Recombinant Chimeric Antibody hCAb as a Novel Anti-human Colorectal Carcinoma Agent

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A new chimeric IgG1 antibody hCAb which could be specifically directed against a cell surface-associated glycoprotein of colorectal cancer cells was prepared by genetic engineering technology in our lab. In this study, we explored the potential therapeutic mechanisms and described the evaluation of hCAb directed against colorectal cancer. The standard ^{51}Cr release assay showed that like many other clinically validated IgG1 monoclonal antibodies, hCAb primarily acts by antibody-dependent cell-mediated cytotoxicity (ADCC). The maximal cell lysis of ADCC induced by hCAb was over 50% in the presence of peripheral blood mononuclear cells (PBMCs). Moreover, in vivo studies showed potent antitumor effects in nude mice with SW480 and Hce-8693 tumor xenografts. The treatment with hCAb induced a dramatic reduction (over 70%) in tumor volume in comparison to untreated control group. Furthermore, during the period of treatment, the animals treated by hCAb did not show signs of wasting or other visible signs of toxicity. No obvious tissue damage in vital organs was detected. The chimeric antibody hCAb may be a promising candidate in the treatment of human colorectal cancer. This study can provide a reference for the potential application of hCAb in clinical trial.

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INTRODUCTION

The treatment of colorectal cancer has consisted of fluoropyrimidine-based chemotherapy for over 50 years. However, although the initial responses to chemotherapeutic regimens are frequently positive, their duration is often brief and the majority of patients die shortly after relapsing. Unlike chemotherapeutics, antibody-based therapies can be designed to target tumor cells specifically via the recognition of antigens overexpressed on their surface (1). The use of monoclonal antibody (mAb) as a cancer therapeutics agent has shown interesting results in phase I/II clinical trials, and several mAbs that predominantly act by antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) have been approved for the treatment of cancer patients. These include chimeric IgG1 mAb rituximab (Rituxan) binding to the B-cell differentiation antigen CD20 for the treatment of B-cell lymphomas (2,3), humanized IgG1 mAb trastuzumab (Herceptin) targeting HER-2 (human epithelial growth factor receptor type 2) (4), and humanized IgG1 alemtuzumab (Campath) targeting the differentiation antigen CD52 for the treatment of B-cell chronic lymphocytic leukemia (5-7). Several other mAbs are currently at advanced stages of clinical development. The treatment for human colorectal cancer remains pessimistic, making the discovery and development of a novel anti-human colorectal carcinoma antibody both necessary and significant.

Murine monoclonal antibody CAb was generated in our laboratory to fight against human colorectal cancer. Our previous studies show that CAb could be specifically directed against a cell surface-associated glycoprotein of colorectal cancer cells (8) and possessed high specificity to human colorectal cancer (9). These results showed that CAb might possess therapeutic potentiality in the treatment for human colorectal cancer. The successful preparation of the high specificity and affinity anti-human colorectal cancer antibody provides a new approach for specific detection and therapy of colorectal cancer. However, murine mAb does not constitute an ideal therapeutic agent. Its inherent immunogenicity in patients had hindered its long-term administration in immunosuppressive therapy (10), which resulted in rapid clearance of the antibody and reduced tumor targeting with subsequent dosing (11). This has led to the development of chimeric or humanized antibodies. We reconstituted the murine monoclonal antibody to a chimeric version by genetic-engineering technology. The chimeric antibody hCAb consists of the murine variable regions, which bring about antigen recognition, fused to the constant or effector part of human antibody (12). Meanwhile, hCAb has less immunogenicity than murine antibody CAb, enabling repeated antibody administration and giving improved capacity to recruit cytotoxic cells and complement. These improvements have contributed to the increased therapeutic efficacy of murine antibody CAb.

In this study, we present the in vitro and in vivo characteristics of the chimeric antibody hCAb. Our study focuses not only on the potential therapeutic mecha-

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nisms, but also on anti-tumor activity in murine tumor xenograft models. hCAb might serve as an effective and safe new therapeutic in the treatment for human colorectal cancer.

MATERIALS AND METHODS

Cell Culture

The various human colorectal cancer cell lines (Hce-8693, SW480, and HR8348) were cultured in DMEM medium supplemented with 10% fetal bovine serum at 37°C in a humidified 5% CO_2 atmosphere.

