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Journal of Cellular Biochemistry 113:3810–3822 (2012)

Journal of Cellular Biochemistry

Molecular Mechanism of a Novel CD59-Binding Peptide sp22 Induced Tumor Cells Apoptosis

Bing Li,[1*] Mei-Hua Gao,[2] and Xian-Ming Chu[3]

[1]*Department of Biology, Medical College of Qingdao University, Qingdao 266021, China*
[2]*Department of Immunology, Medical College of Qingdao University, Qingdao 266021, China*
[3]*Department of Cardiology, Affiliated Hospital of Medical College of Qingdao University, Qingdao 266100, China*

ABSTRACT

Some short peptides discovered by phage display are found to be able to inhibit cancer growth and induce cancer cell apoptosis. In this study, a novel cancer-targeting short peptide which was composed of 22 amino acids (ACHWPWCHGWHSACDLPMHPMC, abbreviated as sp22) and specifically bound to human CD59 was screened from a M13 phage display library so as to counteract tumor immune escape activity. The mechanism of exogenous sp22 peptide in inducing apoptosis of MCF-7 cells was investigated. The results suggested that sp22 could lower CD59 expression level, downregulate Bcl-2 expression, activate Fas and caspase-3, and finally increase apoptotic cell numbers of MCF-7 cells. However, sp22 had no obvious influence on normal human embryonic lung cells. In addition, the effects of endogenous sp22 gene on CD59 expression and NKM cell apoptosis were explored using the recombinant plasmid sp22-PIRES. It showed that sp22 gene was efficiently expressed in transfected NKM cells. Compared with normal NKM cells, NKM cells transfected with sp22 displayed reduced mRNA and protein expression levels of CD59, increased sensitivity to complement-mediated cytolysis, decreased cell survival ratio, changes of the expression of apoptosis associated proteins, increased number of apoptotic cells and the appearance of apoptotic morphology. The results suggested that sp22 protein could bind to CD59 and inhibit the expression of CD59. The cytolytic activity of complement on tumor cells strengthened and apoptosis signal was stepwise transferred which might be a potential way to kill tumor cells. J. Cell. Biochem. 113: 3810–3822, 2012.

KEY WORDS: sp22; CD59; SHORT-PEPTIDE; APOPTOSIS; PHAGE DISPLAY LIBRARY; TUMOR CELLS

Nonspecific anti-cancer chemotherapy has no tumor-targeting effect and will kill normal and cancer cells, causing severe side effects in many patients. To solve this problem, targeted drug delivery systems have received great attention [Lammers et al., 2008; Penate Medina et al., 2011]. A key factor of a targeted drug delivery system is the discovery of targeting molecules that can specifically recognize tumor and cancer cells [Molek et al., 2011]. Phage display technology is a powerful approach to screening targeting molecules, such as peptides, for cancer cells or tumor blood vessel endothelial cells [Smith, 1985; Scott and Smith, 1990; Liu et al., 2011]. Many novel angiogenic vessels and homing peptides have been isolated recently using this method [Lee et al., 2007; Yang et al., 2008]. Furthermore, phage display can screen cancer-binding peptides regardless of whether the receptor is known or not [Pasqualini and Ruoslahti, 1996; Pasqualini et al., 1997; Brummelkamp et al., 2002]. In addition to drug delivery systems, tumor-targeting peptides are also employed in diagnosis or radiotherapy by delivering radionuclide [Mier et al., 2005; Howell et al., 2007; Zitzmann et al., 2007]. Phage display technology has been demonstrated to be a powerful tool for screening useful ligands that are capable of specifically binding to biomarkers on the surface of tumor cells. Some peptides discovered by phage display are even able to inhibit cancer growth and induce cancer cell apoptosis [Chen et al., 2009; Wu et al., 2010].

Human CD59 is a M_r 18–20 kDa protein and anchored through glycanphosphatidylinositol to the cell membrane [Ninomiya et al.,

Bing Li and Mei-Hua Gao contributed equally to this work.

Grant sponsor: National Natural Science Foundation of China; Grant number: 81001346; Grant sponsor: Medical Health Science and Technology Development Plan Project of Shandong Province; Grant number: 2011HZ023; Grant sponsor: Science and Technology Development Plan of Qingdao City; Grant number: 2012-1-3-5-(4)-nsh.

*Correspondence to: Prof. Bing Li, Department of Biology, Medical College of Qingdao University, 38 Dengzhou Road, Qingdao 266021, P.R. China. E-mail: libing_516@yahoo.com.cn

Manuscript Received: 28 April 2012; Manuscript Accepted: 16 July 2012
Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 20 July 2012
DOI 10.1002/jcb.24258 • © 2012 Wiley Periodicals, Inc.

1992]. The mature form of the CD59 polypeptide is consisting of 77 amino acids starting with Leu and terminating with Asn [Sugita et al., 1989; Sugita et al., 1993] and folded through five intra-chain disulfide bonds. The functions of CD59 protein are mainly involved in three aspects. First, CD59 functions as an inhibitor of the C5b-9 membrane attack complex (MAC) of human complement. CD59 interacts with both the C8 and C9 components of MAC to inhibit formation of membrane-inserted C9 polymer, thereby restricting the cytolytic activity of the C5b-9 complex. Through these interactions, CD59 protects human blood, vascular and tumor cells from injury or lysis by human complement [Meri et al., 1990; Rollins and Sims, 1990]. Second, CD59 acts as the second signal stimulant, inducing the activation of T lymphocytes and taking part in the regulatory course of immunoreaction [Treon et al., 2000]. Third, CD59 is the ligand of CD2 that can associate with CD59. CD59–CD2 complex activates T cells, then guides adhesion of T and T cells or T and other tissue cells, and further regulates the growth of tissue cells [Zaltzman et al., 1995].

CD59 is widely spread in human body and expressed on the surface of most tissue cells under normal conditions, such as monocytes, red cells, granular cells, heart, liver, spleen, kidney and vena umbilicalis endothelial cells [Hideshima et al., 1990]. Studies show that the density of CD59 molecules on the cell surface changes in some disease. The absence or low-level expression of CD59 may be partially responsible for the pathogenesis of some autoimmune diseases, such as diabetes, multiple sclerosis, and AIDS [Seifert et al., 1992; Weiss et al., 1992; Seya et al., 1993; Vakeva et al., 1993; Venneker et al., 1994], whereas up-regulation of CD59 expression is also reported in some inflammatory diseases, such as alzheimer, ulcerative colitis, and rheumatoid arthritis [McGeer et al., 1991; Uesu et al., 1995; Wang et al., 2002]. Some researchers have already discovered which CD59 molecules have a high expression in most malignant tumors.

In this study, a novel cancer-targeting short peptide specifically binding to human CD59 was screened from an M13 phage display library so as to counteract tumor immune escape activity. Furthermore, the molecular mechanism of apoptosis induced by the novel CD59-binding peptide sp22 was investigated in tumor cells including breast cancer cells (MCF-7) and stomach cancer cells (NKM).

MATERIALS AND METHODS

MATERIALS

Phage peptide library kit (including random 12 peptide library, receptor bacteria ER 2738, and sequencing primer 5′-HOCCCTCA-TAGTTAGCGTAACG-3′) was from New England BioLab Company (London, UK). RPMI1640 medium was from HyClone Company. Horseradish peroxidase (HRP)-conjugated rabbit anti-goats IgG (H + L) were from Beijing Zhongshan Life Technologies Ltd (Beijing, China). Rabbit anti-NKM polyclonal antibody was made by using conventional method in our laboratory. Biotinylated rabbit anti-human Fas/caspase-3/Bcl-2/CD59 monoclonal antibody, Reverse Transcriptional (RT)-PCR kit, TUNEL kit, NEB kit, BIOZOL total RNA extraction kit, all restriction endonucleases, and *Taq* polymerase were from Sigma Aldrich Company (St Louis, MO). Human embryonic lung (HEL), breast cancer cell line (MCF-7), stomach cancer cell line (NKM), and Chinese hamster ovary cell line (CHO) with high expression of CD59 were purchased from American Type Culture Collection (Manassas, VA). pIRES plasmid was from Promega (Madison, WI). T4 DNA ligase and DMEM were from Gibco BRL Life Technologies (Gaithersburg, MD). Trypsin and G418 antibiotic were from Amersco (San Francisco, CA). Lipfectamine-2000 was from Invitrogen™ Life Technologies (Carlsbad, CA). Triton X-100, IPTG/X-gal, and PEGS000 were from Sangon (Shanghai, China).

