparing with the BEL-7402 cells transfected with vector only. The CD80-transfected cell line was also found able to activate T cells which subsequently induced cell lysis of SMMC-7721, QGY-7703 and parent BEL-7402 cell lines as detected by cytotoxicity assay. It can be concluded that the cytotoxicity was due to MHC class I restricted CD8⁺ cytotoxic T lymphocytes, but not natural killer (NK) cells, since this cytotoxic effect could be blocked by anti-MHC class I antibody and the cytotoxicity was shown very low in NK-cell-sensitive K562 cell line. Electroporation of CD80 cDNA into human HCC cells could increase the expression of the functional CD80 molecules and enhance the immunogenicity of the genetically-modified HCC cells to activate T cells for targeting 3 HCC cell lines in an HLA-restricted manner.

Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer death in China (1) and ranks as the fifth most frequent malignancy in the world, accounting for almost 1 million deaths annually (2,3). HCC rarely responds to conventional cancer treatment such as radiation therapy or chemotherapy, and usually causing death within several months of detection (4). Several types of immunotherapy including lymphokine-activated killer (LAK) cell injections have been used for HCC treatment. However, no satisfactory results were obtained (5-8), as HCC is generally considered a very non-immunogenic cancer.

Accumulating evidence suggests that the lack of ability of the host immune system to eradicate tumors is not totally due to the absence of recognizable tumor antigens, but rather the inability of these antigens to stimulate an effective immune response (9). To overcome this problem, an approach involves the engineering of tumor cells to express the costimulatory molecules of the B7 family. Tumor-specific T lymphocytes recognize peptides derived from proteins synthesized by tumor cells and present them on their cell surface by MHC molecules (10). However, T cells require 2 activating signals to express full effector functions (11). Signal 1 is generated when the T cell receptor (TCR) interacts with the MHC-peptide complex, and signal 2 is provided by co-stimulatory molecules expressed by professional antigen-presenting cells (APCs). In the absence of co-stimulation, T-cell receptor antigen interactions appear to induce an anergic state in T cells (12). CD80, one of the co-stimulatory molecules, may need to synergize with other co-stimulatory molecules such as ICAM-1 to reach a critical activation threshold in the responding T cells. One of the most characterized reactions involving co-stimulatory molecules is the interaction of ICAM-1, expressed on APC with LFA-1 presented on T cells (13).

Although many cancer strongly express HLA class I antigens (which is generally the first signal), they do not express co-stimulatory molecules, particularly in HCC (14), thus failing to activate tumor-specific T lymphocytes due to lack of the second signal (i.e. signals from co-stimulatory molecules) (15-17). This finding has provided a rationale for the introduction of genes encoding co-stimulatory molecules

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into tumor cells to increase their immunogenicity and vaccination potential. Among the different co-stimulatory molecules, CD80 molecules are of particular interest, since they are predominantly expressed on professional APCs, and play an integral role in activating T cells by interacting with T cell-receptors (18,19), CD28 molecules (20), and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) (21).

In the present study, the human CD80 gene was cloned into a mammalian expression vector using a human spleen cDNA library as a template. The constructed plasmid was stably transfected into the ICAM-1⁺ human HCC BEL-7402 cells by electroporation. Our results showed that the CD80 transfectant could prime naïve T cells to induce MHC class I restricted lysis of targeted HCC cells expressing shared tumor antigens with the transfected cell line. We believe that the CD80 transfected cell line may be a promising approach of priming HCC patient's T cells to target autologous tumors which express shared tumor antigens. This study raises the possibility of an alternative immunotherapy against poorly immunogenic HCC.

Materials and methods

Cell lines and cell culture. Human HCC SMMC-7721, QGY-7703, BEL-7402 and human erythroleukemia, K562, cell lines were cultured in RPMI-1640 medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), 2 mM L-glutamine (Gibco), and 100 units/ml penicillin and 100 µg/ml streptomycin (Gibco) in a humidified atmosphere of 5% CO₂ at 37°C.

cDNA cloning and transfection. To construct the CD80 expression plasmid, full length cDNA of human CD80 was subcloned into multi-cloning sites of a eukaryotic pcDNA3 expression vector. The recombinant CD80 plasmid was designated as pcDNA3/B7-1, which contained a human cytomegalovirus (HCMV) promoter for high-level transcription in mammalian cells, and a neomycin resistance gene for drug selection. BEL-7402 cells were transfected with either pcDNA3 or pcDNA3/B7-1, respectively, and G418-resistant clones were isolated and amplified after G418 selection (0.2 mg/ml, Calbiochem). Cells transfected with pcDNA3/B7-1 were named BEL-7402/B7-1. Cells transfected with pcDNA3 vector only, designated as BEL-7402/pcDNA3 cells, served as the control for the experiment.