Construction and Production of the Human-mouse Chimeric Antibody-hCAb

CAb, a murine IgG1 against the human colorectal cancer monoclonal antibody, was recently developed in our laboratory (13). In our previous paper (14), we reported the construction and production of human-mouse chimeric antibody hCAb. Briefly, the variable region genes of the murine monoclonal antibody CAb were introduced into the expression vectors pYR-GCEVH and pYR-GCEVL, which contain the cDNA encoding the human constant regions. The chimeric antibody was produced by transfecting dihydrofolate reductase-deficient Chinese hamster ovary cells (dhfr$^-$CHO) with the recombinant vector using lipofectamine transfection reagent following the manufacturer's instruction. Stable transfectants were selected in the presence of G418 (Sigma) at a concentration of 200 μg/mL, and were subjected to amplification (stepwise increments in methotrexate (MTX, Sigma) level, such as 3×10^{-8}, 10^{-7}, 10^{-6} M). Expression of the antibody was determined in the culture medium by quantitative ELISA. Finally, the recombinant antibody, secreted by transfected CHO cells, was purified from culture medium by affinity chromatography on a protein A-Ceramic Hyper D^{R}F column (BioSepra).

Flow Cytometry Analysis

To determine hCAb's affinity to human colorectal cancer cells, 3 groups of colorectal cancer cell lines (Hce-8693, SW480, and HR8348, 5×10^5 cells) were incubated with 10 μg of hCAb or CAb or unrelated control anti-cytomegalovirus IgG in the dark for 1 h on ice. Cells were then washed and labeled with FITC-conjugated anti-human IgG (1:500 dilutions, hCAb group) or FITC-conjugated anti-mouse IgG (1:500 dilutions, CAb group and unrelated control group) for 30 min on ice in the dark, respectively. Three washes were carried out again before cytometric analysis. Data were processed using the Cell Quest software (Becton-Dickinson, USA).

Western Blot Analysis

Furthermore, we evaluated hCAb's specificity to human colorectal cancer cells by Western blot analysis, SW480 and Hce-8693 cells were cultured for 72 h respectively. Then cells were harvested, washed twice with cold PBS, and lysed in buffer (150 mM NaCl, 50 mM Tris-HCL, 2 mM EDTA, 1% NP-40, and pH 7.4), containing protease inhibitors. Equal amount of protein (30 μg/lane) from lysate were subjected to SDS-PAGE under reducing conditions on 10% acrylamide gels. After SDS-PAGE, proteins were transferred to an ImmobilonTM-P Transfer Membrane (Millipore Corporation, USA). Then, to block nonspecific binding, the membrane was incubated in PBS with 0.1% Tween-20 (T-PBS) containing 5% nonfat skim milk for 1h. Subsequently, the membrane was incubated with chimeric antibody HCAb overnight at 4°C, washed in T-PBS and incubated with peroxidase-conjugated anti-human IgG (Fc specific) antibody (Sigma) for 2 h. Protein on the membrane was visualized using enhanced chemiluminescence (ECL plus Western blotting detective system, Amersham, UK).

Isolation of PBMCs from Blood

Peripheral blood mononuclear cells (PBMCs) were prepared as described elsewhere (15). Briefly, buffy coats from standard blood donations were diluted by adding PBS and were carefully placed on top of 10 mL of lymphocyte separation medium (Bio Whittaker) in 50-mL tubes. Tubes were then centrifuged at 800*g* for 20min at room temperature. Thereafter, the PBMCs were recovered from the plasma-medium interface and were washed several times with RPMI 1640 until the supernatant was clear.

In Vitro Antibody-dependent Cell-mediated Cytotoxicity Assay

The capacity of chimeric antibody-hCAb to induce effector cell-dependent lysis of tumor cells was evaluated by the standard ^{51}Cr release assay as previously described (15-18). To more accurately evaluate the results, several controls were included: 1, an additional control chimeric antibody (rituximab, anti-CD20 chimeric antibody) was chosen that does not react with colorectal cancer cells; 2, a cell line (the human glioblastoma cells U251) were used that is negative for hCAb; 3, another control assay in the absence of PBMC was also performed. Target cells (1×10^6 cells, SW480 or Hce-8693 or control U251 cells) were labeled for 2 h with 200 μCi of $Na_2{}^{51}CrO_4$ (Amersham Biosciences) at 37°C, washed 3 times and were resuspended in culture medium (1×10^5 cells/mL). Labeled cells were dispensed in V-bottomed 96-well plates (5×10^3 cells, in 50 μL/well) and preincubated (RT, 1 h) with 50 μL of 10-fold serial dilutions of hCAb or CAb or control antibody Rituximab in culture medium, ranging from 100 μ/mL to 0.001 ng/mL (final concentrations). Thereafter, PBMC (2.5×10^5 cells/well), resulting in a final volume of 200 μL, were added to the wells, and cells were incubated at 37°C overnight. The next day, supernatants were collected and ^{51}Cr release from triplicates was measured. Percent specific lysis was calculated as 100% × (sample release – spontaneous release)/(maximal release – spontaneous release); where maximal ^{51}Cr release was determined by adding 5% SDS to target cells, and spontaneous release was measured in the absence of sensitizing antibody and effector cells.

