P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wcjd@public.bta.net.cn www.wjgnet.com

World J Gastroenterol 2002;8(4):619-623
World Journal of Gastroenterology
 ISSN 1007-9327

• GASTRIC CANCER •

Identification of tumor associated single-chain Fv by panning and screening antibody phage library using tumor cells

Yong-Zhan Nie, Feng-Tian He, Zhi-Kui Li, Kai-Chun Wu, Yun-Xin Cao, Bao-Jun Chen, Dai-Ming Fan

Yong-Zhan Nie, Feng-Tian He, Zhi-Kui Li, Kai-Chun Wu, Yun - Xin Cao, Bao-Jun Chen, Dai-Ming Fan, Institute of Digestive Diseases, Xijing Hospital, Fourth Military Medical University , Xi' an 710032, Shaanxi Province, China
Supported by the National "863" Strategy for Science (102-10-01-06) and National Natural Science Foundation of China, No. 39525020
Correspondence to: Dai-Ming Fan, Institute of Digestive Diseases, Xijing Hospital, Fourth Military Medical University, Changle West Road, Xi' an 710032,Shannxi Province China. fandaim@fmmu.edu.cn
Telephone: +86-29-2539041 **Fax:** +86-29-2539041
Received 2001-06-12 **Accepted** 2001-09-14

Abstract

AIM:**To study the feasibility of panning and screening phage-displaying recombinant single-chain variable fragment (ScFv) of anti-tumor monoclonal antibodies for fixed whole cells as the carriers of mAb-binding antigens.**

METHODS: **The recombinant phage displaying libraries for anti-colorectal tumor mAb MC3Ab, MC5Ab and anti-gastric tumor mAb MGD1 was constructed. Panning and screening were carried out by means of modified fixation of colorectal and gastric tumor cells expressed the mAb-binding antigens. Concordance of binding specificity to tumor cells between phage clones and parent antibodies was analyzed. The phage of positive clones was identified with competitive ELISA, and infected by *E.coli* HB2151 to express soluble ScFv.**

RESULTS:**The ratio of positive clones to MC3-ScF-MC5-ScFv and MGD1-ScFv were 60 %, 24 % and 30 %. MC3-ScFv had M_r 32 000 confirmed by Western blot. The specificity to antigen had no difference between 4 positive recombinant phage antibodies and MC3Ab.**

CONCLUSION:**The modified process of fixing whole tumor cells is efficient, convenient and feasible to pan and screen the phage–displaying ScFv of anti-tumor monoclonal antibodies.**

Nie YZ, He FT, Li ZK, Wu KC, Cao YX, Chen BJ, Fan DM. Identification of tumor associated single-chain Fv by panning and screening antibody phage library using tumor cells.*World J Gastroenterol* 2002; 8(4) :619-623

INTRODUCTION

Tumor specific or associated antibodies *in vivo* diagnosis and treatment in tumor patients have been sought recently[1-4]. Most tumor-specific or tumor-associated antibodies have been obtained by the approach to immunizing animals with tumor cells, which inevitably cause allergic reaction against animal antibodies[5,6]. To miniaturize animal antibodies is an efficient way to decrease the rejection and allergy reaction. Gene engineering methods, especially phage display(PD), have great advantages[7-9]. It is the best way that purified tumor antigens (TA) were coated to capture recombinant antibodies in phage antibody library[10-12]. Unfortunately, many TA corresponding to tumor specific antibodies have not yet been isolated and purified, even not yet identified[13,14]. It hinders the production of miniaturizing tumor-specific antibodies specific to un-isolated TA. It was speculated that the whole tumor cells which expressed TA might have been considered for replacement of TA. However, It was reported that the panning and screening of PD was non-specific by means of replacing TA with whole tumor cells[8]. This could be attributed to the much lower antigen density and much complicated antigens. Nevertheless, significant progress on the methods has been made, allowing the utilization of PD using whole tumor cells[15,16]. But this utilization is just limited to screen new unknown recombinant antibodies. In this study, we modified the fixing conditions of whole cells for panning and screening phage libraries constructed for the unique monoclonal antibodies such as anti-colon cancer MC3, MC5mAb and anti-gastric cancer MGD1 mAb [13,17-21], and cell ELISA for screening ScFv clone. The results were satisfactory.