CELL CULTURE

CHO, HEL, MCF-7, and NKM cells were cultured in DMEM medium supplemented with 10% fetal bovine serum and antibiotics (100 mg/ml streptomycin and 100 U/ml penicillin) at 37°C with 5% CO_2.

SCREENING OF PHAGE DISPLAY PEPTIDE LIBRARY

CHO cells were incubated in DMEM medium with 1 g/L bovine serum albumin (BSA) in the six-well culture plate at 37°C for 1 h. Stock solution of 12-mer phage display peptide library (titer was 2×10^{16} pfu/L) was added and incubated at 37°C for another hour. After wash in cold phosphate-buffered saline (PBS), glycine buffer was added and placed on ice for 15 min. The eluants were collected and incubated with Triton X-100 at room temperature for 2 h in order to rupture the cells. The phages were counted, amplified, and purified. After six rounds of screening, titer was determined and 10 blue plaques were randomly selected in IPTG/X-gal agar plate for further amplification and purification.

COMPETITION ASSAY OF SELECTED PHAGES WITH THE WILD-TYPE M13 PHAGE

After six rounds of screening, 16 phages were randomly amplified and mixed with the wild-type M13 phages at the ratio of 1:1. The CHO cells were added with constant proportional mixtures in a 12-well culture plate followed by washing, screening, and lysis process.

$$\text{Relative affinity of the amplified phages} = \frac{\text{Input M13 phages} \times \text{Output phages of peptide library}}{\text{Output M13 phages} \times \text{Input phage of peptide library}}.$$

IDENTIFICATION OF POSITIVE CLONES BY ELISA ASSAY AND DNA SEQUENCING

The aforementioned purified phage clones (the titer was 1.0×10^{15} pfu/L, 50 μl/well) were cultured in ELISA plate overnight. After sealing with BSA, the plate was in turn incubated with 100 μl cell lysis buffer, goat anti-human CD59 Ab (1:400), and HRP-conjugated rabbit anti-goats IgG (1:5,000). The assays were developed with *O*-phenylenediamine substrate, and the optical density (OD) at 490 nm was measured. The original phage library was used as a negative control.

Positive phage clones were purified with NEB kit, and then single stranded DNA was extracted and identified by agarose gel electrophoresis. DNA sequencing was performed by Sangon.

DESIGN OF A SHORT PEPTIDE SEAL SPECIFIC TO CD59 ACTIVE SITES

A sequence of 22 peptides (abbreviated as sp22) was designed as follows: ACHWPWCHGWHSACDLPMHPMC. This sequence was synthesized by Beijing Scilight Biotechnology Ltd Co.

THE EFFECTS OF sp22 PROTEIN ON THE CD59 EXPRESSION OF MCF-7 AND HEL CELLS BY ELISA

MCF-7 cells were harvested and incubated in 96-well plate at 37°C overnight. The cells were treated with 100 μl different concentrations of sp22 protein liquid (0, 20, 40, 60, 80, and 100 mg/L) for 3 days, and then with 100 μl HRP-conjugated goat anti-human CD59 antibodies (1:800) at 37°C for 1 h. The substrate showed color by DAB in dark place for 20 min. Subsequently 2 mol/L sulfuric acid was added to terminate the color reaction. The OD 490 nm was measured. HEL cells acted as normal control and treated with the same procedure.

THE EFFECTS OF sp22 ON THE GROWTH OF MCF-7 AND HEL CELLS BY 3-(4,5-DIMETHYLTHIAZOL-2-YL)-2,5-DIPHENYL TETRAZOLIUM BROMIDE MTT ASSAY

MTT assay was applied to determine the effect of sp22 on the growth of MCF-7 and HEL cells. The cells (1×10^6 cells/ml) were incubated in triplicate in a 96-well culture plate, in 5%CO_2, at 37°C for 48 h. The cells were treated with different concentrations of sp22 protein solutions (0, 20, 40, 60, 80, and 100 mg/L), respectively. Three days later, cells were incubated in 5% MTT at 37°C for 4 h. Dimethyl sulphoxide (DMSO) was added with gentle shaking for 10 min, and OD at 570 nm was measured.

THE EFFECTS OF sp22 ON APOPTOTIC-RELATED PROTEINS (FAS, BCL-2, AND CASPASE-3) IN MCF-7 AND HEL CELLS BY IMMUNOHISTOCHEMISTRY (IHC)

MCF-7 and HEL cells (2×10^5 cells/well) were incubated in a 24-well plate (each well having a small cover glass) in the absence or presence of sp22 (0, 20, 40, and 60 mg/L) for 48 h. The cover glasses were taken out and fixed by 4% polyformadehyde/0.1 M PBS for 30 min. After washed with distilled water, the cells were treated with 30% H_2O_2:methanol (1:50) at room temperature for 30 min and washed again. The expression quantities of proteins (Fas, Bcl-2, and caspase-3) were measured by IHC. In brief, the cells were incubated, in turns in the following liquids: blocking buffer, the biotinylated rabbit anti-human Fas/Bcl-2/caspase-3 monoclonal antibody, avidin-biotin complex (ABC), and diaminobenzidine (DAB). The experiments were performed for three times on the same conditions and the results were observed under a light microscope. The average optical density (AOD) of 100–200 positive cells was randomly measured with 10× objective, and the average percentage of positive cells (APCP) was obtained by measuring positive cells and total tumor cells in random 10 high power fields with 40× objective. Protein expression quantities were determined by positive level index (PLI) = APCP × AOD [Niu et al., 2004].

APOPTOSIS DETERMINATION BY TUNEL ASSAY

MCF-7 and HEL cells treated with increasing concentrations of sp22 (0, 20, 40, and 60 mg/L) were harvested, washed with PBS, and then incubated in 1% paraformaldehyde for 30–60 min and fixed in 70% ethanol. DNA breaks were labeled, in turns, by TdT enzyme, bromodeoxyuridine triphosphate (BrdUTP) and fluorescein labeled anti-BrdU antibody. Total DNA was counterstained using propidium iodide/RNase A solution by using APO-BRDU apoptosis kit.

DESIGNING OF THE GENE SEQUENCE OF sp22

Intact sp22 DNA sequence (sp-dsDNA) was synthesized by adding initiator codon, terminator codon, *Nhe*I restriction enzyme digestion site and protection bases, as well as *Eco*RI restriction enzyme digestion site and protection bases. The sp22 gene sequence was designed as follows: 5′-ctagctagcATGGCATGTCATTGGCCTTGGTGTCATGGATGGCACTCAGCATGTGATCTACCAATGCACCCAATGTGCTAAgaattccg-3′.

CONSTRUCTION AND IDENTIFICATION OF RECOMBINANT VECTOR sp-piRES

A pair of oligonucleotide primers 5′-CTAGCTAGCATGGCATGTCATTGGCCTTGGTGTCATGGATGGCACTCAGCATGT-3′ and 5′-CGGAATTCTTAGCACATFGGGTGCATTGGTAGATCACATGCTGATGCCATCCA-3′ were designed according to the nucleotide sequence of sp-dsDNA. PCR was performed under the following conditions: first, predenaturation at 94°C for 2 min; second, 35 cycles of denaturation at 94°C for 30 s, annealing at 46°C for 1 min and elongation at 68°C for 2 min; followed by a final extension at 68°C for 5 min. The PCR products were isolated by electrophoresis on a 3% agarose gel. The 89 bp amplicon was purified, and then the amplicon and 6,000 bp pIRES vector were ligated with T4 ligase at 16°C overnight. The recombinant vector sp-pIRES was transformed into *E. coli* JM109, and three clones were randomly selected by blue-white screening. The desired sp-pIRES recombinant plasmid was identified by PCR, double digestion of *Nhe*I and *Hin*dIII, and DNA sequencing.

TRANSFECTION OF RECOMBINANT PLASMID sp-piRES INTO NKM CELLS

NKM cells were incubated in 24-well plates (2×10^8 cells/well) overnight. After washing with DMEM medium for two times, NKM cells were transfected with recombinant plasmid sp-pIRES and empty plasmid pIRES as control by cationic liposome (Lipfectamine-2000). The positive transfectants probably containing desired recombinant plasmid sp-pIRES were screened by adding G418. After 10 days, the monoclonal cells were proliferatively cultured. NKM cells transfected with sp-pIRES were called spNKM and NKM cells transfected with pIRES were called pNKM.