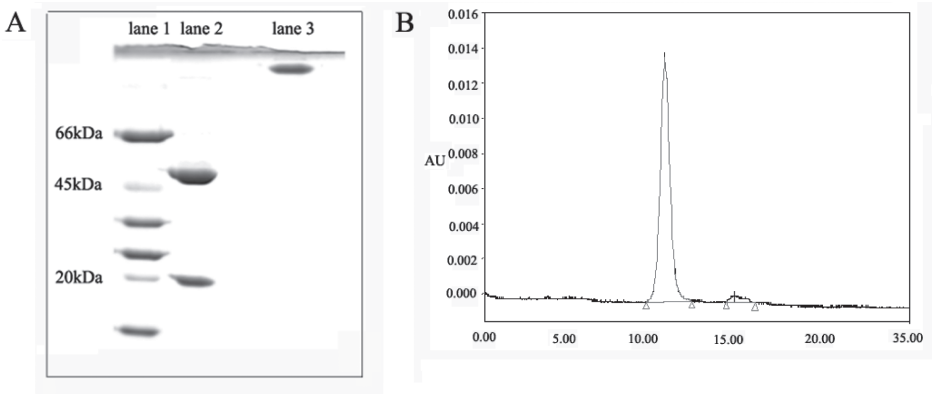

Figure 1. HPLC chromatogram and SDS-page of purified chimeric antibody hCAb. A: SDS-page of purified hCAb. hCAb was run under reducing (lane 2) and non reducing (lane 3) conditions. Molecular weight standards were in lane 1; B: HPLC chromatogram of hCAb.

In vivo Anti-tumor Activity Studies

Nude female BALB/c *nu/nu* mice (6-week-old, 18.5-20.2 g) were used throughout the studies. All procedures involving animals and their care were conducted in accordance with the regulations for animal welfare in China. The Ethics Committee of the Fourth Military Medical University approved all experiments.

For therapy trials, SW480 or Hce-8693 (8×10^6 cells) were suspended in 0.1 mL normal saline and injected subcutaneously (day 0) in the right flank of the mice, respectively. At day 5, when tumors started to appear, the mice of every xenograft modal were divided into 4 groups (6 in each group). At day 10, when tumors were clearly detectable, hCAb dissolved in normal saline was administered intraperitoneally at doses of 2.0 mg/kg of body weight for 5 times at 72-h intervals. Control groups included: left-untreated group, injected intraperitoneally with identical volumes of normal saline; chemotherapy group, 5-fluorouracil administered intraperitoneally at doses of 20 mg/kg of body weight for 5 times from day 10 to day 14; and unrelated control antibody group, rituximab administered intraperitoneally at doses of 2.0 mg/kg of body weight for 5 times at 72 h intervals. During the period of treatment, tumor sizes (V) were measured with calipers and calculated by the formula of rotational ellipsoid $V = A\times B^2/2$ (A is the axial diameter, B the rotational diameter) (18). Moreover, the inhibition rate (IR) of tumor growth was calculated according to the following formula: IR = [(Mean tumor volume of nontherapeutic control – Mean tumor volume of therapeutic group)/(Mean tumor volume of nontherapeutic control)] × 100%.

Furthermore, toxicity was monitored principally by loss in body weight, the white blood cell (WBC) count, and platelet counts. Hematoxylin and eosin stain was performed to reveal tissue damage in such vital organs as the heart, liver, spleen, lung, and kidney in nude mice.

Statistical Analysis

Results were expressed as the mean ± SD. The data were analyzed for significance by Wilcoxon rank sum test, and results were considered significant if $P < 0.05$.

RESULTS

Construction and Characterization of the Human-mouse Chimeric Antibody hCAb

We generated the human-mouse chimeric antibody hCAb by fusing the Ig variable region with a human IgG1 Fc domain. After amplification in MTX, and purification by affinity chromatography, the final yield of the chimeric antibody was over 100 mg/L (Figure 1B) (13). When hCAb was analyzed by SDS-PAGE (Figure 1A), it was shown under reducing conditions, the size of heavy chains are approximately 50 kDa, the size of light chains approximately 21 kDa as expected. And a band of 140 kDa was under nonreducing conditions. These results confirmed the correct structure of the individual Ig chains, and demonstrated the correct polypeptide assembly of the heavy and light chains in the production of the recombinant Ig molecules.

hCAb Possesses the High Specificity and Affinity to Colorectal Cancer Cell Lines

The specific binding of hCAb with colorectal cancer cells was evaluated by flow cytometry. To more accurately describe the results, 2 parameters were used: the mean fluorescence intensity (MFI) and the percentage of positive staining cells. Figure 2 shows hCAb's binding ability to colorectal cancer cell lines. These results confirm that the chimeric antibody-hCAb possesses the high specificity and affinity to colorectal cancer cell lines. And the mouse mAb CAb shows similar binding characteristics. However, the unrelated control anticytomegalovirus IgG can't bind with colorectal cancer cell lines. Moreover, western blot also confirmed that hCAb can bind with human colorectal cell lines. The molecular weight (Mol.) of target antigen is approximate 200 KDa (Figure 3).

hCAb is a Very Potent Inducer of ADCC In Vitro

Besides the high specificity and affinity to colorectal cancer cell lines, therapy by hCAb may also operate by inducing immune effector-mediated killing of tumor cells. Therefore, we tested whether hCAb can induce ADCC.

