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• ORIGINAL RESEARCH •

Construction and selection of the natural immune Fab antibody phage display library from patients with colorectal cancer

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Abstract

AIM: **To construct the natural immune Fab antibody phage display libraries of colorectal cancer and to select antibodies related with colorectal cancer.**

METHODS: **Extract total RNA from tissue of local cancer metastasis lymph nodes of patients with colorectal cancer. RT-PCR was used to amplify the heavy chain Fd and light chain κ and the amplification products were inserted successively into the vector pComb3 to construct the human libraries of Fab antibodies. They were then panned by phage display technology. By means of Dot immunoblotting and ELISA, the libraries were identified and the Fab phage antibodies binding with antigens of colorectal cancer were selected.**

RESULTS: **The amplified fragments of Fd and κ gained by RT-PCR were about 650 bp. Fd and κ PCR products were subsequently inserted into the vector pComb3, resulting in a recombination rate of 40% and the volume of Fab phage display library reached 1.48×10^6. The libraries were enriched about 120-fold by 3 cycles of adsorption-elution-multiplication (panning). Dot immunoblotting showed Fab expressions on the phage libraries and ELISA showed 5 clones of Fab phage anti bodies which had binding activities with antigens of colorectal cancer.**

CONCLUSION: **The natural immune Fab antibody phage display libraries of colorectal cancer were constructed. They could be used to select the relative antibodies of colorectal cancer.**

Subject headings colorectal neoplasms, immunology, bacteriophages, antibody library

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INTRODUCTION

The incidence of colorectal cancer is growing in China. It has been clear that the prognosis of colorectal cancer is related to early diagnosis and treatment[1-15]. Since the development and evolution of the colorectal cancer is one kind of complicated pr ocedures involving multi-genes, multi-factors and multi-steps, there have been no highly peculiar molecular pathologic changes and tumor[16-39]. A major focus of cancer immunology is on the isolationof antibodies that react selectively with human tumor cells[40,41], because the antibodies could have important applications for targeting diagnostic and therapeutic agents to tumors and for identifying tumorigenic antigens[42-47]. The established approach has been to generate large panels of monoclonal antibodies (mAb) from mice immunized with human tumor cells and to screen the antibodies for reactivity against the tumor[48-50]. Despite the enormous efforts put on this approach, few antibodies that react preferentially with human tumors, and none that react specifically with one type of tumor (such as colorectal cancer), have been reported. Further attempts to isolate more specific and high affinity antibodies will require improved methods of generating and selecting antibodies against human tumors[51]. One is the introduction of method for synthesizing virtually the entire repertoire of a person's antibody genes of variable regions by PCR technique and for expressing the encoded antibodies on the surface of a phage vector[52]. The resulting phage antibody library can be panned to select and clone rare antibodies on the basis of their binding specificities[53,54]. It can resolve the problems of generating humanization mAb by hybridoma approach. Humans can be immunized or are immune to many antigens (including tumor antigens) but only local lymph nodes, a productive source of antibody-producing cells, is readily available. Here we describe the construction of the natural immune phage display libraries expressing Fab antibodies derived from local cancer metastasis lymph nodes of co lorectal cancer patients and report the initial results of panning the libraries for anti-colorectal cancer antibodies.

MATERIALS AND METHODS

Vector, E.coli and helper phage

The vector (pComb3), about 4894 base pairs, contains ampicillin resistance (Ampr) gene, start sites of plasmid CO1E1 and f1 phage replication. The expression of inserting fragments is control led by the same LacZ promoter on its upstream sequence. *E.coli* XL1-Blue contains tetracycline re sistance (Tetr) gene on its gene type of Tn10. Helper phage (VCSM13) contains kanamycin resistance (Kanar) gene with valency of 10^{15} pfu·L^{-1}. It is amplified in SOC culture medium and preserved in 4℃.

Lymph nodes total RNA preparation

Lymph nodes in mesenterium were resected during surgical operation on patients with colorectal cancer and preserved in liquid nitrogen immediately. The nodes of patients (case number 260280, 260583 and 260476) were defined as tumor metastatic lymph nodes by pathological examination. One hundred mg of each node was used to

extract total RNA by the standard method of guanidinium isothiocyanate.