SCREENING OF TRANSFECTED POSITIVE CELL CLONES BY RT-PCR

Total RNA of spNKM and pNKM cells were extracted, respectively. A pair of oligonucleotide primers was designed as follows: 5′-CATGTCATTGGCCTTGGTGT-3′ (upstream) and 5′-ACATTGGGTGCATTGGTAGA-3′ (downstream). Total RNA acted as templates and reverse transcription reaction was performed at 42°C for 45 min. Then PCR was performed under the following conditions: predenaturation at 94°C for 3 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 49°C for 50 s, and elongation at 68°C for 1 min; followed by a final elongation step at 72°C for 5 min. PCR products were separated by agarose gel electrophoresis.

DETERMINATION OF CD59 mRNA EXPRESSION LEVEL BY RT-PCR

Total RNA of spNKM and pNKM cells was isolated, respectively, then reverse transcribed. The forward primer of CD59 was 5′-CTGCCATFCAGGTCATAGCC-3′ and the reverse primer was 5′-GAGAAATGGAGTCACCAGCA-3′. The forward primer of GAPDH was 5′-CGTGGAAGGACTCATGACCA-3′ and the reverse primer was 5′-TCCAGGGGTCTTACTCCTTG-3′. CD59 reaction was carried out as follows: predenaturation at 94°C for 3min; then 32 cycles of 94°C for 30 s, 47°C for 52 s, and 68°C for 1 min; followed by 70°C for 5 min. GAPDH reaction was as follows: predenaturation at 94°C for 3 min; then 25 cycles of 94°C for 30 s, 45°C for 1 min, and 68°C for 1 min; followed by 68°C for 5 min. PCR products were quantified using Tanon Image Software. RT-PCR was performed for six times on the same conditions, and the bands were scanned by gray scanner and analyzed by BandScan strip analysis software. CD59 levels were normalized with respect to GAPDH levels and grayscale ratio of CD59/GAPDH was calculated.

DETERMINATION OF CD59 PROTEIN CONCENTRATION BY WESTERN BLOT

The spNKM, pNKM, and control NKM cells were harvested, respectively, washed twice with cold PBS, lysed for 2 h on ice, and centrifuged at 4°C to remove insoluble materials. Protein concentrations were determined by BCA assay. The lysate supernatant was separated using 12% and 5% SDS–PAGE gel electrophoresis and transferred to polyvinylidene fluoride (PVDF) membranes. The membrane was incubated, first, with mouse anti-human CD59 and mouse anti-human β-actin antibodies, respectively, and, second, with HRP-conjugated goat anti-mouse secondary antibody. The bands were scanned and analyzed by BandScan strip analysis software. CD59 levels were normalized with respect to β-actin levels and grayscale ratio of CD59/β-actin was calculated.

COMPLEMENT-MEDIATED CYTOLYSIS BY ELISA AND ASSESSMENT OF PROLIFERATIVE ABILITY BY MTT ASSAY

NKM, pNKM, and spNKM cells (1×10^5/ml) were incubated with rabbit anti-NKM polyclonal antibody and serial dilutions of fresh human serum in medium for 60 min at 37°C in 96-well plates. The plates were added with 5% MTT for 4 h, and then centrifuged at 1,500 rpm for 10 min. The supernatant was discarded and DMSO was added for 20 min, then the plates were read on a multi-well scanning spectrophotometer with a test wavelength of 490 nm. The anti-NKM polyclonal antibody was replaced with equal volume of DMEM, which acted as a control group.

$$\text{The percentage of cell lysis} = \frac{\text{OD 490 of control group} - \text{OD 490 of experiment group}}{\text{OD 490 of control group}}.$$

Three kinds of cells were incubated in 96-well plates (10^4 cells/well) for 24 h. MTT (5 mg/ml) was added for 4 h and removed, and then acidic isopropanol was added. The OD of the solution was measured at 570 nm.

APOPTOTIC-RELATED PROTEINS (FAS, Bcl-2, AND CASPASE-3) BY IHC

Expressions of Fas, Bcl-2, and caspase-3 proteins in NKM, pNKM, and spNKM cells were determined by IHC. Detailed steps referred to "The Effects of sp22 on Apoptotic-Related Proteins (Fas, Bcl-2, and Caspase-3) in MCF-7 and HEL Cells by Immunohistochemistry" Section.

APOPTOSIS DETERMINATION BY TUNEL ASSAY AND ELECTRON MICROSCOPE

NKM, pNKM, and spNKM cells were harvested and detected by TUNEL assay. Detailed steps referred to "Apoptosis Determination by TUNEL Assay" Section.

Three groups of cells were harvested, resuspended, and fixed in 2.5% glutaraldehyde for 2 h. After washing with PBS, the cells were postfixed in 1.5% osmium tetroxide, dehydrated through a graded alcohol, embedded in Epon 812, thin-sectioned, and stained with uranyl acetate and lead citrate. Ultrathin sections were placed on grids and examined with a Zeiss EM 10 transmission electron microscope.

STATISTICAL ANALYSIS

Results are expressed as mean ± standard error of mean (SEM). Statistical analysis was performed by using the SPSS 17.0 package. Differences among the groups were evaluated by employing paired *t*-test and a $P < 0.05$ was considered to be statistically significant.

RESULTS

SCREENING OF PHAGE PEPTIDE LIBRARY

In the six rounds of screening process, through each round of the elution, amplification and screening, the percentage of phage clones which have low affinity with target cells gradually decreased, while the phage clones which have high affinity with target cells were collected and cultured little by little. So the recovery rate gradually raised (Table I).

COMPETITION BINDING ASSAY OF SELECTED PHAGES WITH THE WILD-TYPE M13 PHAGE

After six rounds of screening, 16 randomly chosen monoclones and phage library competed with the wild-type M13 phage, respectively. The results showed the affinities of 13 randomly chosen monoclones and wild-type M13 phage were higher (the affinities of phage 2, 4, and 11 were lower than 10, not shown). After sixth round of selection, the affinities gradually increased from 1 to 40 (Table II).

DETERMINATION OF POSITIVE CLONES BY ELISA

The affinities of 13 phage clones and human CD59 were measured by ELISA. The result illustrated that eight clones (phage 1, 5, 8, 9, 13,

TABLE I. Screening of the Specific Peptide of CD59 From 12 Phage Library

Round	Input (pfu)	Output (pfu)	Recovery (%)
1	2.0×10^{13}	2.0×10^{5}	1.00×10^{-6}
2	5.0×10^{12}	3.8×10^{6}	7.60×10^{-5}
3	4.0×10^{12}	4.2×10^{7}	1.05×10^{-3}
4	4.0×10^{12}	1.1×10^{9}	2.75×10^{-2}
5	4.0×10^{12}	1.5×10^{9}	3.75×10^{-2}
6	4.0×10^{12}	1.8×10^{9}	4.50×10^{-2}

TABLE II. Competitive Binding Assay of 13 Phage Clones and Phage Peptide Library Screened Through Six Rounds

Number	Binding forces	Number	Binding forces
Phage 1	38	Phage 13	32
Phage 3	25	Phage 14	38
Phage 5	19	Phage 15	42
Phage 6	20	Phage 16	45
Phage 7	28	Round 2	1
Phage 8	32	Round 3	12
Phage 9	31	Round 4	23
Phage 10	28	Round 5	35
Phage 12	33	Round 6	40

14, 15, and 16) had high affinities with CD59 (absorbance value >0.6), and the phage library functioned as a negative control (Fig. 1).

DNA SEQUENCING AND THE DEDUCTION OF AMINO ACID SEQUENCES

The single stranded DNA of eight positive clones were extracted and sequenced. Then the amino acid sequences were deduced. Results illustrated that the amino acid sequences of six clones (phage 5, 8, 9, 13, 14, and 15) were identical (HSACDLPMHPMC), while the other two clones (phage 1 and 16) were slightly different (HSACDLLMHPMC and HSACDLPKAPWC). All of these three screened amino acid sequences had nine hydrophobic residues (Table III).