We chose 2 different human colorectal cancer cell lines of various antibody targeted antigen densities. Freshly isolated human PBMC were used as effector cells at an E:T ratio of 50:1, and ^{51}Cr release

A

Cell line	Organ	Tumor	hCAb		CAb		anti-cytomegalovirus IgG	
			MFI (mean±SD)	positive percentage	MFI (mean±SD)	positive percentage	MFI (mean±SD)	positive percentage
Hce-8693	Cecum	Adenocarcinoma	146.93±6.72	92.34±2.41	133.31±4.75	93.45±3.14	3.42±0.72	6.20±1.53
HR8348	Rectum	Adenocarcinoma	90.34±5.63	70.42±3.51	87.31±6.46	72.76±3.15	1.31±0.14	7.76±0.92
SW480	Colon	Adenocarcinoma	87.45±4.65	65.83±4.91	84.46±6.75	62.47±3.68	0.75±0.21	5.31±0.68

B

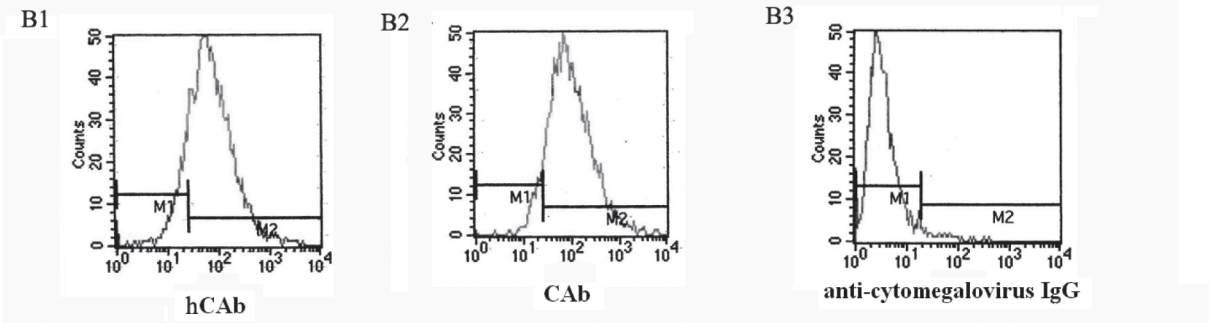

Figure 2. Flow cytometry analysis of hCAb's binding ability to colorectal cancer cell lines. The FACS Results were measured in 3 independent experiments and expressed as the mean ± SD. A: The FACS data; B: Detection of hCAb's binding ability to Hce-8693 cell lines by FCM. Hce-8693 cells were stained with hCAb (B1), CAb (B2) or anti-cytomegalovirus IgG (B3), respectively, and then were analyzed by flow cytometry.

was measured for assessing cytotoxicity. Figure 4 shows representative example of ADCC dose-response curves derived from SW480 and Hce-8693 cells. Apparently, hCAb is a very potent inducer of ADCC. The maximal specific lysis was observed 51.98% and 58.36% for SW480 cells and Hce-8693 cells, respectively. This result suggests that ADCC might play an important role in anti-tumor activity of this chimeric antibody in vivo. Meanwhile, because the effector parts of murine antibodies are homologous to those of humans, CAb possesses some of weaker capacity for induction of ADCC. And there was no induction of ADCC in control groups (Figure 4).

Anti-tumor Activity in Murine Tumor Xenograft Models

The in vitro experiments described above demonstrate potential mechanisms for inhibition of tumor growth. We further evaluated hCAb's potential therapeutic roles using 2 mouse tumor models, which are more relevant to the clinical condition than the in vitro assays because the xenograft models include three-dimensional tumor growth, controlled by growth factors, tumor vascularization, distribution of the antibody in the body, and presence of immune effector systems.