MATERIALS AND METHODS

Cell lines

Gastric tumor cell lines[2, 10] KATO-III, AGS, MKN-45, GC803, SGC7901, colorectal tumor cell lines W480, HT-29, CoCa-2, and human fibroblast cells were grown in RPMI 1640 or DMEM supplemented with 100 mL· L^{-1} new born bovine serum (NBS). All cell lines were grown adherently except KATO-III.

Construction of phage ScFv libraries

mRNA was isolated from the corresponding antibodies hybridoma cells. VH and VL cDNA were amplified with RT-PCR and linked with ScFv by linker DNA to form ScFc DNA, which then were inserted into plasmid PCANBSE. Plasmid DNA was transformed into *E.coli* strain TG1. ScFv-phage was induced by superinfection with helper phage M13KO7.

Cells fixation

The fixed cells were used for libraries panning and as antigens of cell ELISA. Methods reported by Ridgway *et al*[8] were used with the following modifications. Fixation of suspending cells: the cells were washed with PBS, resuspended, and transferred to 96-well enzyme-labeled plates at (4-5)×10^5 cells/well. The volume of cell suspension was no less than 300 μl each well. Otherwise, the cells would be distributed unevenly during centrifugation. The plates were centrifuged for 12 min at 1 200 r· min^{-1}, and the supernatants were discarded immediately without disturbing the pellets. The plates were allowed to dry at 37 ℃ for 15-20 min. Into each well, 2.5 g· L^{-1} glutaraldehyde prepared with 60 μl of 0.1 mol· L^{-1} PBS was added. Twelve min later, the fixative solution was discarded. The cells were washed 5 times by PBS. The plates were blocked with 100

g· L^{-1} skimmed milk powder overnight at 4 ℃. Coating of suspending cells for library panning: the cells were plated into 6-well plates at(1-1.5)×10^7 cells/well. The cell suspension volume was no less than 7mL in each well. The rest procedures were as described above. Fixation of adherent cells: the cells were plated into 96-well plates at 0.2×10^4 cells/well. The cells were allowed to incubate 48-72 h. When the cells were 80 % confluent, the medium was removed. The plates were washed twice with prewarmed PBS and dried at 37 ℃ for 20 min. The cells were fixed for 8 min as described above.

Detection of intracellular peroxidase

The fixed cells were divided into 2 groups. Cells in one group were treated with 3 mL· L^{-1} H_2O_2 prepared with methanol and washed 3 times with PBS. The plates were blocked with 50 g· L^{-1} skimmed milk powder overnight at 4 ℃ (or 37 ℃ for 2h). Cells in another group were treated with blocking solution directly. After the blocking solution was removed, the plates were washed 3 times with PBS containing 0.5 g· L^{-1}Tween20, and OPD substrate(50 μl/ well) was added to develop color. thirty min later, the color development was terminated with 2 mol· L^{-1} sulfuric acid. *A*490 were read. The well without substrate was designed as background control. Negative control and blank control were also designed.

Panning of phage libraries

The TG1 recombinant phage antibodies and soluble ScFv secreted by *E.coli* HB2151 were obtained according to the kit instructions (Pharmacia Biotech) [7]. Wash the 6 well plate coated with tumor cells three times with PBS, empty it completely after each wash. Fill the plate completely with blocking buffer to block any remaining sites on the plate surface. Incubate at room temperature for 1 h. Wash the flask three times with PBS, and empty it completely after each wash. Prepare 14 mL of blocking buffer containing 1 mL· L^{-1} thimerosal or 100 mL· L^{-1} sodium azide as a preservative. Dilute the 16 mL of PEG-precipitated recombinant phage with 14 mL of blocking buffer and incubate at room temperature for 10-15 min. Add 20 mL of the diluted recombinant phage to the plate and incubate for 2 h at 37℃. Wash the plate 20 times with PBS and 20 times with PBS containing 10 mL· L^{-1} Tween 20. Empty the plate completely each time. To isolate colonies for small-scale rescue, reinfect *E. coli* TG1 cells with bound phage directly in the panning vessel and plate the reinfected cells. Subsequent rounds of panning are to be performed. Add the entire 10 mL of log-phase TG1 cells to the flask or panning vessel. Incubate with shaking at 37 ℃ for 1 h. Transfer the entire 10 mL from the panning vessel into a sterile 50 mL disposable polypropylene centrifuge tube. To the cell suspension, add ampicillin to 0.1 g· L^{-1}, and glucose to a final concentration of 200 g· L^{-1}. Also add 4×10^{10} pfu of M13KO7 volume of stock to add = 4×10^{13} pfu ÷ M13KO7 pfu/L. Incubate the culture for 1 h at 37℃ with shaking. Sediment the cells by centrifugation and complete the rescue. The selection produre was repeated 4 times before isolated clones were tested by ELISA.