Amplifying Fd and κ chain genes of antibodies by RT-PCR

Total RNA (20-50 μg) was added to 60pmol primer of *Oligo(dt)* and heated at 65℃ for 10 min. The mixture was then used in a 20 μl reverse transcription reaction containing 200 μmol·L^{-1} each dNTPs and 20 U of reverse transcriptase (Promega), which was incubated at 37℃ for Ih. The RNA-cDNA mixture (5 μL) was then used in 50 μL PCR reaction mixture containing all four dNTPs at 60 μmol·L^{-1}, 5 U of Taq polymerase (Promega), and 50 pmol·L^{-1} of appropriate 5' and 3' primers[55,56]. VK1a and VK3a are 5' primers for amplification of the κ chain with the Sac I site f or cloning into the vector pComb3. CK1a is a 3' primer corresponding to the 3' end of the light chain κ, *Xba* I site. VH1a and VH3a are 5' primers for the heavy chain (Fd), *Xho* I site. CG1z is the 3' primer for the Fd and corresponds to part of the hinge region, *Spe*I site.

$V_κ$5' primers: VK1a, 5'-GACATCGAGCTCACCCAGTCTCCA -3';
$V_κ$3a, 5'-GAAATTGAGCTCACGCAGTCTCCA-3';
$V_κ$3' primers: CK1a,
5'-GCGCCGTCTAGAACTAACACTCTCCCCTGTTGAAGCTCTTTG-TGACGGGCAAG-3';
V_H (Fd) 5' primers: VH1a, 5'-CAGGTGCAGCTCGAGCAGTCTGGG-3';
V_H3a, 5'-GAGGTGCAGCTCGAGGAGTCTGGG-3';
V_H (Fd) 3' primers: CG1z, 5'-GCATGTACTAGTTTTGTCACAAGATTTGGG -3'.

The reaction mixtures were then subjected to 35 rounds of amplification (PE/Cetus thermal cycler) at 94℃ for 1 min, 52℃ for 1.5 min and 72℃ for 2 min followed by a final incubation at 72℃ for 10 min. An aliquot of the reaction mixture (5 μL) was run on a 10 g·L^{-1} agarosegel.

Cloning heavy chain Fd into pComb3

The Fd fragment product of PCR (isolated by agarose gel electrophoresis) was cut with an excess of the restriction enzymes *Xho* I and *Spe* I and typically about 350 ng was ligated with 2 μg of *Xho/Spe* I -linearized pComb3 vector (isolated by agarose gel electro phoresis) in a total volume of 150 μL with 10 U of ligase (Promega) at 16℃ for 15 h. Following ligation, DNA was precipitated at -20℃ for 2 h by the addition of 15 μL of 3 mol·L^{-1} sodium acetate (pH 5.2), and 330 μL of ethanol. DNA was pelleted by microcentrifugation at 4℃ for 15 min. The DNA pellet was resuspended in 20 μL of water and transformed into 150 μL XL1-blue (porated by calcium chloride). After transformation, XL1-blue samples (10, 1, 0.1 μL) were withdrawn for plating to determine the rate of transformation. The insertion of target Fd fragments were detected by PC R from the plasmids extracted from several random XL1-blue monoclones. The plasmids with Fd insertion were named p+Fd.

Cloning light chain κ into p+Fd and antibodies Fab libraries construction

The κ fragment product of PCR and recombined p+Fd (isolated by agarose gel electrophoresis) were cut with an excess of the restriction enzymes *Sac* I and *Xba* I. The ligation, transformation, *E.coli* amplification and determination of the transformation rate were same as described above. The insertion of target κ fragments was detected by digestion with *Sac* I / *Xba* I from the plasmids extracted from several random XL1-blue monoclones. The Fab fragments' insertion was detected by digestion with *Xho* I and *Xba* I. The plasmids with Fd together with κ insertion were named Fd+κ. Helper phage VCSM13 (10^{12} pfu) was added to XL1-blue samples contained Fab gene libraries. Following the superinfection, the primer Fab phage display libraries of two patients were constructed, named K1 and K2, and preserved at 4℃.

Panning of the primer Fab phage display libraries

The colorectal cancer antigens were derived from fresh cancer tissues of patients 1, 2, and 3 by sonicating the tissues (each mass 500 mg). The supernatants were named Ag1, Ag2 and Ag3. Colon cancer cell line of Lovo (10^9 ·L^{-1}) was sonicated also, and its supernatants were named Lovo. The wells of a microtiter plate were coated overnight at 4℃ with the antigen supernatants above respectively (antigens were diluted with 0.1 mol·L^{-1} bicarbonate buffer, pH9.6). Cancer tissue antigens coated the microtiter plate wells in mixture of Ag1 and Ag2. Lovo cells of 200 μL(0.5×10^8·L^{-1}) were attached to the wall of the microtiter plat e wells, and 50 μL mixture of K1 and K2 was added to the wells coated with antigens respectively. The panning procedure is a modification[57] of that originally described by Parmley and Smith. The phage binding with antigens re-infected the XL1-Blue by eluting from the wells. A round of panning was finished after superinfection by VCSM13. Following 3 rounds of panning, the percent yield of phage was determined as (no. of phage eluted / no. of phage applied) ×100.