COMPARISON OF DEDUCED AMINO ACID SEQUENCES AND PROTEIN DATA BANK (PDB)

Three sequences were submitted to the PDB (http://pir.georgetown.edu/) for comparison, and the identical homology sequence was not found. Because several key amino acids motif played an important role in the process of proteins interaction, we took each successive six amino acids as a group of peptides from the above three sequences. Compared with the PDB, matching proteins were enzymes, receptors, signal transduction proteins, etc. (Table IV). With reference to the sequence of amino acids, a short peptide composed of 22 amino acids (sp22, ACHWPWCHGWHSACDLPMHPMC) was designed.

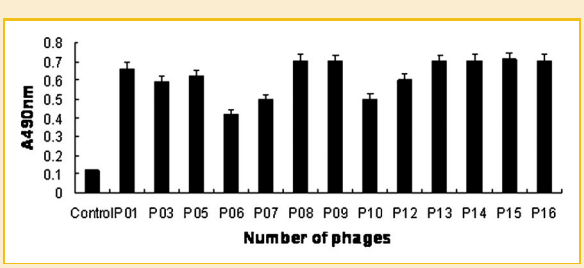

Fig. 1. Detection of affinities of 13 phage clones and CD59. The affinities of 13 phage clones and CD59 were measured by ELISA. If the absorbance value >0.6, the phage clones were selected as positive clones. The phage original library functioned as a negative control (absorbance value = 0.108).

TABLE III. Amino Acid Sequences Deduced From the DNA Sequences

Number of phage clones	Amino acid sequence	Number of hydrophobic residues
1	HSACDLLMHPMC	9
5, 8, 9, 13, 14, 15	HSACDLPMHPMC	9
16	HSACDLPKAPWC	9

THE INHIBITORY EFFECTS OF sp22 ON CD59 EXPRESSION BY ELISA

OD 490 nm value was in direct proportion to the CD59 expression level. With the increasing of sp22 concentrations from 0 to 100 μg/ml, the quantities of CD59 protein binding to CD59 antibody gradually lowered in MCF-7 cells. Simultaneously, sp22 had no significant effect on HEL cells (Fig. 2). This result revealed sp22 could compete with CD59 Ab to combine with CD59 and prohibit the expression of CD59 protein which functioned to protect tumor cells from attacking of MAC.

THE INHIBITORY EFFECTS OF THE sp22 ON MCF-7 AND HEL CELLS PROLIFERATION

MTT assay was adopted to determine the effect of sp22 on the cells growth. The results demonstrated that with the elevation of sp22 concentration, the OD value was gradually lowered which indicated the survival number of MCF-7 cells was gradually decreasing (Table V). The differences were all statistically significant ($P < 0.05$). However, the effects of different concentrations of sp22 on HEL cells were not significant ($P > 0.05$). The results indicated that sp22 had

TABLE IV. Matching Proteins by Comparison of Peptide Fragments with PDB

Peptide fragments	Protein including peptide fragment
HSACDLPMHPMC	
-HSACDL-	Trypsin/chymotrypsin inhibitor; nipped-B protein; immunolectin-A precursor; glycoprotein G1 and G2; alpha-amylase; radical S-adenosyl methionine domain containing 1
-SACDLP-	Similar to chromosome 10 open-reading frame 22; DNA-topoisomerase II
-ACDLPM-	Bifunctional molybdenum cofactor biosynthesis protein
-PMHPMH-	Gluconate kinase; neprilysin-like peptidase; soluble secreted endopeptidase
HSACDLLMHPMC	
-DLLMHP-	DNA polymerase III; LPS alpha 1,3-glucosyltransferase
-SACDLL-	Transcriptional regulator; cholecystokinin receptor
-ACDLLM-	Transient receptor potential channel 5; tumor necrosis factor type 1 receptor associated protein
-LMHPM-	Aspartate ammonia-lyase
HSACDLPKAPWC	
-DLPKAP-	Probable ATP-binding ABC transporter protein; acetyl-CoA transporter; translation initiation factor-like protein
-LPKAPW-	PHD finger-like protein
-PKAPW-	Endothelin B receptor-like protein-2 precursor; insulin receptor-related protein precursor

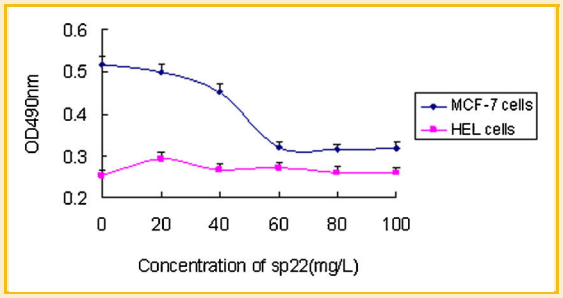

Fig. 2. The effects of different concentrations of sp22 on CD59 expression in MCF-7 and HEL cells. Cells were incubated with increasing concentrations of sp22 for 3 days, and then given HRP-conjugated CD59 antibody. After 1 h, the amount of CD59 bound to the cells was determined by ELISA. OD 490 nm was in direct proportion with the CD59 protein level. Results are expressed as mean ± SEM. Differences among the groups were evaluated by employing paired *t*-test and a $P < 0.05$ was considered to be statistically significant.

the property to hold back the growth of MCF-7 cells, and a concentration-dependent manner existed.

EFFECTS OF sp22 ON EXPRESSION OF FAS/CASPASE-3/BCL-2 IN MCF-7 AND HEL CELLS

The effects of sp22 on expression quantities of apoptosis associated proteins including Fas, Bcl-2, and caspase-3 proteins in MCF-7 tumor cells and HEL normal cells were determined by immunochemistry (IHC). The membrane and cytoplasm of positive cells were dyed brownish yellow. PLI had a positive relationship with protein expression levels. Results illustrated that, compared with untreated MCF-7 cells, Fas protein quantities expressed in MCF-7 cells treated with sp22 were significantly higher than untreated MCF-7 cells (Fig. 3A), simultaneously Bcl-2 protein quantities were apparently lowered (Fig. 3B). With the increasing of sp22 concentrations, the caspase-3 protein quantities were significantly increased (Fig. 3C). However, in HEL cells, the expression levels changed little (Fig. 3D). These results suggested sp22 had a strong efficacy of promoting pro-apoptotic Fas and caspase-3 proteins expressions, and inhibiting anti-apoptotic Bcl-2 expressions in MCF-7 cells. But sp22 had no use on expression of these proteins in HEL cells.

TABLE V. Inhibition Ratios of sp22 on Proliferation of MCF-7 and HEL Cells

Groups	OD570	*P*
Untreated HEL	0.517 ± 0.031	
HEL+20 mg/L sp22	0.492 ± 0.028	>0.05
HEL+40 mg/L sp22	0.488 ± 0.029	>0.05
HEL+60 mg/L sp22	0.494 ± 0.023	>0.05
HEL+80 mg/L sp22	0.505 ± 0.015	>0.05
HEL+100 mg/L sp22	0.490 ± 0.019	>0.05
Untreated HeLa	0.534 ± 0.031	
HeLa+20 mg/L sp22	0.493 ± 0.021	
HeLa+40 mg/L sp22	0.337 ± 0.017	<0.05
HeLa+60 mg/L sp22	0.171 ± 0.020	<0.01
HeLa+80 mg/L sp22	0.160 ± 0.021	<0.01
HeLa+100 mg/L sp22	0.151 ± 0.019	<0.01

APOPTOSIS DETERMINATION BY TUNEL ASSAY

The inducing effects of sp22 on MCF-7 cells apoptosis were determined by TUNEL assay. Compared with control MCF-7 cells, fluorescence density gradually strengthened in the MCF-7 cells treated with increasing concentration of sp22 (Fig. 4A). However, the changes of fluorescence density were small in HEL cells treated with different concentrations of sp22 (Fig. 4B). The results suggested that sp22 gene could induce the apoptosis of MCF-7 cells, but had no use on HEL cells.

IDENTIFICATION OF RECOMBINANT PLASMID sp-piRES BY PCR AND DOUBLE DIGESTIONS

The PCR products of recombinant vector sp-pIRES contained a 63 bp of clear strip by electrophoresis on a 3% agarose gel (Fig. 5A). In addition, sp-pIRES was digested with *Eco*RI and *Hin*dIII, and a 406 bp of clear strip was obtained by electrophoresis on a 1% agarose gel (Fig. 5B). The results confirmed that recombinant vector sp-pIRES was successfully constructed.