Cell ELISA

Detection of parent antibodies activity[22,23]: cellular endogenous peroxidase activity was rather low, so treatment of cells with H_2O_2 was unnecessary. The procedures were as follows. mAb were added to the blocked wells (50 μl/well). The plates stood for 1h at 37℃. The cells were washed 5 times by PBST. fifty μ L horseradish peroxidase (HRP) conjugated sheep anti-mice IgG was added to each blocked well. The plates stood for 1h at 37 ℃. Cells were washed 5 times with PBST. OPD substrate (50 μl/well) was added to develop color. Ten to twenty min later, *A*490 was read. Normal mice IgG and PBS served as negative and blank controls respectively.

Screening of recombinant phage antibodies or recombinant soluble ScFv clones: recombinant phage antibodies (M13KO7 as negative control) or soluble antibodies bearing anti-E-tag label protein displayed on phage served as the first antibody. Incubation condition was set as 2h at room temperature, gently shaking at 120 r· min^{-1} with cradle. Correspondently, rabbit anti-M13-HRP polyclonal antibody (1:5 000, Pharmacia) or mouse anti-E-tag IgG (1:1000, Pharmacia) were chosen as the second antibody, for the latter sheep anti-mouse IgG HRP, should be used. The rest procedures were as described above.

Concordance of the specificity to tumor cells between the positive recombinant phage clones and parent mAb: the blocked cells were coated as described above (including tumor cells or normal cells expressing antigen highly, lowly and blankly). Pairs of ScFv and parent antibodies were added to all cell plates respectively. A values for each well were read by the same method. Correlation coefficient was obtained through correlation analysis[24-26].

Competitive ELISA

The aim was to screen positive clones with high affinity[27-29]. The cells were coated as described previously. ScFv-phage supernatants or recombinant soluble ScFv (50 μl/well) were added to plates after co-incubation with 1/4 volume of 200 g· L^{-1} skimmed milk powder for 15min. The plates stood for 1hr at 37 ℃. Twenty-five μl corresponding mAb was added to each well. After standing for 1h at 37 ℃, the plates were washed. Fifty μl HRP conjugated sheep anti-mouse IgG were added to each well. After standing for 1 h at 37 ℃, the plates were washed 5 times with PBST. 50 μl OPD substrate were added to each well to develop color. *A*490 were read 10-20 min later. M13KO7 served as negative control. Inhibition rate (%)=1-(*A*490 for experimental well/*A*490 for control well)× 100%.

Western blot

Routine methods were applied[30-33].

Statistical methods

The data were analyzed with SPSS software package.

RESULTS

Detection of parent antibody activity with cell ELISA

The least dilution for MC3mAb was 25 mg· L^{-1}, for MC5mAb was 50 mg· L^{-1}, and for MGD1mAb was 12.5mg· L^{-1}. *A*490 were all more than 0.600, indicating that the mAb used in the experiment were active.

Construction of the ScFv antibody libraries

The VH, VL and ScFv DNAs were about 340, 320 and 750 bp respectively(Figures 1,2).

Panning of phage libraries with coated cells

When suspending cells were plated, if the cell suspensions adding to each well were not enough (<5 mL) or the cell number in each well was too many (>2×10^7 cells/well), the cells would be distributed unevenly during centrifugation. In 4 outer wells, the cells were found to be distributed in a half-moon shape, which was inconvenient for next fixation and panning steps. To avoid the above problems, we added no less than 7 mL cell

suspensions to each well with (1-1.5)×10^7 cells/well, and speeded centrifugation to 1 200 r· min^{-1} gradually. The TG1 containing recombinant phage recovered after 4 rounds of panning were plated on SOB-AG (A: ampicillin ,G: glucose). After 14 h, dense clones were observed.

Screening of recombinant phage antibodies or recombinant soluble clones with cell ELISA

Sixty clones were selected randomly from each panned library. The positive clones, whose *A*490 were over 0.300 (positive control), were counted. The ratio of positive clones were obtained. Soluble expression of *E.coli.* HB2151 was carried out using the positive clones. Its positive rate was determined by cell ELISA and Western blot. The result is shown in Table 1.