Dot immunoblotting analysis of Fab displaying[58]

Five μL library of each round of panning was added to nitrocellulose (NC) filter (with diameter of 0.5 cm). After being dried at room temperature, the NC filters were blocked in 50 mL·L^{-1} de-lipid milk and then react with the biotinylated anti-human IgG (1:100 dilution) and alkaline phosphatase (AP)-avidin at room tempe rature successively. Finally the NBT+BCIP was added and color developed. The control unit was the suspension of the phage contained vacant vector pComb3.

ELISA analysis of Fab displaying[59]

The wells of a microtiter plate were coated overnight at 4℃ with the antigen supernatants Ag1, Ag2, Ag3 and Lovo respectively (antigens were diluted in 1:100 with 0.1 mol·L^{-1} bicarbonate buffer, pH9.6). The suspension of K1 and K2 (50 μL each) was added to the wells respectively, and cultivated at 37℃. Following 3 washes with TBS buffer, 25 μL of a 1:100 dilution of biotinylated goat anti-human IgG and HP-conjugated biotin were added succes sively and incubated at 37℃. Finally 50 μL of TMB was added and color development was moni tored at 490 nm. The A490 values of positive clones were higher than that of neg ative clones at least two folds. After 3 rounds of panning, 5 clones of positive XL1-blue were superinfected with VCSM13 and the supernatants of Fab displaying were prepared. FiftyìL of the Fab display supern atants were added to the wells (coated with antigens of Ag1, Ag2, Ag3 and Lovo) respectively, and ELI SA analysis was applied by the method described above.

RESULTS

Amplifying the fragments of Fd and κ chain genes of antibodies by RT-PCR

The total RNAs of the lymph nodes defined as tumor metastasis in mesenterium of patients with colorectal cancer were isolated. The integrity of RNAs was shown by alkaline denaturing agarose gel. Their purity (A_{260}/A_{280}) reached 2.04. Fd and κ immunoglobulin chains were PCR-amplified after reverse transcription from this RNA. For amplifying Fd and κ, two 5'primers and one 3'primer were used respectively which contained some sites of the restriction enzymes corresponding to the vector pCom b3. The PCR amplification of Fd and κ was about 650 bp (Figure 1).

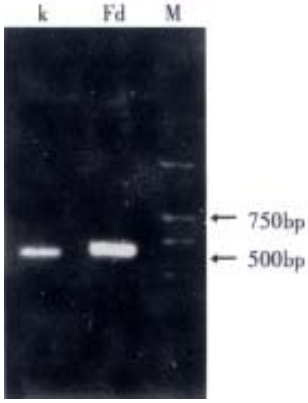

Figure 1 PCR products of Fd and κ.

Construction and identification of recombinant p+Fd

The recombinants of p+Fd were constructed by cloning Fd fragments into pComb3. After transforming XL1-blue, the Fd gene libraries were formed. And the quantity of the transformants reached 5.53×10^6. After extracting the plasmids from eight random XL1-blue monoclones, the insertion of target Fd fragments was detected by PCR with the primers same as that in amplifying Fd described above. Four of eight PCR could amplify the fragments of 650 bp (Figure 2). This meant that the recombination frequency was 50%, so the practical volume of Fd library was 2.77×10^6.

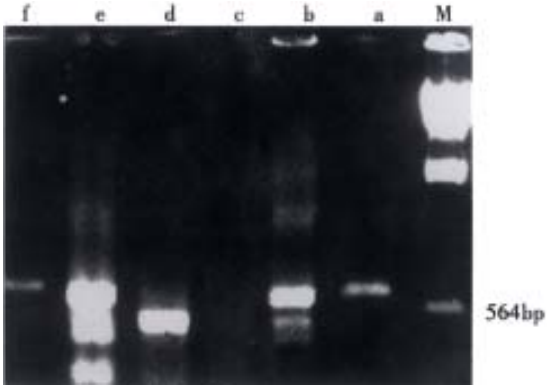

Figure 2 Fd insertion by PCR identification.