In cloning of human CD80 cDNA, marathon-ready human spleen cDNA (Clontech) was used as the template for polymerase chain reaction, using specific oligonucleotides encompassing the entire open reading frames. The following PCR primers were used: human CD80 sense 5'-AGCTGG ATCCAGCATGGGCCACACACGGAGG-3', human CD80 antisense 5'-CGATCTCGAGCTATACAGGGCGTACAC TTTC-3'.

Polymerase chain reaction was performed with the following profile: 60 sec at 95°C, 60 sec at 60°C, and 90 sec at 72°C for 30 cycles, followed by a last 10 min cycle at 72°C. The PCR products were subcloned into pcDNA3-Neo mammalian expression vector (Invitrogen). For transfections, 10⁷ exponentially growing tumor cells were resuspended in 0.5 ml of serum free ice-cold RPMI-1640 medium containing

2 µg of BglII linerized plasmid DNA pcDNA3/B7.1 and pcDNA3, transferred to 0.4-mm electroporation cuvettes (Bio-Rad, Richmond, CA) and put on ice for 5 min. Then the cells were electroporated at 250 V, 960 µF using a Bio-Rad gene pulser™ (Bio-Rad). The cuvettes were put on ice for 10 min after electroporation. Cells were plated in 100 mm-plates and incubated at 37°C for 48 h. Selection was then applied with 200 µg/ml G418 (Calbiochem). Fresh medium and neomycin were refreshed every 3 days. After 30-day culture in selection medium, the BEL-7402 cells expressing CD80 were sorted on a FACS Vantage cell sorter (Becton-Dickinson) (22) and the CD80 expression was evaluated by flow cytometry analysis.

Isolation of peripheral blood mononuclear cells and preparation of human T cells. Peripheral blood mononuclear cells (PBMC) were isolated from freshly collected blood of healthy donors (Hong Kong Red Cross Blood Transfusion Centre) by Ficoll-Hypaque (1.077 g; Pharmacia) density gradient centrifugation. The PBMC were washed with PBS and resuspended in RPMI-1640 supplement with 10% FBS. They were plated in 6-well tissue culture plates (Falcon) at a density of 2x10⁶ cells per well and incubated at 37°C for 2 h prior to experimentation.

After 2 h-incubation, the non-adherent PBMCs were removed by washing with PBS, and the T cells were isolated from these non-adherent PBMCs by nylon-wool separation according to the protocol stated by the manufacturer (Polysciences).

HLA typing. The total DNA of SMMC-7721, BEL-7402, QGY7703 and BEL-7402/B7-1 cells were isolated using DNA isolation kit (Puregene®). The HLA alleles of the 4 cell lines were identified by the Bag Histo Type SSP kits (Bag-Germany).

Flow cytometry. The CD80 expression was performed after transfection. The cells were detached by 5 ml of 5 mM EDTA in PBS and washed with PBS twice. The cells were incubated with murine monoclonal antibodies against HLA-ABC (MHC class I; Pharmingen), CD80 (Pharmingen), CD86 (Pharmingen) or ICAM-1 (Pharmingen) at 4°C for 1 h. After washing the cells with PBS twice, the cells were incubated with FITC-conjugated goat anti-mouse IgG (Zymed) in PBS with a 1:50 dilution and incubated for 30 min at 4°C. The cells were washed again with PBS twice and then resuspended in 0.5 ml 2% paraformaldehyde. Cells with the isotypical mouse Ig (Pharmingen) acted as a negative control.