SCREENING OF sp22-TRANSFECTED CELL CLONES BY RT-PCR

Total RNA of spNKM and pNKM cells was extracted, respectively, and RT-PCR was carried out. The results showed a clear amplification band (63 bp) by 3% agarose gel electrophoresis and suggested positive spNKM cells transfected with sp22 were successfully selected (Fig. 6).

DETECTION OF CD59 mRNA EXPRESSION IN spNKM CELLS BY RT-PCR

The expression quantities of CD59 mRNA in spNKM cells were determined by RT-PCR. Results illustrated both GAPDH (548 bp) and CD59 band (232 bp) were observed by 3% agarose gel electrophoresis (Fig. 7A). The grayscale ratio of CD59/GAPDH in spNKM cells was significantly lower than that in NKM and pNKM cells ($P < 0.05$) (Fig. 7B). These results suggested that sp22 could bind and block the active site of CD59 could reduce CD59 mRNA quantities.

IDENTIFICATION OF CD59 PROTEIN EXPRESSION BY WESTERN BLOT

Two protein bands (42 kDa for β-actin and 20.5 kDa for CD59) were shown by Western Blot using mouse anti-human CD59 antibody and β-actin antibody (Fig. 8A). The grayscale ratio of CD59/β-actin was determined to show CD59 protein level. The grayscale ratio in spNKM cells was obviously lower than that in NKM and pNKM cells, and the differences were statistically significant ($P < 0.05$). Meanwhile, there were no evident differences between pNKM and NKM cells (Fig. 8B). These results showed that CD59 protein was expressed at a high level in NKM cells and pNKM cells, while sp22 could cause the reduction of CD59 protein quantities in spNKM cells, which indicated when sp22 was overexpressed in NKM cells, CD59 was downregulated.

DETERMINATION OF COMPLEMENT-MEDIATED CYTOLYSIS BY ELISA AND CELLS PROLIFERATIVE ABILITY BY MTT ASSAY

The sensitivity to complement-mediated cytolysis was compared between NKM, pNKM, and spNKM cells. The results showed that the percentage of cell cytolysis in spNKM cells was consistently higher than that in the other two kinds of cells, which indicated that

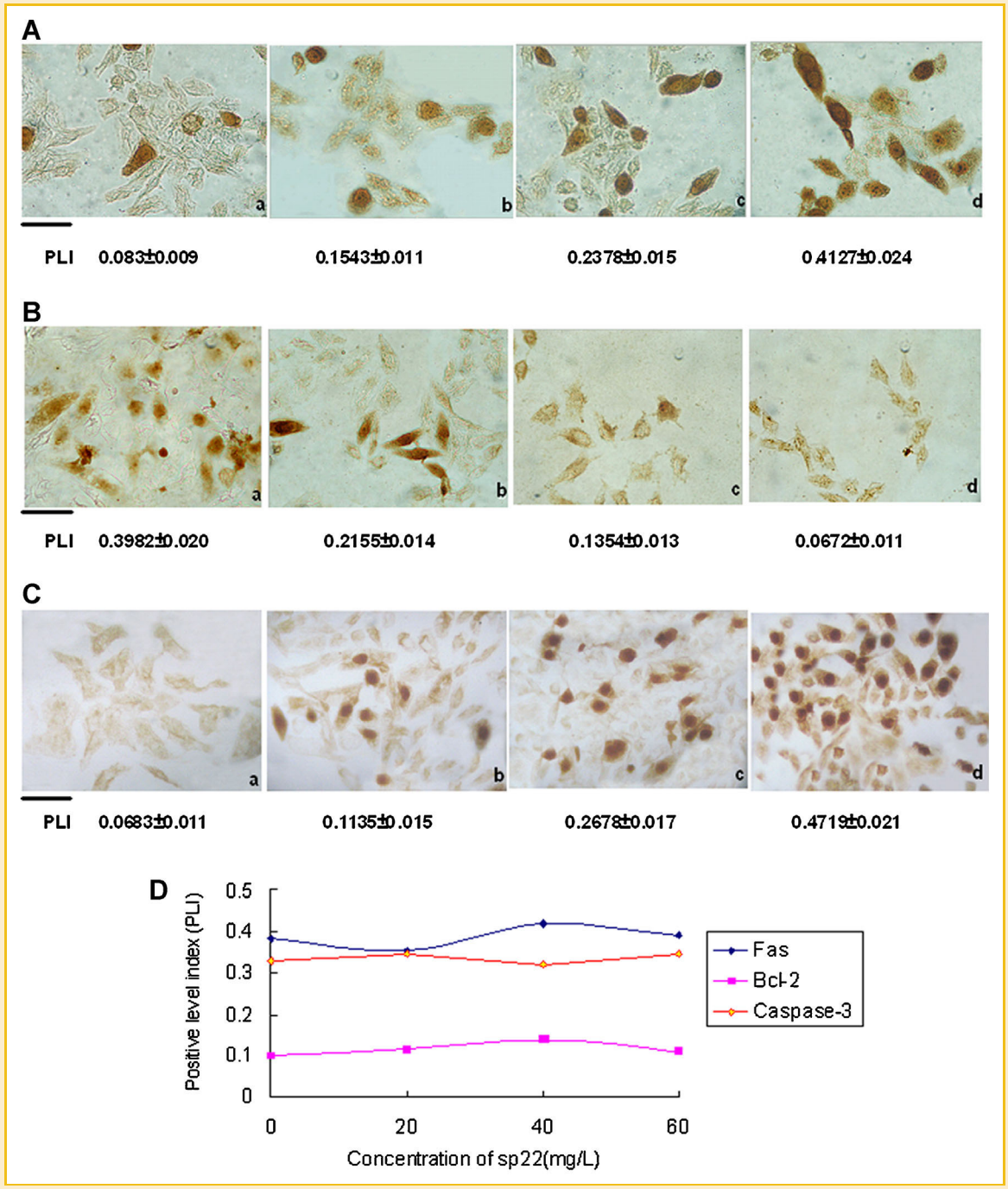

Fig. 3. Expression levels of Fas, Bcl-2, and caspase-3 proteins in MCF-7 and HEL cells (×400). MCF-7 cells were incubated in the absence or presence of sp22 (0, 20, 40, and 60 mg/L) for 48 h, fixed and treated with 30% H_2O_2:methanol (1:50). The expression quantities of proteins were measured by IHC. In brief, MCF-7 cells were incubated, in turns in the following liquids: blocking buffer, (A) the biotinylated rabbit anti-human Fas monoclonal antibody (or [B] the biotinylated rabbit anti-human Bcl-2 monoclonal antibody or [C] the biotinylated rabbit anti-human caspase-3 monoclonal antibody), ABC, and DAB. The membrane and cytoplasm of positive cells were dyed brownish yellow. PLI value represented the proteins expression quantities. (a) Untreated MCF-7 cells; (b) MCF-7 cells treated with 20 mg/L sp22; (c) MCF-7 cells treated with 40 mg/L sp22; and (d) MCF-7 cells treated with 60 mg/L sp22. Scale bar, 15 μm. (D) Expression levels of Fas, Bcl-2, and caspase-3 in HEL cells. Treatment approaches were same with MCF-7 cells.

the protective effects of CD59 against complement lysis were functionally degraded after NKM cells were transfected with sp-pIEPS. Compared with NKM cells and pNKM cells, complements had a high killing ratio for spNKM cells. The differences were significant ($P < 0.05$) (Fig. 9A). In order to determine the effect of sp22 gene on the growth of NKM cells, MTT assay was adopted. Results demonstrated that NKM cells transfected by sp-pIEPS were significantly fewer than those untransfected and transfected by pIEPS, implicating that sp22 gene transfection might be a new way for gene therapy of cancer cells (Fig. 9B).