Concordance of binding specificity of phage library and parent antibody to tumor cells

Taking MC3-ScFv clone19 as example, the binding specificity of ScFv and parent antibody MC3 to AGS, SW480, SGC7901 fibroblasts were analyzed as shown in Table 2. *A*490 of parent antibodies were set as Y axis, and that of ScFv as X axis. Correlation analysis revealed r =0.991,P<0.01, primarily indicating the concordance of binding specificity of MC3-ScFv clone 19 and parent antibody to tumor cells. Other clones of MC3-ScFv displayed similar characteristics to MC3-ScFv clone 19.

Table 1 Ratio of positive recombinant phage antibody clone and soluble ScFv

	Recombinant phage antibodies		Soluble ScFv	
	Ratio of positive clone (%)	Ratio of competitive clone (%)	Ratio of positive clone (%)	Ratio of competitive clone (%)
MC3 ScFv	60	8	30	8
MC5 ScFv	24	6	18	4
MGD1 ScFv	30	4	10	4

Table 2 Concordance for MC3-ScFv-19 and it's parental mAb specificity to cells

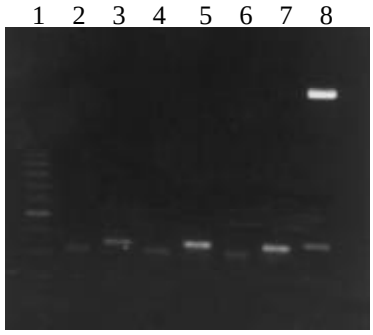
Cell lines	MC3-ScFv 19	MC3 Ab
AGS	1.403±0.132	0.929±0.187
SW480	0.921±0.201	0.647±0.132
SGC7901	0.257±0.045	0.259±0.078
Fibroblast	0.325±0.106	0.297±0.056

Screening of positive ScFv clones with competitive ELISA

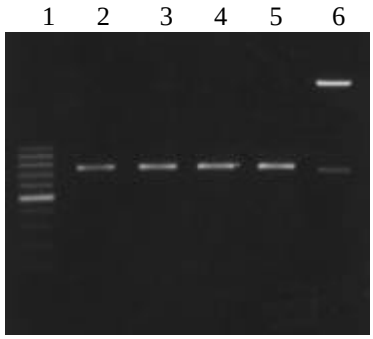
Inhibition rate over 30 % was put as criteria[8]. Positive clones from all libraries were obtained as shown in Table 1. As an example ,inhibition rates of 4 positive recombinant phage MC3 antibodies were 56.2 %, 53.6 %, 49.7% and 46.7 %. And inhibition rates of their soluble ScFvs were 41.5 %, 36.9 %, 33.7 % and 21.6 % respectively.

Western blot

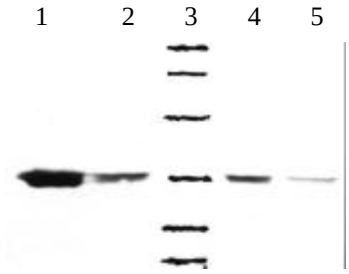
Soluble products expressed by *E.coli*. HB2151 were all about M_r 32 000, consistent with the expected molecule weight of soluble ScFv. The result is shown in Figure 3.



Lane 1: 100bp DNA ladder (100-1 000 bp); lane 2, 4,6: VL DNA; lane 3,5,7: VH DNA;
lane 8: VH DNA marker(2.7 kb, 350 bp)
Figure 1 VH and VL DNA amplified by RT-PCR



Lane 1: 100bp DNA ladder (100-1 000 bp); lane 2, 3,4,5: MC5, MC3, MGD1, MGD1 ScFv DNA;
lane 6 ScFv DNA marker (2.7 kb, 750 bp)
Figure 2 ScFv DNA amplified by RT-PCR



1,2,4,5: Four posotive clones of MC3 ScFv, 3: Low molecular weight marker(14.4, 20.1, 31.0, 43.0, 66.2, 97.4)KD
Figure 3 Western bloting of soluble MC3-ScFv clones