T cell proliferation assay. The stimulatory effect of CD80-transfected BEL-7402 cells (BEL-7402/B7-1) on T cells was determined by T cell proliferation assay (4). BEL-7402/B7-1 and the control cell line BEL-7402/pcDNA3 (BEL-7402 transfected with vector only) were irradiated with 30 Gy of γ-radiation under a blood irradiator (Noridon Gama-Elite) and co-cultured for 96 h with T cells which had been isolated from nylon wool. After 96 h, 1 µCi tritium thymidine was pulsed for 18 h and then the cells were harvested. The amount of tritium incorporated in the DNA was detected by liquid scintillation counter (Beckman LS6500).

Enzyme-linked immunosorbent assay (ELISA). For the determination of γ interferon (IFN- γ) in the supernatant of the cultured T cells, T cells were co-cultured with BEL-7402/B7-1 and BEL-7402/pcDNA3 respectively in a 24-well tissue culture plate. The supernatant was harvested at day 2, 4 and 6, respectively, and stored at -20°C. When feasible, the supernatant was assayed for the presence of IFN- γ using ELISA kits (Pharmacia) according to the manufacturer's protocol.

In vitro cytotoxicity test. The functional assay of CD80 transfected cells was determined by cytotoxicity test. BEL-7402/B7-1 and BEL-7402/pcDNA3 were irradiated with 30 Gy and then co-cultured with fresh human T cells which were isolated from nylon column at a 10:1 ratio (T cells: target cells) in the presence of 20 U/ml hIL-2 (Shanghai Hua Xin High Biotechnology Inc) for 14 days in 24-well tissue culture plates at 37°C in a humidified atmosphere containing 5% CO₂. At days 3 and 6 of culture, 1 ml of culture medium was removed and replaced with 1 ml of fresh complete medium containing hIL-2 (20 U/ml). After 7 days of culture, the same number of irradiated BEL-7402/B7-1 and BEL-7402/pcDNA3 cells as in the previous priming were added to the wells, respectively. Cytotoxic effector lymphocytes were harvested after 14 days of incubation and subjected to cytotoxicity assay of stimulated cells. The stimulated T cells were isolated using Ficoll gradient centrifugation to remove the dead cell debris and used as effector cells in the LDH cytotoxicity assay. SMMC-7721, QGY-7703, BEL-7402 and K562 cells (10⁴ cells per well) were used as target cells in the assay. After washing the effector cells and target cells by assay medium (RPMI-1640 with 1% BSA), the effector cells were co-cultured with target cells in a 96-well round bottom plate (Falcon) for 6 h at 37°C in a humidified atmosphere containing 5% CO₂. In the indicated experiment, anti-MHC class I mAb (W6/32; Pharmingen) was incubated with the target cells for 30 min at 37°C before addition of effector cells. After 6 h, the plates were centrifuged and the supernatant was transferred to another flat-bottom ELISA plate (Falcon). Lactate dehydrogenase (LDH) detection mixture (100 μ l) (Roche) was added to each well and incubated in the dark for 30 min at room temperature. The absorbance of the samples was measured by an ELISA reader at 490 nm and the reference wavelength was 630 nm. The spontaneous release of LDH by target cells or effector cells was assayed by incubation of target cells in the absence of effector cells and vice versa. The maximum release of LDH was determined by incubation of target cells in 1% Triton X-100 in assay medium. The percentage of cell mediated cytotoxicity was determined by the following equation: cytotoxicity (%) = [(effectors and targets mix - effectors control - spontaneous)/(maximum - spontaneous)] x 100.

Statistical analysis. Statistical analysis of the CTL assay was performed with a 2-tailed Student's t-test. Values of p<0.1 were regarded as significant.

Results

CD80 and MHC class I expression in CD80 transfected BEL-7402 cells. The expression of surface molecules on BEL-7402/B7-1 and BEL7402/pcDNA3 was examined by flow

cytometry (Fig. 1). As shown in Fig. 1a, BEL-7402/B7-1 cells expressed CD80 molecules, MHC class I molecules and adhesion molecule ICAM-1, but did not express B7-2. In contrast, BEL-7402/pcDNA3 expressed MHC class I molecules and ICAM-1 only, but not CD80 or B7-2 (Fig. 1b). Furthermore, we examined whether transfection of human CD80 gene into BEL-7402 cells had any effect on T cell proliferation, but we found that BEL-7402/B7-1 and BEL-7402/pcDNA3 cells exhibited the same growth rate as BEL-7402 cells, thus it did not seem to affect cell proliferation (data not shown). The level of CD80 expression by the stable transfected BEL-7402/B7-1 cells remained consistent for up to 6 months.