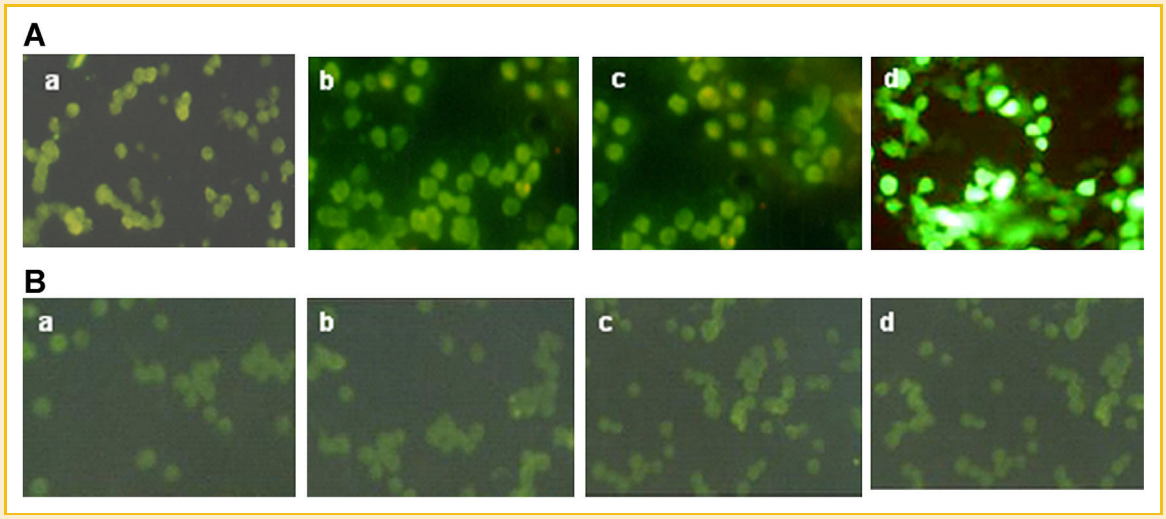

Fig. 4. Apoptosis determination by TUNEL assay. (A) MCF-7 cells and (B) HEL cells were harvested, washed, and then incubated in 1% paraformaldehyde and fixed in 70% ethanol. DNA breaks were labeled, in turns, by TdT enzyme, BrdUTP, and fluorescein labeled anti-BrdU antibody. Total DNA was counterstained using propidium iodide/RNase A solution. Fluorescence intensity was an index to judge cell apoptosis number. (a) Untreated MCF-7 cells; (b) MCF-7 cells treated with 20 mg/L sp22; (c) MCF-7 cells treated with 40 mg/L sp22; and (d) MCF-7 cells treated with 60 mg/L sp22.

EXPRESSION OF FAS, Bcl-2, AND CASPASE-3 PROTEINS BY IHC

The pro-apoptotic Fas and caspase-3 protein quantities in spNKM cells were significantly higher than NKM and pNKM cells, but the anti-apoptotic Bcl-2 protein levels were lower. The differences were significant, $P < 0.05$ (Table VI). The changes of apoptosis-related proteins suggested NKM cell death might be via apoptosis pathway.

APOPTOSIS DETERMINATION BY TUNEL ASSAY AND ELECTRON MICROSCOPE

The inducing effects of sp22 on NKM cells apoptosis were determined by TUNEL assay and electron microscope. Compared with control NKM group, fluorescence density in the spNKM groups was obviously strengthened. The results suggested that sp22 gene could induce the apoptosis of NKM cells (Fig. 10A). Electron microscope studies revealed the presence of morphological changes such as loss of microvilli, chromatin condensation into dense granules or blocks, and nuclear rupture, all of which were characteristics of cells undergoing apoptosis. On the contrary, control NKM cells exhibited morphologically normal and no sign of apoptosis appeared (Fig. 10B). These results further confirmed that sp22 could induce apoptosis in NKM cells in vitro.

DISCUSSION

Targeted therapy selectively transports therapeutic or cytotoxic agents into tumor by means of special vectors, which can specifically recognize and bind to the target cells such as tumor

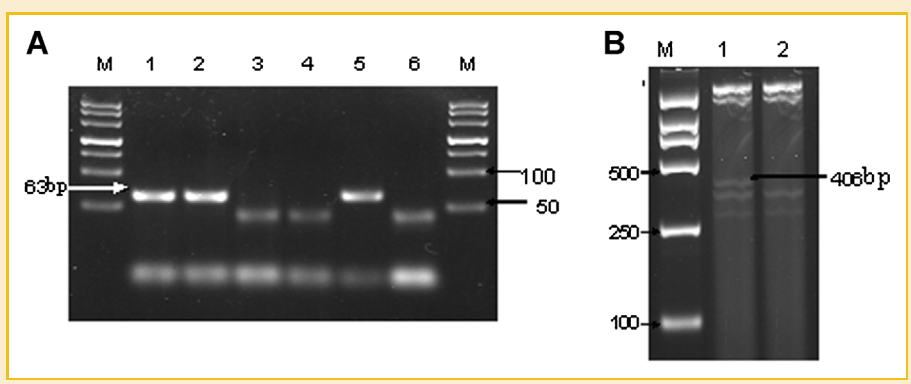

Fig. 5. Identification of recombinant plasmid sp-pIRES by PCR and double digestions. The recombinant vector sp-pIRES was transformed into *E. coli* JM109, and three clones were randomly selected by blue-white screening. The desired sp-pIRES recombinant plasmid was identified by PCR and double digestion. (A) Electrophoresis graph of recombinant plasmid sp-pIRES by PCR. M: DL 500 marker; Lines 1 and 2: sp-pIRES; Lines 3 and 4: negative control (pIRES); Line 5: positive control (sp-dsDNA); and Line 6: blank control (H_2O). (B) Electrophoresis graph of recombinant plasmid sp-pIRES digested with double digestions (*Eco*R I and *Hin*dIII). M: DL 2,000 marker; Line 1: sp-pIRES; and Line 2: pIRES.

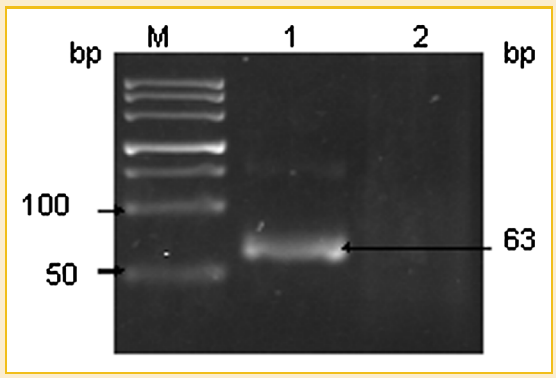

Fig. 6. Detection of sp22 in spNKM and pNKM cells. M: DL 500 marker; Line 1: spNKM; and Line 2: pNKM. Total RNA of spNKM and pNKM cells was extracted, respectively. RT-PCR was performed and products were separated by agarose gel electrophoresis.

cells, thereby greatly improving the therapeutic efficacy and reducing toxicity [Askoxylakis et al., 2005; Bockmann et al., 2005; Ferrieu-Weisbuch et al., 2006; McWhirter et al., 2006]. Polyclonal antibodies, antibody Fabs, monoclonal antibodies, and genetic engineering antibodies have been used as vector in most of the traditional targeted therapies. The traditional targeted treatments are confronted with a number of problems, such as the relatively large-sized molecules of conjugates that prevent them from penetrating the biological barriers of tumor cells. Small peptides as vectors will have great advantages [Beasley et al., 2002; Sharon et al., 2005; Zitzmann et al., 2005]. The peptide library screening can help to overcome some disadvantages associated with the traditional target therapies [Lu et al., 1995; Fei et al., 2004; Landon et al., 2004; Hu et al., 2006; Samoylova et al., 2006].

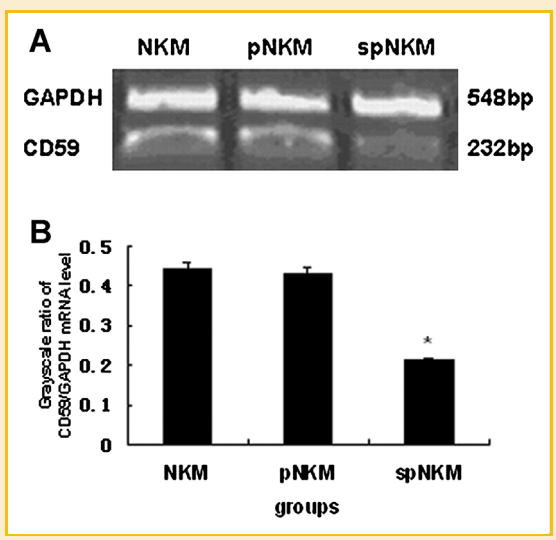

Fig. 7. Detection of CD59 mRNA quantities in spNKM cells by RT-PCR. (A) Electrophoretogram of CD59 and GAPDH. Total RNA of spNKM and pNKM cells was isolated, respectively. RT-PCR was performed to detect CD59 and GAPDH. Line 1: NKM; Line 2: pNKM; and Line 3: spNKM. (B) The grayscale ratios of CD59/GAPDH mRNA level. The bands were scanned by gray scanner and analyzed by BandScan strip analysis software. CD59 levels were normalized with respect to GAPDH levels and the grayscale ratios of CD59/GAPDH represented the quantities of CD59 mRNA. $^{*}P < 0.05$, compared with NKM and pNKM cells.