DISCUSSION

Phage display (PD) has many advantages in preparing minimized mouse and man antibodies. But the operation has met great difficulties due to severe conditions. The most difficult to satisfy is purified antigen, which is necessary for library panning and clone screening[32-35]. Recently, whole cells have been used in PD to obtain tumor-associated or tumor-specific antibodies. The results are encouraging. Kupsch *et al*[32] obtained human melanoma specific antibody by panning of a phage library using melanoma cells as negative screening, peripheral mononuclear cells as negative screening. Ridgway *et al*[8] cloned a human anti-CD55 ScFv by subtractive panning of a phage library using tumor and nontumor cells. In their studies,

they panned the known libraries to obtain new tumor specific ScFv, so most possible integrity of tumor antigen should have been retained, so they used live cells in panning and cell ELISA. However, the procedures were complicated, and the specialized equipments were required. Particularly, only tumor-associated membrane antigens could be obtained, as for intracellular antigens, the method was powerless[36-38]. The defects could be avoided by using fixed cells. But fixation procedures could destroy antigens partially, resulting in loss of some information. In the present report, we have studied the amenability of fixed cells to phage panning in PD technique.

Since development of mAb technique in the 1980s, many tumor specific or associated mAb have been cloned. However, most tumor antigens have not been identified and purified, and most mouse mAb had great molecular weight, and heterogeneity, which limited the application[39-42]. So, it is necessary to minimize some specific mAb. We constructed recombinant phage displaying libraries for anti-colorectal tumor mAb MC3Ab, MC5Ab and anti-gastric tumor mAb MGD1. Panning was carried out using fixed coated colorectal and gastric tumor cell lines. The positive recombinant phage clones were screened by means of cell ELISA. The concordance of the specificity to tumor cells with parent mAb was analyzed. The affinity of positive clones with tumor antigens was detected with competitive ELISA. The molecules and product amounts were determined by Western blot. All results suggested that fixed cells could be used to panning tumor-associated mouse ScFv using PD.

However, only the following conditions were satisfactory, it successful manipulation was confirmed. Activity of antigen should not be influenced during cell fixation procedures[43-45]. If the structure of antigen determinant cluster were changed during the fixation procedures, the fixation procedures should be modified or the fixative should be changed. We got satisfactory results using coating cells and cell ELISA with the following modifications. Cell lines highly expressing target antigens were used. Adherently or half-adherently grown cells were used because they easily plated and distributed evenly after plating. Fixation with glutaraldehyde could be half shortened. Cell lines highly expressing endogenous peroxidase were excluded to avoid destruction of lipoprotein antigens caused by methanol and H_2O_2 blocking[46-48]. So previous detection of endogeneous peroxidase activity of cell lines is very important; Fixation condition with glutaraldehyde should be modified. Kupsch *et al*[32] recommended that the cells should be fixed with glutaraldehyde at concentration of 5 $g \cdot L^{-1}$ for 30 min in cell ELISA. But we found that the results were more satisfactory with glutaraldehyde at concentration of 2.5 $g \cdot L^{-1}$ for 12 min for suspending cells and for 9min for adherent cells. Other precise conditions, such as centrifugation condition of suspending cells, drying prior to fixation, should also be paid great attention to[49,50]. Application limits: Firstly, fixed cells could be applied to minimization and gene engineering of known mouse mAb. The phage libraries were constructed by using hybridoma. Panning of phage libraries and screening of positive clones were feasible theoretically and practically by using cell lines which strongly expressed these mAb-binding antigens. Secondly, fixed cells might be also applied to panning of large human, mouse phage libraries to obtain unknown tumor specific antibodies. Although fixation procedures might destroy antigens partially so as to lose some information, application of live cells also had disadvantages, such as unfeasibility of screening of intracellular antigens. In present study, gastric tumor specific antigen corresponding to MDG1-ScFv did belong to intracellular antigens, which further indicating the amenability of the fixed cells.

ACKNOWLEDGMENT

We would like to thank Prof. Bo-Rong Pan from Oncology Center, Xijing Hospital for his great contributions to this article.