Stimulation of T cell proliferation by BEL-7402/B7-1 cells. To determine whether the BEL-7402/B7-1 cells are more effective in stimulating T cells than BEL-7402/pcDNA3, the irradiated BEL-7402/B7-1 cells were co-cultured with normal donor T cells isolated by nylon wool column from non-adherent PBMCs. As a control, T cells were co-cultured with irradiated BEL-7402/pcDNA3 cells. T cells were incubated with the cell lines for 96 h to test the proliferation of T cells. The results show a dramatic stimulation of T cells by the BEL-7402/B7-1 cells (Fig. 2A). The BEL-7402/pcDNA3 cells stimulated T cell proliferation slightly (Fig. 2A), perhaps due to the presence of some APCs in the T cells isolated from nylon wool. These findings demonstrate that transfection of human costimulatory element CD80 into HCC BEL-7402 cells results in an increase in the immunogenicity of the BEL-7402 cells and stimulation of a specific T cell response, whereas transfection of the vector pcDNA3 into BEL-7402 did not increase the immunogenicity of BEL-7402 cells.

Activation of specific T cells to secrete IFN- γ by BEL-7402/B7-1. Another indication that CTLs are functionally activated is the secretion of IFN- γ . T cells from healthy donors isolated with nylon wool column were cocultured with BEL-7402/B7-1 or BEL-7402/pcDNA3 cells. The supernatant was harvested at day 2, 4 and 6, respectively and assayed for IFN- γ secretion. T cells incubated with irradiated BEL-7402/B7-1 cells exhibited a high level IFN- γ secretion compared with the BEL-7402/pcDNA3 (Fig. 2B).

Generation of MHC class I restricted antitumor CTLs targeting 3 HCC cell lines using T cells primed with BEL-7402/B7-1. T cells incubated with irradiated BEL-7402/pcDNA3 cells exhibited a low level of target cells lysis (Fig. 3B and D). In contrast, T cells stimulated with irradiated BEL-7402/B7-1 cells were more effective in inducing cytotoxicity towards 3 targeted HCC cells (Fig. 3A and C). In a subset of experiments, effector T cells from the healthy donor primed with BEL-7402/B7-1 cells were also tested for cytotoxic activity against natural killer (NK) cell susceptible K562 target cells. The results showed that the cytotoxic activity of the effector cells against K562 cells is lower than that against the 3 HCC cells, implying that this cytotoxicity is not due to the NK cells.

To determine whether the lysis of tumor targets had an MHC-restricted component, anti-HLA-ABC antibody (W6/32) was added at a final concentration of 10 μ g/ml to BEL-7402 cells. As shown in Fig. 2C and D, lysis of the BEL-7402 cells

Figure 1. Flow cytometry analysis of BEL-7402/B7-1, BEL-7402/pcDNA3, SMMC-7721, QGY-7703, BEL-7402 and K562 cells. Curves were plotted with the relative cell number against the log fluorescence intensity. The profile of positive stained (open histogram) is superimposed over the profile of negative control (shaded histogram). a, BEL-7402/B7-1 cells express HLA-ABC, B7-1 and ICAM-1. b, While BEL-7402/pcDNA3 cells express HLA-ABC and ICAM-1 only. c, d, e and f, SMMC-7721, QGY-7703, BEL-7402 and K562 cells all express HLA-ABC and ICAM-1 only.

Figure 2. A, Stimulation of T cells by BEL-7402/B7-1. T cells were cultured with BEL-7402/B7-1 cells (◆) or BEL-7402/pcDNA3 cells (■) at the indicated ratios of T cells to stimulators (S). After 96 h incubation, uptake of [3 H]-thymidine was measured during 18 h incubation. The results are expressed as mean of 3 separate assays $\pm$ SEM. B, Normal donor T cells were co-cultured for 6 days with irradiated BEL-7402/B7-1 or BEL-7402/pcDNA3 cells at a ratio of 10:1. The cell-free supernatants were analyzed by enzyme-linked immunosorbent assay for levels of interferon- γ (IFN- γ). Data represent the mean of 3 separate assays $\pm$ SEM. C and D, Decrease in cytotoxicity of effector cells caused by anti-HLA-ABC antibody. The target cells (BEL-7402 cells) were pre-incubated with anti-MHC class I molecule antibody (1:50 dilution) and then assayed for lysis. CTL activity was determined by LDH release. (C and D, sample obtained from donor 1 and 2, respectively).