Construction and identification of Fab gene recombinant

The recombinants of Fd+κ were constructed by cloning ê fragments into p+Fd. After transforming XL1-blue, the Fab gene libraries were formed and the quantity of the transformants reached 7.4×10^6. Following extracting the plasmids from ten random XL1-blue monoclones, the insertion of target κ fragments was detected by digestion with *Sac* I and *Xba* I, and five of these plasmids discharged the fragments of 650bp (Figure 3). By digesting with *Xho* I and Xba I, four of these plasmids discharged the fragments about 2.4 kb (Figure 4). The recombination frequency of fragments Fd together with κ insertion was 40%, so the practical volume of Fab library was $7.4\times10^6\times50\%\times40\% = 1.48\times10^6$.

ELISA of binding activity of colorectal cancer antigens and Fab displaying on primer phage libraries

The activity of colorectal cancer antigens binding with K1 (or K2) was identified by ways of ELISA with control unit of wash buffer. The primer phage display libraries integrated with the colorectal cancer antigens of not only its immune derivation, but also other immune derivation (Table 1).

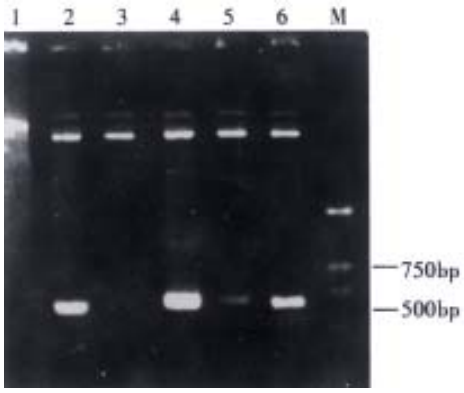

Figure 3 Identification of κ insertion by *Sac* I/*Xba* I digestion.

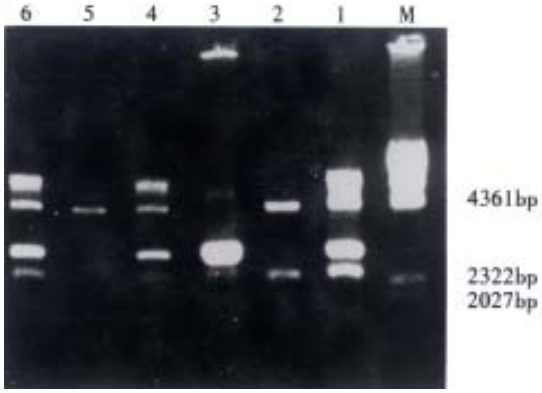

Figure 4 Identification of κ and Fd insertion by *Xho* I/ *Xba* I digestion.

Table 1 Binding of Fab displayed by prime phage display libraries with colorectal cancer antigens (A_{490})

	Ag1	Ag2	Ag3	Lovo	Wash buffer
K1	1.285	1.496	1.332	0.326	0.105
K2	1.324	1.552	1.352	0.322	0.122
BC	0.115	0.105	0.110	0.092	0.087

Panning and identification of the Fab phage display libraries

After 3 rounds of panning, the libraries were enriched about 120-fold. Dot immunoblotting applied by the system of AP-goat anti-human IgG showed that there were Fab expressions on the phage lib raries of each panning round (Figure 5). There was no obvious color development in the control units (C). The supernatants of Fab displaying prepared from 5 clones of positive XL1-Blue after 3 rounds of panning showed the significant binding activity with the antigens related with c olorectal cancer by ELISA analysis (Table 2). The control units were the rat colon antigens and wash buffer.

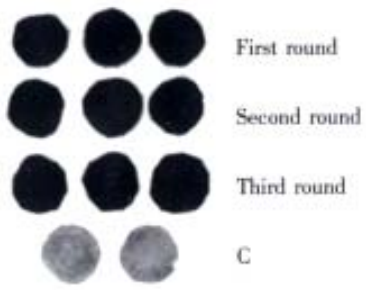

Figure 5 Dot immunoblotting analysis.

Table 2 Binding activity of phage antibodies with antigens (A_{490})

Phage antibodies	Ag1, Ag2 and Ag3	Lovo	Rat colon antigens	Wash buffer
Clone 1	1.236	1.042	0.121	0.096
Clone 2	1.117	0.873	0.110	0.100
Clone 3	1.450	1.329	0.182	0.121
Clone 4	1.106	0.912	0.106	0.087
Clone 5	1.345	1.024	0.090	0.083