As shown in Figure 5, although mice in the untreated control group developed steadily growing tumors, all mice treated with hCAb, were fully protected against tumor growth. In SW480 xenograft model, the tumors growth rate in the untreated control group was very rapid; with mean volume quadrupling times was 22.1 days, and the mean volume quadrupling times in 5-fluorouracil and rituximab group were 27.6 days and 21.6 days, respectively. In contrast, the tumor growth rate in the hCAb treated group showed growth delay; tumor volume never quadrupled. Meanwhile, similar results were observed in Hce-8693 xenograft model. Treatment with hCAb induced a dramatic reduction in tumor volume. When compared with the untreated control group, the inhibition rate (IR) of tumor growth in hCAb group was 70% and 73% in SW480 and Hce-8693 models, respectively, which shows the therapeutic efficacy of hCAb to Hce-8693 model was better than that to SW480 model. On the other hand, rituximab possessed no therapeutic action.

During the period of treatment, the animals did not show signs of wasting or other visible signs of toxicity, (Figure 6). Microscopic observation revealed no obvious tissue damage in such vital organs as the heart, liver, spleen, lung, and kidney in any nude mice. On the other hand, although 5-fluorouracil possessed some therapeutic action (reduction approximately 40% in tumor volume), it also caused myelosuppressive side effects, such as violent decrease of WBC and platelets. These results showed that hCAb possessed more efficient and safer therapeutic action in the treatment for human colorectal carcinoma when compared with 5-fluorouracil.

DISCUSSION

Targeted therapies are the focus of much research in oncology. After the development of imatinib for the treatment of chronic myeloid leukemia, biological

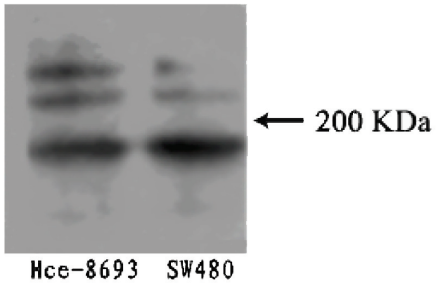

Figure 3. Western blot analysis of hCAb's specificity to colorectal cancer cell lines. Two cell lines (SW480 and Hce-8693) were chosen. Western blot confirmed that hCAb can bind with human colorectal cell lines. The molecular weight of the target antigen is approximately 200 KDa.

therapies that target tumor-associated antigens give hope for improvement of survival in many cancers (19). The use of monoclonal antibodies could be another approach to improve prognosis of patients with cancer (20). This study explored a new therapeutic agent for human colorectal cancer. We investigated the characteristic of chimeric antibody hCAb and its potential therapeutic mechanisms.

In the treatment of colorectal cancer, the most commonly targeted antigens include epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), carcinoembryonic antigen (CEA), TAG-72, A33, Ep-CAM, $EphB_2$, and so on. Moreover, several novel drugs like cetuximab and bevacizumab, whose target antigens are EGFR and VEGF, respectively, have been approved by the US Food and Drug Administration (FDA) for the treatment of metastatic colorectal cancer (21-23). These antibody agents such as cetuximab and bevacizumab have improved the treatment for human colorectal cancer, however, the therapy remains challenging, especially taking into consideration the toxic effects and high costs of these medication. One of the limitations of these antibody agents is the necessity of identifying a particular receptor or target. When compared with these antibody agents, hCAb has several advantages. First, hCAb possesses high specificity to