by the effector cells was reduced. The ability of the antibody to block HLA-class I-mediated lysis was demonstrated by addition of W6/32 antibody to the target cells. Pre-incubation of the target cells with W6/32 antibody resulted in abrogation of BEL-7402 cells lysis. This finding indicates that the killing effect was MHC class I-restricted. The lysis of SMMC-7721,

QGY-7703 and BEL-7402 cells were therefore due to CD8 $^+$ T cells, not NK-cells which lyse target cells in a non-specific and MHC molecule unrestricted manner. Furthermore, the HLA-typing result shows that BEL-7402, BEL-7402/B7-1 and SMMC-7721 cells are all A68 in locus A (Table I). While QGY-7703 cells are A24 and A68 in locus A (Table I). These

Figure 3. Stimulation of antitumor CTL cells by BEL-7402/B7-1 cells. T cells from 2 donors were incubated with BEL-7402/B7-1 (A and C) or BEL-7402/pcDNA3 (B and D) cells at a ratio of 10:1 for 14 days in the presence of 20 U/ml hIL-2. The stimulated T cells were incubated with 3 HCC cell lines SMMC-7721, QGY-7703, BEL-7402 and NK-cells sensitive cell line K562, respectively, at the indicated ratios. Percentage cytotoxicity was determined by LDH released. In the (T cells:S) -ratio S is equal to 1. Differences between paired cultures were compared by 2-tailed Student's t-test for paired samples ($p<0.05$).

data shows that the target cells have the same HLA molecules in at least 1 locus.

Discussion

Identification of tumor-associated anti-gens (TAAs) (23) has prompted the development of different strategies for anti-

tumor vaccination, aimed at inducing specific recognition of TAAs in order to elicit a persistent immune memory that may eliminate residual tumor cells and protect recipients from relapses (24). It was assumed that tumors must have some surface antigens, which are presented only on cancer cells, not on normal cells. Specific TAAs have been used as targets in immunotherapy. Using 1 or 2 TAAs in TAA-based

Table I. HLA typing results of SMMC-7721, BEL-7402, QGY-7703 and BEL-7402/B7-1 cells at 3 locus A, B and DRB1.

	A		B		DRB1	
SMMC-7721	68	-	18	72	010x	-
BEL-7402	68	-	72	-	010x	030x
QGY-7703	24	68	35	72	010x	030x
BEL-7402/B7-1	68	-	72	-	010x	030x

(-), HLA molecules are outside the range detected by the kit.

therapy can be used with partial success (24), but greater success is anticipated if the vaccine encompasses a broader array of tumor antigens (24). We assume that human hepatocellular carcinoma may share some of the antigens which have been identified as being able to be targeted by cytotoxic T lymphocytes. For example, human melanoma antigen MAGE-1 and MAGE-3 are expressed in 70% of HCC patients (25). The melanoma cell lines express shared melanoma antigens which can be recognized by a T cell clone (26). We assume that there are some shared HCC antigens expressed among the HCC cell lines. We found that all of the HCC cell lines we used are positive for expression of MAGE-1 and MAGE-3 (data not shown). In this experiment we attempted to use a laboratory established HCC cell line to activate human T cells to become CTLs and then to target 3 other HCC cell lines which may express the shared antigens with the first cell line.

Tumor cells usually do not induce immune responses but rather escape from the systemic immune mechanism. This is thought to result from the lack of MHC molecules (27), and costimulatory molecules (28) in tumor cells. The necessary components for effective T cell stimulation by antigen include recognition of the peptide which is presented by MHC molecules and interaction with co-stimulatory molecules on the antigen-presenting cell such as dendritic cells (4). We found that 3 human HCC cell lines SMMC-7721, QGY-7703 and BEL-7402 strongly express HLA class I, but do not express CD80 molecules on their surface (Fig. 1). These results suggest that lack of costimulatory molecules CD80 rather than lack of MHC expression may be the cause of the inability of the HCC cells to induce antitumor immunity.