DISCUSSION

Fd parts and κ immunoglobulin chains (650 bp) were PCR-amplified after reverse transcription from the RNAs, which were isolated from the lymph nodes de fined as tumor metastasis in mesenterium of patients with colorectal cancer. For mimicking the diversity of the antibody genes, we used 5' primers based on the leader exon and the N terminus of first framework (FR1) sequences according to their relatively constant regions, and 3' primers within the constant regions during amplifying the V genes of immunoglobulin Fab. To maximize complementarity, degeneracy was incorporated into the primers, or different primers were designed for different families of antibody V genes. For cloning of the amplified DNA into expression vectors, rare restriction sites were introduced within the PCR primers. Following mixing the 2-group of Fd PCR products, we cloned them into the vector pComb3. Then the 2-group of κPCR products were inserted into the recombinants of p+Fd. Therefore the gene libraries of humanization antibody were constructed, which contained parts of 2 -subgroup of heavy chains and 2-subgroup of κ chains. Previously reports had shown that the volume of the phage display libraries was 1.0×10^7 at most constructed by this vectors and ways[57]. In review, the volume was 10^6-10^7 according to the reports abroad, and about 10^6 according to the domestic reports[60,61]. We reported the volume being 1.48×10^6.

By antibody technology, the mAbs can be prepared by-passing hybridoma approach. Construction of the natural immune antibody library is the method of by-passing unnatural immunization. The natural immune antibody library is constructed from the natural donors, such as lymphocyte B and plasma cell. In our present study, the natural immune Fab antibody phage display libraries were constructed from the lymphocytes in the lymph nodes of 2 patients with colorectal cancer. There have been several studies on the library of immunity antibody in recent years[60-62], but no reports on construction of the natural immune antibody library of human colorectal cancer. Theoretically the humanization antibody library constructed from the lymphocytes B in lymph nodes defined as tumor metastasis of patients with tumor can aim at screening the antibodies, because the genes of heavy and light chains have been rearranged and ligated *Spe* cifically due to immunizing of tumor antigens. By this way the promiscuous work of preparation of antigens by immunizing rats was relieved and the problems of weak antigens, heterologica l antibodies, low fusion, instability and low antibodies products of hybridoma were resolved. From the natural immune antibody library, a few of phage antibodies immunized by some foreign antigens (such as bovine serum album, lysozyme, bovine thyroid globulin, etc.) were screened, and some self-antigens (such as tumor necrosis factor-α, carcinoembryonic antigen, CD4, thyroid globulin, etc.). Many of these phage antibodies showed high affinity and specificity, and the potential value for application.

It is important to assemble antibody molecules that have correctly folded heavy and light chains and thus retain antigen-recognition capabilities. Previously it had been demonstrated that Fd chains (comprising V_H and C_{H1}, the variable region and constant domain 1 of the immunoglobulin heavy chain) and κ chains targeted to the periplasm of *E.coli* assemble to form functional Fab molecules. If the Fd chain was anchored in the membrane and concomitantly provided with secreted κ chain, functional Fab molecules could form on the membrane surface facing the periplasm. The coexpression behind the PelB leader sequence of Fd fused to cpIII (the major coat protein of M13 phage) and ê chains lead to membrane anchoring of the Fdc hain and compartmentalization of the κ chains in the periplasm. These two chains could a ssemble *in situ* to allow accumulation of functional Fab on the membrane surface, which, by virtu e of the cpIIIsequences, would be incorporated along the entire length of the filamentous ph age particles on subsequent infection with helper phage. These phages displaying Fab could inf ect *E.coli* again. Following rounds of panning by adsorption-wash-amplification, the phages comprising specific antibody molecules could be enriched[63]. Our study showed that the phage Fab libraries were enriched about 120-fold after 3 rounds of panning. It suggested that the colorectal cancer antigens had selected the phages in the libraries displaying Fab. Dot immunoblotting showed that there were Fab expressions on the phage libraries of each panning round. ELISA analysis showed that the phage displaying Fab had the significant binding activity with the antigens related with colorectal cancer. Considering that nowadays there are few and hypo-specific antibodies related with colorectal cancer, the natural immune Fab antibody phage display libraries of colorectal can be used to select the affinity and specificity antibodies related to colorectal cancer. It is important to diagnose and treat colorectal cancer using these antibodies.