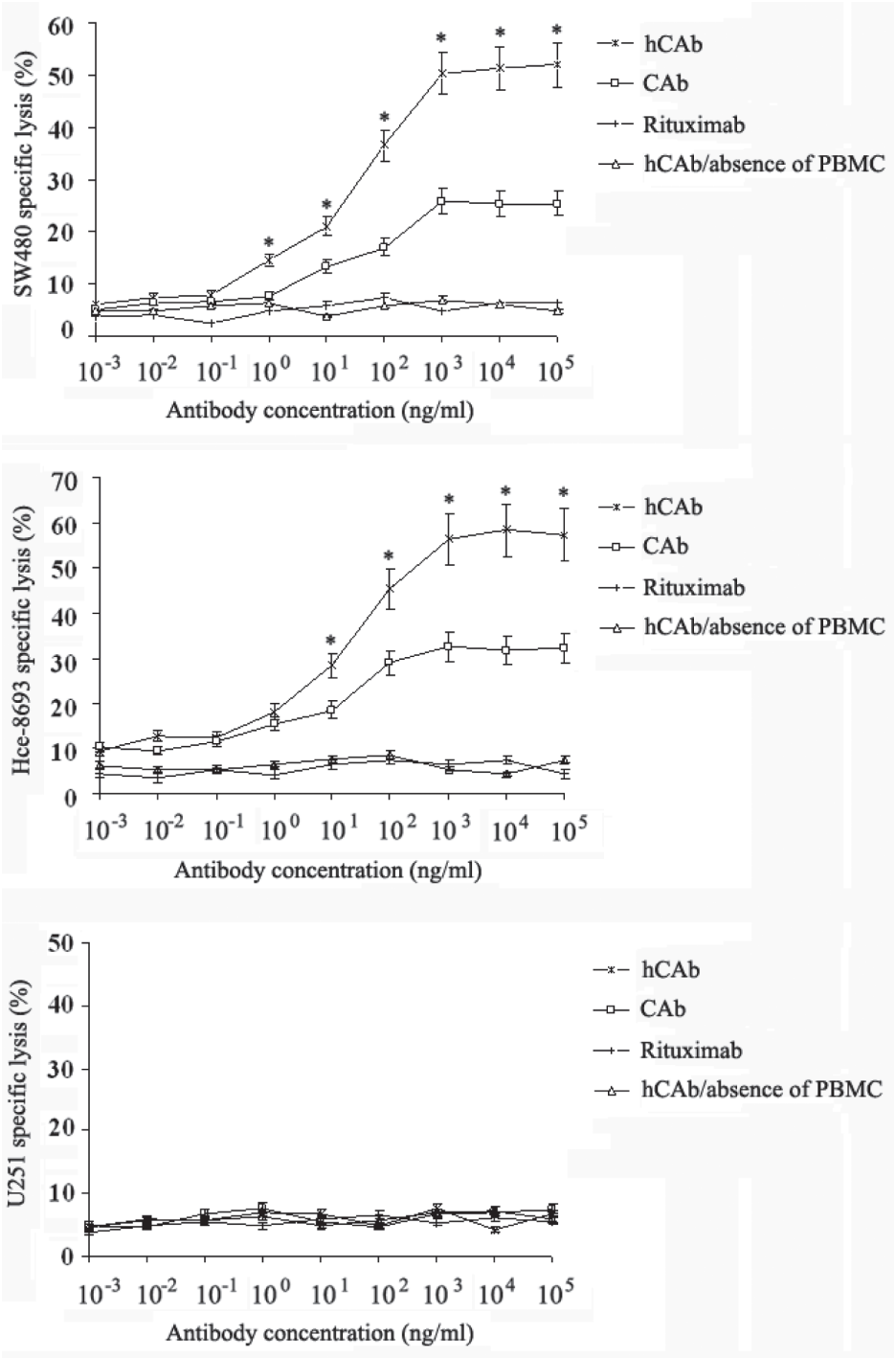

Figure 4. ADCC of hCAb against human colorectal cancer cell lines. Each data point represents the mean± SD specific lysis (%) measured in 3 independent experiments. Percent specific lysis was calculated as 100% × (sample release – spontaneous release)/(maximal release – spontaneous release); where maximal ^{51}Cr release was determined by adding 5% SDS to target cells, and spontaneous release was measured in the absence of sensitizing antibody and effector cells. *Results were considered significant ($P < 0.05$, versus control groups).