In the present study, human CD80 gene was transfected into human HCC cell line BEL-7402 in order to increase its immunogenicity. Previously reported studies using CD80 gene to modify immunogenicity have been reported. Stimulating melanoma cells were modified with cDNA for CD80 by lipofection (29) or by infection using a potentially antigenic retroviral vector (30). In the present study, we extend these findings and it is the first time that a non-antigenic means (electroporation) of direct gene transfer was used to modify the human HCC cell line.

In our T cell proliferation assay, results show that the uptake of radiolabeled ^3H -thymidine was greater in T cells cultured for 96 h with irradiated BEL-7402/B7-1 cells than in T cells cultured with the control cell line BEL-7402/pcDNA3. This means that transfer of human CD80 gene into BEL-7402

cells can increase immunogenicity and cause T cells to have a higher proliferation. This proliferation is positively correlated with the cytotoxicity test (Fig. 3). BEL-7402/pcDNA3 cells stimulated T cell proliferation slightly (Fig. 2A), perhaps due to the presence of some APCs in the T cell isolation from nylon wool which removed the B cells only. These APCs display the allogenic MHC antigen and tumor antigens of the BEL-7402 cells to the T cells and stimulate their proliferation. Furthermore, the T cells activated by BEL-7402/B7-1 secreted much more IFN- γ than that of the control cell line (Fig. 2B). Recent research found that IFN- γ secretion is another indication of CTLs activation (31), demonstrating a Th1 recall response (32).

The purpose of transfecting HCC tumor cell lines with CD80 cDNA was to increase the ability of the tumor to activate anti-HCC T cell cytolytic activity. The results of the present study demonstrate that the cytolytic activity of normal donor T cells was enhanced when T cells were stimulated by HCC cells transfected to express CD80. The stimulated effector cells were able to lyse 3 HCC cells: SMMC-7721, QGY-7703 and BEL7402, respectively. This result implies that CD80 transfected BEL-7402 can activate the naïve T cells by the antigens displayed on its cell surface and that these antigens may be shared among these 3 HCC cell lines.

We found that this cytolytic activity is HLA-class-I restricted, as it was blocked by addition of anti-HLA ABC antibody. Furthermore, the cytotoxic effect of the effector cells on K562 cells is very low compared with its effect on the 3 HCC cell lines. This means that this cytotoxic effect is due to CD8 $^+$ cytotoxic T lymphocytes, not NK-cells.

Although the target cells we used in the experiment have the same HLA molecules in at least 1 locus (Table I), they have mismatched HLA molecules in other loci. Using BEL-7402/B7-1 cells to prime naïve T cells can target all these target cells imply that they have the shared tumor antigens expressed in the same HLA molecule complexes or they formed a peptide-HLA complex structure that may fit into the groove of that T cell receptor. The recent literature shows that T cells recognize antigens in the form of whole peptide-HLA complexes which can fit into the groove of TCR (33). The present study implies that the 3 HCC target cell lines we used have the same molecular structure as the peptide-HLA of BEL-7402/B7-1 cells. Moreover, they have the same HLA molecules in at least 1 locus, thus they may present the shared tumor antigens by the same HLA molecules that can be recognized by the primed T cell receptor.

Recent reports have demonstrated that both CD80 and ICAM-1 are necessary to induce an efficient primary tumor rejection. CD80 alone cannot induce the primary rejection of the nonimmunogenic B16.F1 cell lines (34). Therefore, we transfected the human CD80 gene into the ICAM-1 positive HCC cell line BEL-7402 for our experiment. Moreover, we found that all 3 human HCC cell lines SMMC-7721, QGY-7703 and BEL-7402 cells express ICAM-1 on their surface. The poor immunogenicity of human HCC is likely due to lack of CD80 expression rather than lack of ICAM-1 molecules.

In spite of recent progress in HCC therapy, most HCC cannot be cured with conventional treatment. The need is great for innovative therapeutic methods. Our studies report

that CD80-transfected human HCC cells can elicit potent cytolytic activity by allogenic effectors. We observed significant lysis of 3 HCC cell lines which may be due to display of the shared antigens as the CD80-transfected cell line. Recent reports have shown that allogenic melanoma cells transfected with human CD80 cDNA can function as stimulator cells to elicit enhanced cytolytic activity by allogenic PBMC (13). Therefore, using an allogenic cell line to activate the patient's PBMC for cancer immunotherapy may be a future trend as it does not require as much time and effort as does isolating the patient's cancer cells to establish the patient's cell line. The present study shows that the HCC cells BEL-7402/B7-1 share some of the antigens which can activate the T cells to target other HCC cells (SMMC-7721, QGY-7703, BEL-7402) express the same shared antigens. We hope that this idea can be evaluated in clinical trials as it may be more cost-effective than the immunotherapy currently used.