In 1975, with the emergence and development of hybridoma technique, people had a lot of hope for the mAb as "bio-missile" to cure cancer. However, because of the humanization of mAb, especially the problem of tumor escape, there is not yet a clinical successful report. Recently, the overexpression of CD59 gene has been discovered in many kinds of tumor cells in intestine, ovarian or prostate. Moreover, researches revealed that CD59 was closely related to out-of-control growth and malignant transformation of tumor. It is reported CD59 overexpressed in most solid malignancy and CD59 expression level is closely correlated with malignant transformation. So CD59 has become researching hot spot in immune escape and aversion.

Our studies focused on screening a novel CD59-ligand peptide sp22 by phage peptide display technique and investigating the effects of sp22 on cancer cell growth and apoptosis.

The ligand peptides found by phage peptide display technique have been successfully applied in the fields of early cancer diagnostics and chemotherapy. The random sequence oligonucleotides were inserted into a specific site of phage DNA, then random sequence peptides or proteins were expressed and exposed on the phage surface. As organs and tumors carry unique marker molecules, phage display can be used to screen organ- or tumor-specifc peptide sequences for targeted drug delivery [Pasqualini and Ruoslahti, 1996; Lee and Ge, 2009; Molek et al., 2011]. Phage display library screening also enables the investigation of ligand–receptor interactions because a map of ligand or receptor-binding sites can be constructed on the basis of selected peptide sequences [Wrighton et al., 1996; Yanofsky et al., 1996; Cwirla et al., 1997; McConnell et al., 1998; Giordano et al., 2001; Hetian et al., 2002; Schooltink and Rose-John, 2005; Su et al., 2005; Tipps et al., 2010]. Conveniently, the small size of the selected peptide lends itself to the design of non-peptide mimetics with improved characteristics [Ladner et al., 2004; Vrielink et al., 2010].

In the present study, the short peptides specifically binding to human CD59 were screened from an M13 phage display library so as to counteract tumor immune escape activity. CHO cells with high expression of human CD59 acting as target cells, through increasing the input of each round of peptide library and enhancing elution strength, the specificity and affinity of the screening were improved (Table I). Recovery ratios increased by six rounds of competitive binding test (Table II). Then the positive phage clones were screened by ELISA and sequenced. Eight out of 16 phage clones were randomly chosen and identified to have high affinities with CD59 (Fig. 1). After sequencing, three high homologous amino acids were obtained. DNAstar analysis showed that three sequences had some homology with the sequence of human CD2 published in PubMed (HGAAENSLPSPSS). The obtained sequence H x A x x x x x x P x x is helpful for design of short-peptide seal specific to active sites of CD59 which is related to tumor escape (Table III). We imagine that a short peptide can be synthesized in vitro to influence CD59, which may become a new target spot in the treatment of tumor. The newly synthesized short peptide seal should block the tumor escape-related

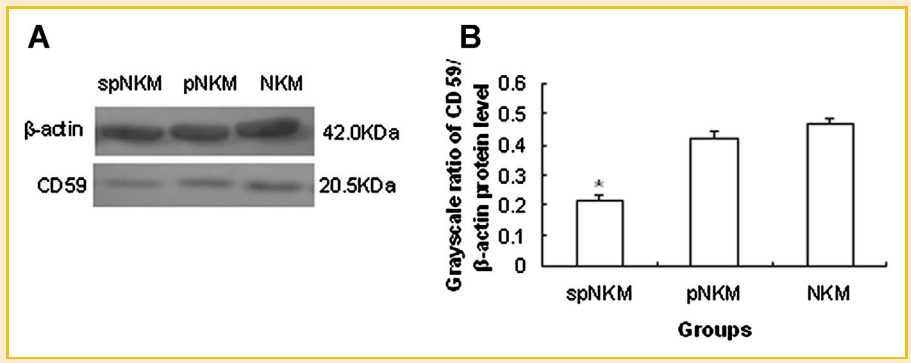

Fig. 8. Identification of CD59 protein expression by Western blot. (A) Western blot. Proteins were transferred to PVDF membranes. The membrane was incubated, first, with mouse anti-human CD59 and mouse anti-human β-actin antibodies, respectively, and, second, with HRP-conjugated goat anti-mouse secondary antibody. Line 1: spNKM cells; Line 2: pNKM cells; and Line 3: NKM cells. (B) The grayscale ratio of CD59/β-actin protein level. The bands were scanned by gray scanner and analyzed by BandScan strip analysis software. CD59 levels were normalized with respect to β-actin levels and grayscale ratio of CD59/β-actin was calculated. $^{*}P < 0.05$, compared with NKM and pNKM cells.

CD59 active site, and may provide new ideas for clinical treatment for carcinoid tumors.

Subsequently, a novel 22 amino acid peptide seal specific to CD59 (abbreviated as sp22, ACHWPWCHGWHSACDLPMHPMC) was designed and synthesized. Results suggested that sp22 could restrain the expression of CD59 molecule on MCF-7 cells, and with the elevation of sp22 concentrations the inhibitory effects were more obvious (Fig. 2). The influence of sp22 on HEL cells was not significant.

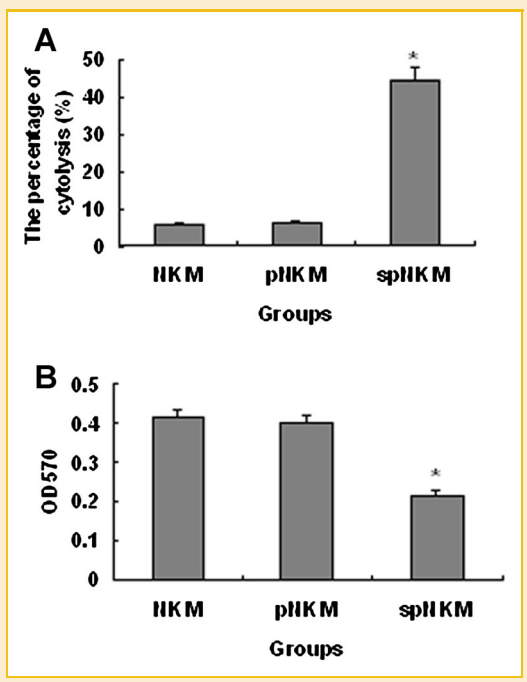

Fig. 9. Determination of complement-mediated cytolysis by ELISA and cells proliferation ability by MTT assay. (A) The percentage of cell cytolysis. Cells were incubated with rabbit anti-NKM polyclone antibody and serial dilutions of fresh human serum in medium. MTT and DMSO were added in turns. The OD 490 nm was measured and the percentage of cell cytolysis (%) was calculated by expressing (OD 490 of control group − OD 490 of experiment group) as a percentage of OD490 of control group. (B) The number of cell survival. The spNKM, pNKM, and control NKM cells were treated with MTT solution and acidic isopropanol in turns. The OD of 570 nm was measured and reflected the number of cell survival. $^{*}P < 0.05$, compared with the NKM and pNKM cells.

As is well known, cell apoptosis has an affinity with the occurrence, development and regression of tumor, therefore an important mechanism to prevent tumorigenesis is the induction of cell apoptosis that takes place continuously in many tissues of our body to remove unwanted, damaged or aberrant cells [Green et al., 1994]. In an effort to gain insight into the biochemical mechanism underlying sp22 induced MCF-7 cells apoptosis, in the present study, we have determined the effects of sp22 on the proliferation and apoptosis of MCF-7 and HEL cells. With the increase of sp22 concentrations, the survival numbers of MCF-7 cells decreased. sp22 was of no distinct effect on HEL cells (Table V). Consequently, the short peptide seal sp22 was proved to possess an anti-tumor activity by holding back expression of CD59 molecule which was related with tumor escape.