REFERENCES

1 Zhou DY, Feng FC, Zhang YL. Comparison of Sham's test for rectal mucus to an immunological test for fecal occult blood in large intestinal carcinoma screening: Analysis of a check-up of 6480 asymptomatic subjects. *Chin Med J*, 1993;106:739-742
2 Jia L, Chen TX, Sun JW, Na ZM, Zhang HH. Relationship between microvesselnsity and proliferating cell nuclear antigen and prognosis in colorectal cancer. *Shijie Huaren Xiaohua Zazhi*, 2000;8:74-76
3 Jiang XL, Quan QZ, Liu T, Dong XC. Recent advances in research of ulcerative colitis. *Shijie Huaren Xiaohua Zazhi*, 2000;8:216-218
4 Arap W, Pasqulini R, Rouslanhti E. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. *Science*, 1998; 279:377-380
5 Gu HP, Ni CR, Zhan RZ. Relationship of expressions of CD15, CD44v6 and nm23H1 mRNA with metastasis and prognosis of colon carcinoma. *Shijie Huaren Xiaohua Zazhi*, 2000;8:887-891
6 Yang JH, Rao BQ, Wang Y, Tu XH, Zhang LY, Chen SH, Oua2Yang XN, Dai XH. Clinical significance of detecting the circulating cancer cells in peripheral blood from colorectal cancer. *Shijie Huaren Xiaohua Zazhi*, 2000;8:187-189
7 Zhou TX, Li JS, Xing LW, You SH. Determination of lipid peroxide and superoxide dismutase in blood and tissue of patients with gastrointestinal cancer. *World J Gastroenterol*, 2000;6(Suppl 3):56
8 Futamura M, Takagi Y, Koumura H, Kida H, Tanemura H, Shimokawa K, Saji S. Spread of colorectal cancer micrometastases in regional lymph nodes by reverse transcriptase-polymerase chain reactions for carcinoembryonic antigen and cytokeratin 20. *J Surg Oncol*, 1998;68:34-40
9 Kobaek-Larsen M, Thorup I, Diederichsen A, Fenger C, Hoitinga MR. Review of colorectal cancer and its metastases in rodent models: comparative aspects with those in humans. *Comp Med*, 2000;50:16-26
10 Liu R, Wang YH, Tang Y, Cao GS. Effect of Octreotide on cell-cycle kinetics and serum CEA level in hepatic metastases of colonic adenocarcinoma. *China Natl J New Gastroenterol*, 1997;3:69-71
11 Khubchandani IT, Karamchandani MC, Kleckner FS, Sheets JA, Stasik JJ, Rosen L. Mass screening for colorectal cancer. *Dis Colon Rectum*, 1989;32:754-758
12 Xu GL, Chen ZM, Zhang WD. The clinical and pathology characteristics of young people colorectal cancer. *Xin Xiaohuabingxue Zazhi*, 1997;5:304
13 Ren DL, Yuan HX, Luo ZB, Lin DS, Fan XH, Zhang SF. Analysis of 186 cases of children colorectal cancer in China. *Xin Xiaohuabingxue Zazhi*, 1997;5:626-628
14 Tannapfel A, Katalinic A, K ckerling F, Wittekind C. The prediction of lymph node metastases in colorectal cancer by expression of the nucleoside diphosphate kinase/nm23-H1 and histopathological variables. *Am J Gastroenterol*, 1997;92:1182-1186
15 Ratto C, Sofo L, Ippoliti M, Merico M, Doglietto GB, Crucitti F. Prognostic factors in colorectal cancer. *Dis Colon Rectum*, 1998;41:1033-