REFERENCES

1 **McCall AM**, Shahied L, Amoroso AR, Horak EM, Simmons HH, Nielson U, Adams GP, Schier R, Marks JD, Weiner LM. Increasing the affinity for tumor antigen enhances bispecific antibody cytotoxicity. *J Immunol* 2001; **166**:6112-6117
2 **Zhang XY**. Some recent works on diagnosis and treatment of gasric cancer. *World J Gastroenterol* 1999; **5**:1-3
3 **Fan DM**, Xiao B, Shi YQ, Ming F, Qiao TD, Chen BJ, Chen Z. A novel cDNA fragment associated with gastric cancer drug resistance was screened out from a library by monoclonal antibody MGr-1. *World J Gastroenterol* 1998; **4**(suppl 2):110-111
4 **Ji F**, Wang WL, Yang ZL, Li YM, Huang HD, Chen WD. Study on the expression of matrix metalloproteinanse-2 mRNA in human gastric cancer. *World J Gastroenterol* 1999; **5**:455-457
5 **Zhou C**, Jiang M, Xu L, Zhen Y.Construction and secretory expression of single-chain Fv fragment M97 with therapeutic potential against tumor invasion and metastasis.*Zhongguo Yixue Kexueyuan Xuebao*1998;**20**:81-88
6 **Liu HF**, Liu WW, Fang DC, Men RP. Expression and significant of proapoptotic gene Bax in gastric carcinoma *World J Gastroentero* 1999;**5**:15-17
7 **Adams Gp**, Schier R. Generating improved single-chain Fv molecules for tumor targeting. *Immunol Methods* 1999; **231**:249-260
8 **Ridgway JB**, Ng E, Kern JA, Lee J, Brush J, Goddard A, Carter P. Identification of a human Anti-CD55 single-chain Fv by subtractive panning of a phage library using tumor and nontumor cell lines. *Cancer Res* 1999;**59**:2718-2723
9 **Rozemuller H**, Chowdhury PS, Pastan I, Kreitman RJ. Isolation of new anti-CD30 scFvs from DNA-immunized mice by phage display and biologic activity of recombinant immunotoxins produced by fusion with truncated pseudomonas exotoxin. *Int J Cancer* 2001;92:861-870
10 **Roovers RC**, van der Linden E, de Bruine AP, Arends JW, Hoogenboom HR. *In vitro* characterisation of a monovalent and bivalent form of a fully humananti Ep-CAM phage antibody. *Cancer Immunol Immunother* 2001;**50**:51-59
11 **Powers DB**, Amersdorfer P, Poul M, Nielsen UB, Shalaby MR, Adams GP, Weiner LM,Marks JD. Expression of single-chain Fv-Fc fusions in Pichia pastoris. *J Immunol Methods* 2001;**251**:123-135
12 **Yuan Y**, Cong W, Xu RT, Wang XJ, Gao H. Gastric cancer screening in 16 villages of Zhuanghe region: a high risk area of stomach cancer in China. *World J Gastroentero* 1998; (Suppl 2):111-112
13 **Shi YQ**, Xiao B, Miao JY, Zhao YQ, You H, Fan DM. Construction of eukaryotic expression vector pBK-fas and MDR reversal test of drug-resistant gastric cancer cells. *Shijie Huaren Xiaohua Zazhi* 1999;**7**:309-312
14 **Wei TY**, Wei MX, Yang SM. Significance of expression of cyclin D1, P16 and preneoplastic lesion tissues. *Shijie Huaren Xiaohua Zazhi* 2000;**8**:234-235
15 **Van E**, Kruif W, Germeraad WT, Berendes P, Ropke C, Platenburg PP, Logtenberg T. Subtractive isolation of phage-displayed single-chain antibodies to thymic stromal cells by using intact thymic fragments. *Proc Natl Acad Sci USA* 1997; **94**:3903-3908
16 **Cai XH**, Garen A. Comparison of fusion phage libraries displaying Vhor sing-chain Fv antibody repertoire of a vaccinated melanoma patient as a source of melanoma-specific targeting molecules. *Proc Natl Acad Sci USA* 1997; **94**:9261-9266
17 **Guo JC**, Li JC, Fan DM, Qiao TD, Zhang XY. Regulation of HSP70 expression in human gastric cancer cell line SGC7901 by gene transfection. *Shijie Huaren Xiaohua Zazhi* 1999;**7**:773-776