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References

1. Stern MC, Umbach DM, Yu MC, London SJ, Zhang ZQ and Taylor JA: Hepatitis B, aflatoxin B1, and p53 condon 249 mutation in hepatocellular carcinomas from Guangxi, People's republic of China, and a meta-analysis of existing studies. *Cancer Epidemiol Biomarkers Prev* 10: 617-625, 2001.
2. Bosch X, Ribes J and Borrás J: Epidemiology of primary liver cancer. *Semin Liver Dis* 19: 271-285, 1999.
3. Munoz N and Bosch X: Epidemiology of hepatocellular carcinoma. In: *Neoplasms of the Liver*. K Okuda and KG Ishak (eds). Tokyo, Springer, pp3-19, 1987.
4. Chan RCF, Xie H, Zhao GP and Xie Y: Dendritomas formed by fusion of mature dendritic cells with allogenic human hepatocellular carcinoma cells activate autologous cytotoxic T lymphocytes. *Immunol Lett* 83: 101-109, 2002.
5. Ichida T, Higuchi K, Arakawa K, Ohta H, Sugiyama K, Miyagiwa M and Nohzawa A: Treatment of hepatocellular carcinoma utilizing lymphokine-activated killer cells and interleukin-2. *Cancer Chemother Pharmacol* 2: 45-48, 1990.
6. Uchino J, Une Y, Kawata A, Wakisaka Y and Hosokawa M: Postoperative chemimmunotherapy for the treatment of liver cancer. *Semin Surg Oncol* 9: 332-336, 1993.
7. Suto T, Fukuda S, Moriya N, Watanabe Y, Sasaki D, Yoshida Y and Sakata Y: Clinical study of biological response modifiers as maintenance therapy for hepatocellular carcinoma. *Cancer Chemother Pharmacol* 33: S145-S148, 1994.
8. Himoto T, Watanabe S, Nishioka M, Tanaka S and Saito M: Combination immunotherapy with OK432, recombinant granulocyte-colony stimulating factor and recombinant interleukin-2 for human hepatocellular carcinoma. *Cancer Immunol Immunother* 42: 127-131, 1996.
9. Boon T, Cerrotini JC, van den Eynde B, van den Bruggen B and van Pel A: Tumor antigens recognized by T lymphocytes. *Annu Rev Immunol* 12: 337-365, 1994.
10. Hellström KE, Hellström I, Hellman S and Rosenberg SA: The Biologic Therapy of Cancer. J.B. Lippincott Co., Philadelphia, pp35-42, 1991.
11. Bretscher P and Cohn M: A theory of self-nonsel discrimination. *Science* 169: 1042, 1970.
12. Gimmi CD, Freeman GJ, Gribben JG, Gray G and Nadler LM: Human T-cell clonal anergy is induced by antigen presentation in the absence of B7 costimulation. *Proc Natl Acad Sci USA* 90: 6586-6590, 1993.
13. Cavallo F, Martin-Fontecha A, Bellone M, Heltai S, Gatti E, Tornaghi P, Freschi M, Forni G, Dellabona P and Casorati G: Co-expression of B7-1 and ICAM-1 on tumor is required for rejection and the establishment of a memory response. *Eur J Immunol* 25: 1154-1162, 1995.
14. Huang J, Cai MY and Wei DP: HLA class I expression in primary hepatocellular carcinoma. *World J Gastroenterol* 8: 654-657, 2002.
15. Talmage DW, Woolnough JA, Hemmingsen H, Lopez L and Laferty KJ: Activation of cytotoxic T cells by nonstimulating tumor cells and spleen cell factor(s). *Proc Natl Acad Sci USA* 74: 4610-4614, 1977.
16. Chen L, Linsley PS and Hellström KE: Costimulation of T cells for tumor immunity. *Immunol Today* 14: 483-486, 1993.
17. Shi M, Wang FS and Wu ZZ: Synergetic anticancer effect of combined quercetin and recombinant adenoviral vector expressing human wild-type p53, GM-CSF and B7-1 genes on hepatocellular carcinoma cells *in vitro*. *World J Gastroenterol* 9: 73-78, 2003.