1049
16 Ji DJ, Cao Y, Zhang YL, Jiang P, Yu N, Feng FC, Zhou DY. Synchronous studies on variations of p53 gene transcriptions and expressions in colorectal carcinomas HT-29 and Lovo cell lines. *Shijie Huaren Xiaohua Zazhi*, 2000;8:77 –79
17 Xu SH, Feng JG, Li DC, Mou HZ, Lou RC. Relationship between CD44in the peripheral blood of patients with colorectal cancer and clinicopathological features. *Shijie Huaren Xiaohua Zazhi*, 2000;8:432-435
18 Yamaguchi A, Urano T, Fushida S, Furukawa K, Nishimura G, Yonemura Y, Miyazaki I, Nakaganara G, Shiku HI. Inverse association of nm23-H1 expression by colorectal cancer with liver metastasis. *Br J Surg*, 1993;68:1020-1024
19 Zhao MF, Mao H, Zheng JX, Yuan YW. Effect of vascular endothelial growth factor on adhesion of large intestine cancer cell HT-29. *Shijie Huaren Xiaohua Zazhi*, 2000;8:646-649
20 Liu H, Wu JS, Li LH, Yao X. The expression of plateletderived growth factor and angio genesis in human colorectal carcinoma. *Shijie Huaren Xiaohua Zazhi*, 2000;8:661-664
21 Xu QW, Li YS, Zhu HG. Relationship between expression P53 protein, PCNA and CEA in colorectal cancer and lymph node metastasis. *World J Gastroenterol*, 1998;4:218
22 Wu BP, Zhang YL, Zhou DY, Gao CF, Lai ZS. Microsatellite instability, MMR gene expression and proliferation kinetics in colorectal cancer with famillial predisposition. *World J Gastroenterol*, 2000;6 :902-905
23 Guo WJ, Zhou GD, Wu HJ, Liu YQ, Wu RG, Zhang WD. Glutathione S-transf erasepi in colorectal cancer cells. *World J Gastroenterol*, 2000;6:454-455
24 Xu L, Lu GY. Analysis of ras p21 and DNA contents in colorectal cancer. *Shijie Huaren Xiaohua Zazhi*,1999;7:706-707
25 Hu JY, Wang S, Zhu JG, Zhou GH, Sun QB. Expression of B7 costimulation molecules by colorectal cancer cells reduces tumorigenicity and in duces anti tumor immunity. *World J Gastroenterol*, 1999; 5:147-151
26 Fang DC, Luo YH, Lu R, Liu WW, Liu FX, Liang ZY. Loss of heterozygosity at APC, MCC and DCC genetic loci in colorectal cancers. *China Natl J New Gastroenterol*, 1995;1:21-24
27 Nakamori S, Kameyama M, Imaoka S, Furukawa H, Ishikawa O, Sasaki Y, Izumi Y, Irimura T. Involvement of carbohydrate antigen sialyl lewisx in colorectal cancer metastasis. *Dis Colon Rectum*, 1997; 40:420-431
28 Zheng CX, Zhan WH, Zhao JZ, Zheng D, Wang DP, He YL, Zheng ZQ. The prognostic value of preoperative serum levels of CEA, CA19-9 and CA72-4 in patients with colorectal cancer. *World J Gastroenterol*, 2001;7:4 31-434
29 Vauthey JN, Dudrick PS, Lind DS, Copeland EM. Management of recurrent colorectal cancer: another look at carcinoembryonic antigen-detected recurrence. *Dig Dis*, 1996;14:5-13
30 Park JG. Genetic diagnosis and management of hereditary nonpolyposis colorectal cancer. *World J Gastroenterol*, 1998;4(Suppl 2):55
31 Luo F, Kan B, Lei S, Yan LN, Mao YQ, Zou LQ, Yang YX, Wei YQ. Study on P53 protein and C-erbB2 protein expression in primary hepatic cancer and colorectal cancer by flow cytometry. *World J Gastroenterol*, 1998;4(Suppl 2):87
32 Wang YX, Ruan CP, Li L, Shi JH, Kong XT. Clinical significance of changes of perioperative T cell and expression of its activated antigen in colorectal cancer patients. *World J Gastroenterol*, 1999;5:181-182
33 Jass JR. Future role of the pathologist in reporting colorectal cancer. *World J Surg*, 1997;21:688-693
34 Wang W, Luo HS, Yu BP. Telomerase and colorectal cancer. *Shijie Huaren Xiaohua Zazhi*, 2000;8:800-802
35 Wang LS, Pan LJ, Chen CL, Li MS, Shun Y, Zhang YL, Zhou DY. Effect of Bifidobacterium on proliferation and apoptosis of experimental large bowel carci noma *in situ*. *Shijie Huaren Xiaohua Zazhi*, 2000; 8:429-431
36 Sheng QG. Relationship between related factors with lymph node metastasis of colorectal cancer. *Shijie Huaren Xiaohua Zazhi*, 2000;8: 654-657
37 Ratto C, Sofo L, Ippoliti M, Merico M, Doglietto GB, Crucitti F. Prognosticfactors in colorectal cancer. Literature review for clinical application. *Dis Colon Rectum*, 1998;41:1033-1049
38 McLeod HL, Murray GI. Tumour markers of prognosis in colorectal cancer. *Br J Cancer*, 1999;79:191-203
39 Yin J, Liang YJ, Wang HZ, Li SQ. Expression of endocrine cells in colorectal cancer and its adjacent mucosa. *Huaren Xiaohua Zazhi*, 1998; 6:315-317