18 **Liu HF**, Liu WW, Fang DC, Liu FX, He GY. Clinical significance of Fas antigen expression in gastric carcinoma. *World J Gastroenterol* 1999; **5**:90-91
19 **Chao CY**, Lie J, Fan DM. Immunohistochemical study of monoclonal antibody MGD-1 in gastric carcinoma. *Histopathology* 1989;**15**:523-529
20 **Xiao B**, Shi YQ, Zhao YQ, You H, Wang ZY, liu XL, Yin F, Qiao TD, Fan DM. Transduction of Fas gene or bcl-2 antisense RNA sensitizes cultured drug resistant gastric cancer cells to chemotherapeutic drugs. *World J Gastroenterol* 1998; **4**:421-425
21 **Cao GD**, Wang SW, Wu SS, Li HF, Zhang WG. Retrovirus mediated antisense RNA to bcl-2 alter the biological behavior of stomach carcinoma MGC-803 cell lines. *World J Gastroenterol* 1998;**4**(suppl 2):45-48
22 **Luo ZB**,Luo YH ,Lu R,Jim HY,Zhang PB, Xu CP. Immunohistochemical study on dendritic cells in gastric mucosa of patients with gastric cancer and precancerous lesions. *Shijie Huaren Xiaohua Zazhi* 2000;**8**:400-402
23 **Xu SH** ,Feng JG. Relationship between CD44 in the peripheral blood of patients with colorectal cancer and clinico-pathological features. *Shijie Huaren Xiaohua Zazhi* 2000;**8**:432-435
24 **Zhang J**, Wang WL, Li Q, Qiao Q. Expression and significance of transforming growth factor-α and its receptor in human primary hepatocellular. carcinoma *Shijie Huaren Xiaohua Zazhi* 1999;**7**:939-941
25 **Du DW**, Zhou YX, Feng ZH, Li GY, Yao ZQ. Study on immunization of anti subcutaneous transplanting tumor induced by gene vaccine. *Shijie Huaren Xiaohua Zazhi* 1999;**7**: 955-957
26 **Zheng CS**, Wang WL,Reng WD,Hu PZ,Chai YB,Ma FC. Promotion of apoptosis of SMMC7721 cells by Bcl-2 ribozyme. *Shijie Huaren Xiaohua Zazhi* 2000;**8**:417-419
27 **Wang CD**, Chen YL, Wu T, Liu YR. Association between lowe expression of somatostatin receptor Ⅱ gene and lymphoid metastasis in patients with gastric cancer. *Shijie Huaren Xiaohua Zazhi* 1999;**7**:864-866
28 **Han FC**, Yan XJ, Hou Y, Xiao LY, Guo YH, Su CZ. Gold immunochromatographic assay for anti-Helicobacter pylori antibody. *Shijie Huaren Xiaohua Zazhi* 1999;**7**:743-746
29 **Cui DX**, Yan XJ, Zhang L, Zhao JR, Jiang M, Guo YH, Zhang LX,Bai XP,Su CZ. Screening and its clinical significance of 6 fragments of highly expressing genes in gastric cancer and precancerous mucosa. *Shijie Huaren Xiaohua Zazhi* 1999;**7**:770-773
30 **Wang YF**, Wu XN, Zhang XQ, Chen XF. Circulating soluble intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in patients with gastric cancer. *Shijie Huaren Xiaohua Zazhi* 1998;**6**:1017-1019
31 **Sun YX**, Chen CJ, Zhou HG, Shi YQ, Pan BR, Feng WY. Expression of c-myc and p53 in colorectal adenoma and adenocarcinoma. *Shijie Huaren Xiaohua Zazhi* 1998;**6**:1054-1056
32 **Kupsch JM**, Tidman NH, Kang NV, Truman H, Hamilton S, Patel N, Newton Bishop JA, Leigh IM, Crowe JS. Isolation of human-specific antibodies by selection of an antibody phage library on melanoma cells. *Clin Cancer Res* 1999;**5**: 925-931
33 **Tordsson JM**, Ohlsson LG, Abrahmsen LB, Karlstrom PJ, Lando PA, Brodin TN. Phage-selected primate antibodies fused to superantigens for immunotherapy of malignant melanoma. *Cancer Immuol Immunother* 2000; **48**:691-702
34 **Marty C**, Scheidegger P, Ballmer-Hofer K, Klemenz R, Schwendener RA. Production of functionalized single-chain Fv antibody fragments binding to the ED-B domain of the B-isoform of fibronectin in Pichia pastoris. *Protein Expr Purif* 2001;**21**:156-164
35 **Sakai A**.Inhibition of endothelial cell adhesion molecule expression with SJC13, an azaindolidine derivative, *in vitro*. *Inflamm Res* 1996;**45**:224-229