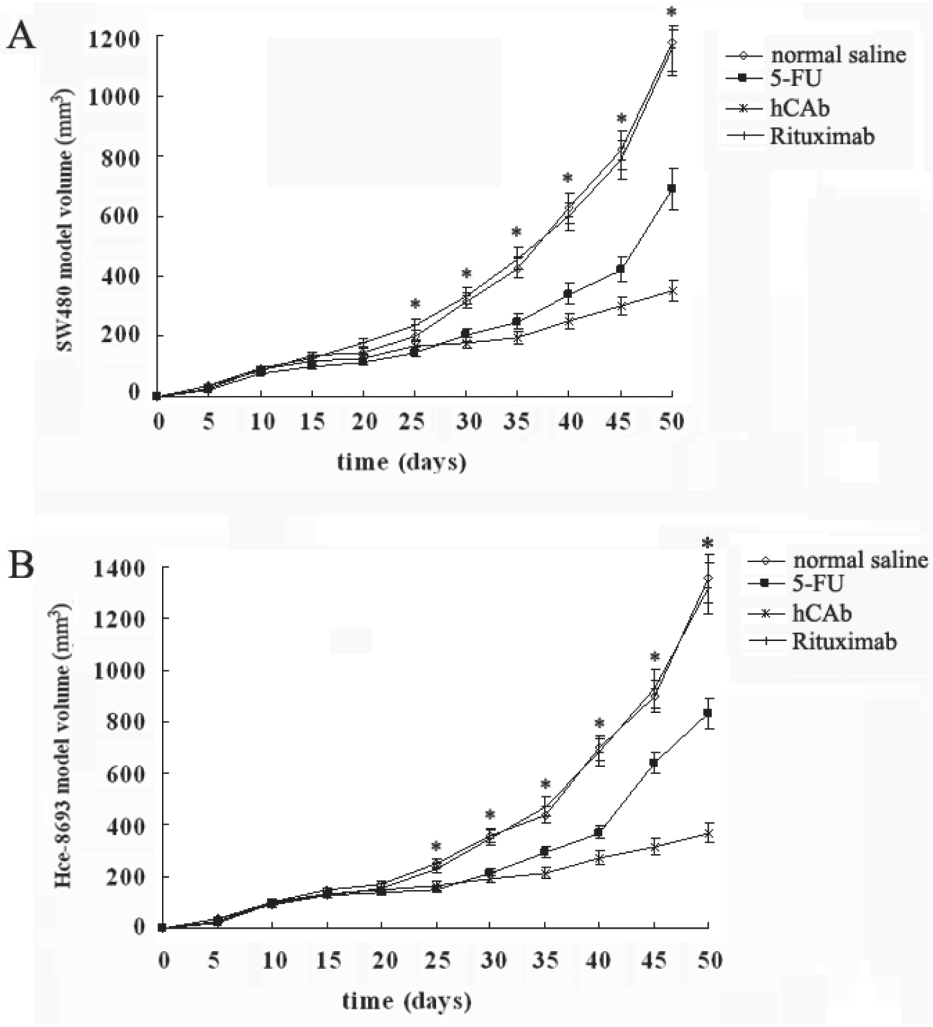

Figure 5. Growth kinetics of xenograft models. Animals were treated with hCAb or 5-fluorouracil or rituximab or left untreated. Data points represent 6 animals in each group. (A: SW480 xenograft model; B: Hce-8693 xenograft model).*Results were considered significant ($P < 0.05$, versus untreated control groups).

human colorectal cancer. The previous immunohistochemistry assay showed that colorectal cancer was strong positive for CAb (+++, 79.3%), gastric adenocarcinoma and lung cancer were weak positive for CAb (+/±, 15%, 23.1% respectively). CAb did not react with normal and embryo tissue/cells, as well as other tumor tissue(9). The chimeric antibody hCAb has similar binding characteristics. These results also demonstrate that the particular antigen of hCAb is both stably and homogeneously expressed by colorectal cancer tissue/cells, and is expressed negligibly in healthy tissue.

However, many other antigens that are recognized by monoclonal antibodies are expressed not only by malignant tissue/cells, but also by healthy tissue/cells. For example, A33 antigen is a transmembrane protein expressed in normal human colonic and small bowel epithelium as well as colon cancer (24). $EphB_2$ is expressed on normal cells and a variety of human cancer cell lines (25). EpCAM is an epithelial cell-adhesion molecule implicated in the transport of calcium across the cell membrane that is expressed in normal epithelial tissues and in numerous tumor including carcinomas of the colon, rectum, pancreas, and stomach (26-28). EGFR, VEGF, CEA and TAG-72 have a broad spectrum beside colon cancer. The toxicity that might arise from targeting these antigens remains to be determined.

Second, recent studies have shown that immunohistochemical staining of hCAb correlates with therapeutic efficacy. That also can be verified by in vitro and in vivo assay. hCAb's binding ability with Hce-8693 cells was stronger than that with SW480 cells. And the maximal specific lysis of ADCC induced by hCAb was observed to be higher for Hce-8693 cells. Meanwhile, the therapeutic efficacy of hCAb to Hce-8693 model was better than that to SW480 model. Nevertheless, many other antibodies don't respond as well. For instance, the observation by Lenz et al. (29) supported recent concern about the reliability of EGFR staining to predict the response to cetuximab therapy. A considerable proportion of patients with high EGFR expression did not respond to cetuximab treatment, and yet patients with low expression of EGFR did respond. Thus, markers are needed to predict responders to EGFR target monoclonal antibody therapy. Third, no circulating antigen of hCAb is detected in the serum of cancer patients, which demonstrates that there is little or no soluble form of the target antigen (to avoid rapid antibody clearance). On the other hand, many other antigens can be detected in the serum of cancer patients. CEA, for example, belongs to the CD66 immunoglobulin superfamily that comprises highly homologous molecules expressed on several normal tissues as well as in the serum of patients, which limits CEA as target antigen, especially in radioimmunotherapy (RIT) (30).

In our previous study (10), to obtain a specific anti-colorectal cancer antibody, we immunized mice with human colorectal adenocarcinoma tissues. Although the traditional method of preparation of monoclonal antibodies is using purified pro-