40 Owens RJ. The genetic engineering of monoclonal antibodies. *J Immunol Methods*, 1994;168:149-165
41 Kohler G, Milstein C. Continuous culture of fused cells secreting antibody of predefined specificity. *Nature*, 1995;256:495-497
42 Fan DM. The clinical application with monoclonal antibodies on digestion neoplasm. *China Natl J New Gastroenterol*, 1993;1:68-70
43 Shi YQ, Xiao B, Miao JY, Li MF, Qiao TD, Chen BJ, Chen Z, Han JL, Zhou SJ, Fan DM. A novel cDNA fragment associated with gastric cancer drug resistance screened from a library by mAb MGr1. *Huaren Xiaohua Zazhi*, 1998;6:656-659
44 Song ZQ, Hao F, Zhang J, Gu CH. Detection of antibodies against hypervariable region 1 in sera from patients with hepatitis C. *Shijie Huaren Xiaohua Zazhi*, 1999;7:666-668
45 Qiu K, Wang BC, Chen ZN, Fang P, Liu CG, Wan WX, Liu YF.^{99}mTc labeled- HAb18 McAb Fab fragment for radioimm unoimaging in nude mice bearing human hepatocellular carcinoma. *World J Gastroenterol*, 1998; 4:117-120
46 Yu SP, Zheng SJ, Zhou HY. Evaluation of colorectal carcinoma screening with fecal monoclonal antibody. *World J Gastroenterol*, 1998;4 (Suppl 2):95
47 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 MGr1. *World J Gastroenterol*, 1998;4(Suppl 2) :110-111
48 Zhang J, Liu YF, Yang SJ, Sun ZW, Qiao Q, Zhang SZ. Construction and expression of mouse/humanized scFv and their fusion to humanized mutant TNFá against hepatocellular carcinoma. *Shijie Huaren Xiaohua Zazhi*, 2000;8:616-620
49 Cai X, Garen A. Anti-melanoma antibodies from melanoma patients immunized with genetically modified autologous tumor cells: selection of specific antibodies from single chain Fv fusion phage libraries. *Proc Natl Acad Sci USA*, 1995;92:6537-6541
50 Hoogenboom H. Antibody phage display technology and its application. *Immunotechnology*, 1998;4:1-20
51 McCafferty J, Griffiths AD, Winter G, Chiswell DJ. Phage antibodies: filamentous phage displaying antibody variable domains. *Nature*, 1990;348:552-554
52 Zheng W, Tan H, Song SB, Lu HY, Wang Y, Yu YX, Yin R. The construction and expression of a fusion protein consisting anti-HBsAg antibody fragment Fab and interferon a in *E.coli*. *World J Gastroenterol*, 2000;6(Suppl 3):83
53 Winter G, Griffiths AD, Hawkins RE, Hoogenboom H. Making antibodies by phage display technology. *Annu Rev Immunol*, 1994;12: 443-455
54 Li J, Wang Y, Li QX, Wang YM, Xu JJ, Dong ZW. Cloning of 3H11 mAb variable region gene and expression of 3H11 human-mouse chimeric light chain. *World J Gastroenterol*, 1998;4:41-44
55 Huse WD, Sastry L, Iverson SA, Kang AS, Alting MM, Burton DR, Benkovic SJ, Lerner RA. Generation of a large combinatorial library of the immunoglobulin repertoire in phage lambda [see com ments]. *Science*, 1989;246:1275-1281
56 Barbas CF, Kang AS, Lerner RA, Benkovic-SJ. Assembly of combinatorial antibody libraries on phage surface: the gene III site. *Proc Natl Acad Sci USA*, 1991;88:7978-7982
57 Barbas CF, Lerner RA. Combinatorial immunoglobulin libraries on the surface of phage (phabs): rapid selection of antigen-specific Fabs. Methods: Comp Methods Enzymol, 1991;2:119-124
58 Wu W, Xu DZ, Yan YP, Zhang JX, Liu Y, Li RL. Evaluation of dot immunogold filtration assay for anti-HAV IgM antibody. *World J Gastroenterol*, 1999;5:132-134
59 Chen B, Zhou SJ, Fan DM. Establishment of a quick ConA-mAb-ELISA for detection of serum MC3-Ag, a novel colorectal cancer-associated antigen. *Xin Xiaohuabingxue Zazhi*, 1995;3(Sup pl 4):12-14
60 Zhao YF, Wang HT, Wang QL, Du ZY, Yang JG, Sun HY, Ma LR, Yin Z. Scree ning of "1F7" idiotypic anti-gp160 antibodies from phage antibody library. Chinese Journal of Biochemistry and Molecular Biology, 1998;14:20-24
61 Wang Y, Hua B, Liu QY, Gao RK, Zhu YC, Chen YP. By passing immunization: Construction of a phage antibody library with randomized CDR3. *Chin J Microbiol Immunol*, 1997;17:449-452
62 Lan FH, Gao H, Liu YF, Chen SM. Construction of a naive IgG phage display library from a normal human individual. *J Fourth Mil Med Univ*, 1999;20:464-467
63 Pini A, Viti F, Santucci A, Carnemolla B, Zardi L, Neri P, Neri D. Design and use of a phage display library. Human antibodies with subnanomolar affinity against a marker of angiogenesis eluted from a two-dimensional gel. *J Biol Chem*, 1998;273:21769-21776

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