36 **Goel A**, Colcher D, Baranowska-Kortylewicz J, Augustine S, Booth BJ, Pavlinkova G, Batra SK.Genetically engineered tetravalent single-chain Fv of the pancarcinoma monoclonal antibody CC49: improved biodistribution and potential for therapeutic application. *Cancer Res* 2000;**60**: 6964-6971
37 **Kang N**, Hamilton S, Odili J, Wilson G, Kupsch J. In vivo targeting of malignant melanoma by 125Iodine- and 99mTechnetium-labeled single-chain Fv fragments against high molecular weight melanoma-associated antigen. *Clin Cancer Res* 2000;**6**:4921-4931
38 **Kuan CT**, Wikstrand CJ, Archer G, Beers R, Pastan I, Zalutsky MR, Bigner DD. Increased binding affinity enhances targeting of glioma xenografts by EGFRvIII-specific scFv. *Int J Cancer* 2000;**88**:962-969
39 **Tur MK**, Huhn M, Sasse S, Engert A, Barth S.Selection of scFv phages on intact cells under low pH conditions leads to a significant loss of insert-free phages. *Biotechniques* 2001;**30**:404-408
40 **Goel A**, Augustine S, Baranowska-Kortylewicz J, Colcher D, Booth BJ, Pavlinkova G, Tempero M, Batra SK.Single-Dose versus fractionated radioimmunotherapy of human colon carcinoma xenografts using 131I-labeled multivalent CC49 single-chain fvs. *Clin Cancer Res* 2001;**7**:175-184
41 **Khare PD**, Shao-Xi L, Kuroki M, Hirose Y, Arakawa F, Nakamura K, Tomita Y, Kuroki M.Specifically targeted killing of carcinoembryonic antigen (CEA)-expressing cellsby a retroviral vector displaying single-chain variable fragmented antibody to CEA and carrying the gene for inducible nitric oxide synthase. *Cancer Res* 2001;**61**:370-375
42 **Boldicke T**, Tesar M, Griesel C, Rohde M, Grone HJ, Waltenberger J, Kollet O,Lapidot T, Yayon A, Weich H. Anti-VEGFR-2 scFvs for cell isolation. Single-chain antibodies recognizing the human vascular endothelial growth factor receptor-2 (VEGFR-2/flk-1) on the surface of primary endothelial cells and preselected CD34+ cells from cord blood. *Stem Cells* 2001;**19**:24-36
43 **Matsumura R**, Umemiya K, Goto T, Nakazawa T, Ochiai K, Kagami M, Tomioka H,Tanabe E, Sugiyama T, Sueishi M. Interferon gamma and tumor necrosis factor alpha induce Fas expression and anti-Fas mediated apoptosis in a salivary ductal cell line. *Clin Exp Rheumatol* 2000;**18**:311-318
44 **Yuan QA**, Yu WY, Huang CF. Construction and expression of a hepatocellular carcinoma specific rodent and its humanized single-chain Fv fragments in *Escherichia coli*. *Shengwu Gongcheng Xuebao* 2000;**16**:86-90
45 **Ozaki H**, Ishii K, Horiuchi H, Arai H, Kawamoto T, Okawa K, Iwamatsu A, Kita T. Cutting edge: combined treatment of TNF-alpha and IFN-gamma causes redistribution of junctional adhesion molecule in human endothelial cells. *J Immunol* 1999;**163**:553-557
46 **Ohizumi I**, Tsunoda S, Taniguchi K, Saito H, Esaki K, Koizumi K, Makimoto H,Wakai Y, Matsui J, Tsutsumi Y, Nakagawa S, Utoguchi N, Ohsugi Y, Mayumi T.Identification of tumor vascular antigens by monoclonal antibodies prepared from rat-tumor-derived endothelial cells. *Int J Cancer* 1998;**77**:561-566
47 **Takami S**, Yamashita S, Kihara S, Ishigami M, Takemura K, Kume N, Kita T,Matsuzawa Y.Lipoprotein(a) enhances the expression of intercellular adhesion molecule-1 in cultured human umbilical vein endothelial cells. *Circulation* 1998;**97**:721-728
48 **Strindhall J**, Lundblad A, Pahlsson P.Interferon-gamma enhancement of E-selectin expression on endothelial cells is inhibited by monensin.Scand *J Immunol* 1997;**46**:338-343
49 **Yamaguchi M**, Suwa H, Miyasaka M, Kumada K.Selective inhibition of vascular cell adhesion molecule-1 expression by verapamil in human vascular endothelial cells. *Transplantation* 1998;**65**:756-757
50 **Zund G**, Nelson DP, Neufeld EJ, Dzus AL, Bischoff J, Mayer JE, Colgan SP.Hypoxia enhances stimulus-dependent induction of E-selectin on aortic endothelial cells. *Proc Natl Acad Sci U S A* 1996;**93**:7075-7080

Edited by Ma JY