18. Schwartz RH: Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. *Cell* 71: 1065-1079, 1992.
19. Liu Y and Linsley PS: Costimulation of T cell growth. *Curr Opin Immunol* 4: 265-270, 1992.
20. Linsley PS, Brady W, Grosmaire L, Aruffo A, Damle NK and Ledbetter JA: Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin-2 mRNA accumulation. *J Exp Med* 173: 721-730, 1991.
21. Linsley PS, Brady W, Urnes M, Grosmaire L, Damle NK and Ledbetter JA: CTLA-4 is a second receptor for the B cell activation antigen B7. *J Exp Med* 174: 561-569, 1991.
22. Wolpert EZ, Petersson M, Chambers BJ, Sandberg JK, Kiessing R, Ljunggren HG and Kärre K: Generation of CD8⁺ T cells specific for transporter associated with antigen processing deficient cells. *Proc Natl Acad Sci USA* 94: 11496-11501, 1997.
23. Sadanaga N, Nagashima H, Mashino K, Tahara K, Yamaguchi H, Ohta M, Fujie T, Tanaka F, Inoue H, Takesako K, Akiyoshi T and Mori M: Dendritic cell vaccination with MAGE peptide is a novel therapeutic approach for gastrointestinal carcinomas. *Clin Cancer Res* 7: 2277-2284, 2001.
24. Renner C, Trumper L and Pfreundschuh M: Tumor vaccines: a new immunotherapeutic approach in oncology. *Ann Hematol* 79: 651-659, 2000.
25. Tahara K, Mori M, Sadanaga N, Sakamoto Y, Kitano S and Makuuchi M: Expression of the MAGE gene family in human hepatocellular carcinoma. *Cancer* 85: 1234-1240, 1999.
26. Robbins PF, El-Gamil M, Li YF, Zeng G, Dudley M and Rosenberg SA: Multiple HLA Class II-restricted melanocyte differentiation antigens are recognized by tumor-infiltrating lymphocytes from a patient with melanoma. *J Immunol* 169: 6036-6047, 2002.
27. Ostrand-Rosenberg S, Roby CA and Clements VK: Abrogation of tumorigenicity by MHC class II antigen expression requires the cytoplasmic domain of the class II molecules. *J Immunol* 147: 2419-2422, 1991.
28. Bodmer WF, Browning MJ, Krausa P, Rowan A, Bicknell DC and Bodmer JG: Tumor escape from immune response by variation in HLA expression and other mechanisms. *Ann NY Acad Sci* 690: 42-49, 1993.
29. Fenton RG, Turcowski-Corrales SM and Taub DD: Induction of melanoma antigen-specific cytotoxic T lymphocytes *in vitro* by stimulation with B7-expressing human melanoma cell line. *J Immunother* 21: 95-108, 1998.
30. Yang S, Darrow TL and Seigler HF: Generation of primary tumor-specific cytotoxic T lymphocytes from autologous and human lymphocyte antigen class I-matched allogenic peripheral blood lymphocytes by B7 gene-modified melanoma cells. *Cancer Res* 57: 1561-1568, 1997.
31. Holmes LM, Li J, Sticca RP, Wagner TE and Wei YZ: A rapid novel strategy to induce tumor cell-specific cytotoxic T lymphocyte responses using instant dendritomas. *J Immunotherapy* 24: 122-129, 2001.
32. Ward S, Casey D, Labarthe MC, Whelan M, Dalgleish A, Pandha H and Todryk S: Immunotherapeutic potential of whole tumour cells. *Cancer Immunol Immunother* 51: 351-357, 2002.
33. Van der Merwe PA and Davis SJ: Molecular interactions mediating T cell antigen recognition. *Annu Rev Immunol* 21: 659-684, 2003.
34. Chen L, McGowan P, Ashe S, Johnston J, Li Y, Hellström I and Hellström KE: Tumor immunogenicity determines the effect of B7 costimulation on T cell-mediated tumor immunity. *J Exp Med* 179: 523, 1994.