This reduction in the survival numbers of MCF-7 cells in the presence of sp22 might be due to either apoptosis or necrosis. Further studies were undertaken on the apoptosis-related factors. Much of the current interest in the process stems from the discovery that tumor can be regulated by certain proto-oncogenes and the tumor suppressor genes. Fas expression has been shown to be involved in the initiation of apoptosis under some conditions. These hinted that in the courses of cellular apoptosis, Fas antigen probably hold a certain synergistic effect [Moller et al., 1993]. Bcl-2 emerged as a new proto-oncogenes interfered with programmed cell death independent of promoting cell division [Grobholz et al., 2002].

TABLE VI. Comparison of Expression Quantities of Fas, Bcl-2, and Caspase-3 Proteins in NKM, pNKM, and spNKM Cells

	PLI		
Groups	Fas	Bcl-2	Caspase-3
NKM	0.1012 ± 0.0103	0.3318 ± 0.0182	0.1552 ± 0.0119
pNKM	0.1224 ± 0.0153	0.3068 ± 0.0215	0.1623 ± 0.0252
spNKM	0.2433 ± 0.0159*	0.1954 ± 0.0354*	0.2597 ± 0.0187*

*$P < 0.05$ vs NKM cells.

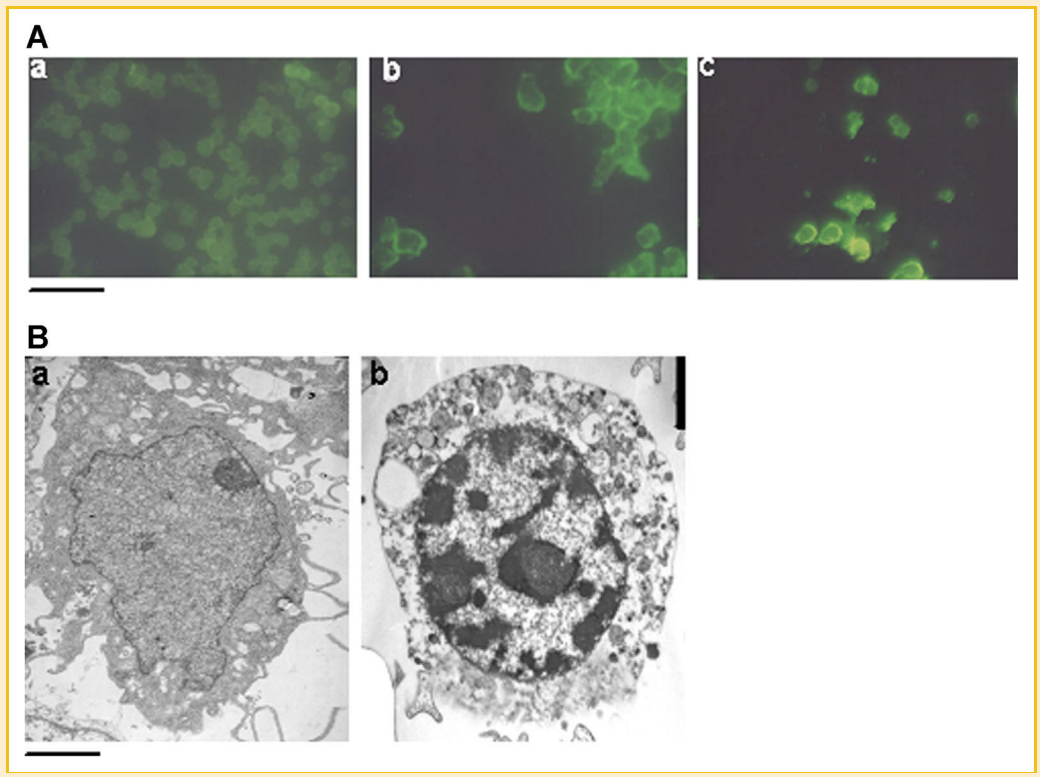

Fig. 10. The inducing effects of sp22 on NKM cells apoptosis by TUNEL assay and Electron microscope. (A) TUNEL assay. NKM, pNKM, and spNKM cells were harvested, incubated in 1% paraformaldehyde and fixed in 70% ethanol. DNA breaks were labeled, in turns, by TdT enzyme, BrdUTP, and fluorescein labeled anti-BrdU antibody. Total DNA was counterstained using propidium iodide/RNase A solution. Fluorescence intensity reflected apoptotic cell numbers. (a) NKM cells, (b) pNKM cells; and (c) spNKM cells. Scale bar, 20 μm. (B) Electron micrographs. Three groups of cells were harvested and fixed in 2.5% glutaraldehyde, and then postfixed in 1.5% osmium tetroxide, dehydrated through a graded alcohol, embedded in Epon 812, thin-sectioned, and stained with uranyl acetate and lead citrate. Ultrathin sections were placed on grids and characteristic morphological changes of apoptosis were examined with a transmission electron microscope. (a) NKM cells and (b) spNKM cells. Scale bar, 3 μm.

Caspases which play a central role in virtually all known apoptotic signal pathways were initiators of apoptosis, and they are regulated by Fas/TNF-R1, mitochondria dysfunction, and TNF-related apoptosis-inducing ligand. Caspase-3 activation results in the cleavage of cellular substrates and eventually leading to apoptosis. So we explored the effects of sp22 on the apoptosis signals of MCF-7 cells. Results suggested, compared with normal cells, sp22 could upregulate expression levels of pro-apoptotic Fas and Caspase-3 proteins, and inhibit anti-apoptotic Bcl-2 protein expression in MCF-7 cells. But the changes of expression quantities of these three kinds of proteins were not obvious (Fig. 3). TUNEL was a convincing approach for cell apoptosis determination. Apoptotic cell numbers were gradually enhanced when MCF-7 cells were treated with increasing concentration of sp22, but the effects of sp22 on HEL cells were not significant. So sp22 induced apoptosis in MCF-7 cells might be through the continuous transduction of tumor apoptosis signals (Fig. 4).

In order to further study how sp22 gene induced NKM cell apoptosis, the DNA sequence of sp22 (sp-dsDNA) was deduced and inserted into the eukaryotic expression plasmid vector pIEPS. The recombinant eukaryotic expression vectors targeted CD59 gene (sp-pIEPS) were successfully constructed (Fig. 5), and then were transfected into NKM cells (Fig. 6). The stable transfectants clones were screened. Subsequently, the effects of sp22 gene on the expression of CD59 in NKM tumor cells were investigated. The quantities of CD59 mRNA and protein decreased in transfected spNKM cells (Figs. 7 and 8).

Human CD59 contains a 4-kD N-linked carbohydrate chain that functions as an inhibitor of the CD5b-9 MAC of human complement, so it can restrict the cytolytic activity of the CD5b-9 complex to protect human blood, vascular and tumor cells from autologous complement attack. We found that the percentage of complement-mediated cell cytolysis increased apparently in spNKM cells. The result revealed that sp22 short peptide seal could inhibit the expression of CD59 which lead to the reduction of ability to avoid complementary attack and the increasing of killing ratio of complement to tumor cells (Fig. 9A). The inhibitory effects of sp22 gene on NKM cells growth were determined. Compared with NKM cells, the cell survival ratio degraded in spNKM cells, and the differences were statistically significant ($P < 0.05$). So sp22 gene transfection could inhibit NKM cell proliferation (Fig. 9B). In addition, expressions of apoptosis-related proteins changed. Fas and caspase-3 proteins increased but Bcl-2 protein expression decreased in NKM cells transfected with sp22 gene (Table VI). Apoptotic cells numbers increased and apoptotic morphology emerged in spNKM cells (Fig. 10). All these results suggested that sp22 could downregulate CD59 expression, promote complement-mediated cytolysis, suppress tumor cells growth, change expressions of

apoptosis-related proteins, cause appearance of morphological apoptotic characteristics, which might be responsible for the apoptosis of NKM cells.

In a word, Sp22, as a novel CD59-binding peptide, can serve as a marker for the early diagnosis of cancer. All these results suggested that sp22 could bind to CD59, inhibit the expression of CD59 molecules on tumor cells surface, increase complement attacking activity to tumor cells, and influence expression of apoptosis associated proteins, which might be a potential way to induce apoptosis of cancer cells. Our results revealed that sp22 peptide can target both breast cancer cell (MCF-7) and stomach cancer cell (NKM) due to its high and specific affinity to CD59 on malignant cells and strong cytotoxic effects. The studies put forward a new idea about exploiting sp22 as an effective approach in cancer therapy and provide a new gene therapy method for cancer patients.

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