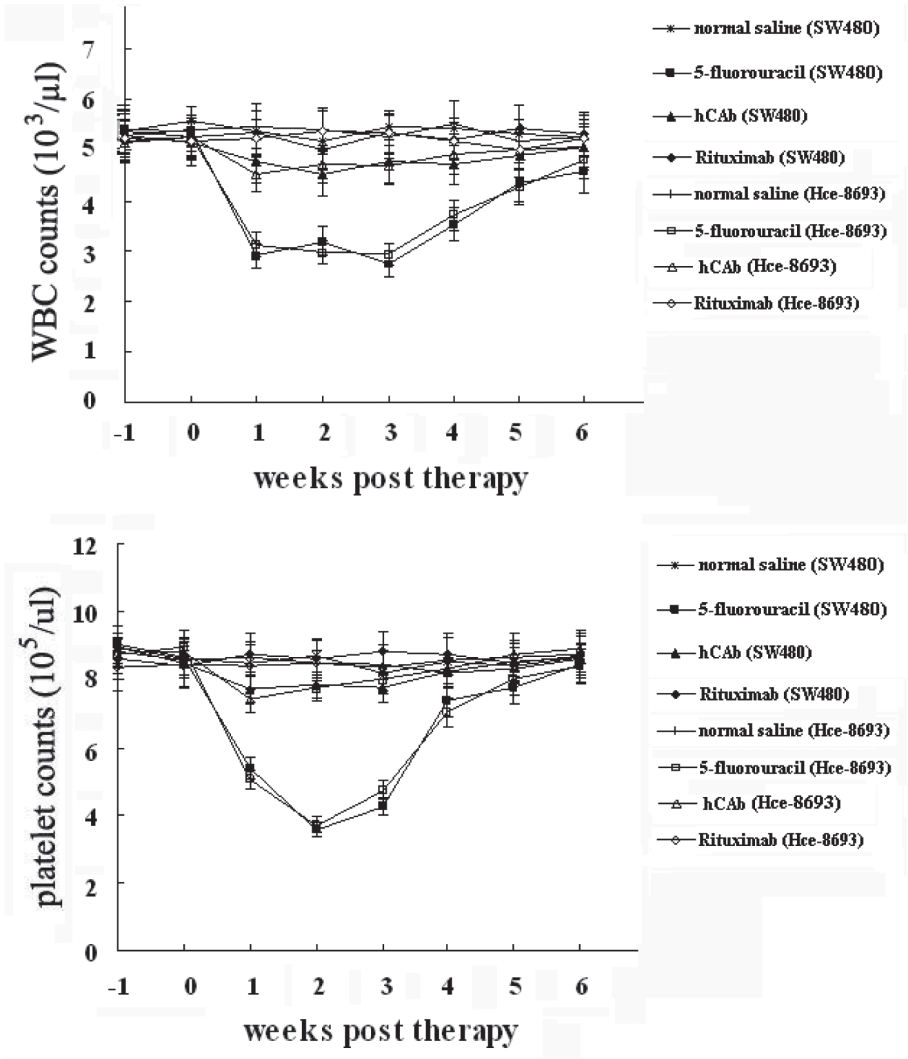

Figure 6. WBC and PLT counts during the period of treatment. WBC and platelet counts were in normal scope when treated by hCAb. However, 5-fluorouracil led to myelosuppressive side effects, such as violent decrease of WBC and platelet counts. 5-fluorouracil possesses much more severe toxicity when compared with hCAb.

teins to immunize mice, the prepared antibodies sometimes can't bind with the natural antigen proteins, because their spatial structures differ from the purified antigens'. Moreover, expression of the protein is time-consuming and labor-intensive. Thus we chose human colorectal adenocarcinoma tissues to immunize mice and obtained a monoclonal antibody CAb that possessed high specificity to human colorectal carcinoma. Furthermore, we have identified CAb's target antigen as a colorectal cancer associated Mol. 200 KDa glycoprotein. The study on the exact structure of the antigen is ongoing.

Previous reports have shown the rodent antibodies are immunogenic in humans, making them less suitable for therapy (31). To make the best use of antibodies, we reconstituted the murine monoclonal antibody CAb to a chimeric version hCAb by genetic engineering technology. Fortunately, the chimeric antibody hCAb not only retains the parent antibody's binding characteristics, but also has the ability to mediate in vitro ADCC, which has been verified by ^{51}Cr release assay. Moreover, we further evaluated hCAb's potential therapeutic roles using mouse models. More data could be gathered using multiple colorectal cancer cell lines with different binding ability to hCAb, therefore in our study we chose 2 different colorectal carcinoma xenograft models. hCAb was effective in both models. The treatment with hCAb induced a dramatic reduction in tumor volumes. Furthermore, during the period of treatment, hCAb possessed more efficient and safer therapeutic action in the treatment for human colorectal cancer when compared with 5-fluorouracil. No weight loss, other visible signs of toxicity, or obvious tissue damages in vital organs were detected. In addition, the WBC and platelet counts were within normal scope. On the other hand, we observed more severe toxicity by 5-fluorouracil which caused myelosuppressive side effects, such as violent decrease of WBC and platelet counts.

Taken together, the data reported here suggest hCAb is a promising new anticancer agent. It is a chimeric immunoagent, hence presumably with reduced or no immunogenicity. In addition, it endures a much longer serum half-life in vivo when compared with its Fab, and it is effective in inhibition of target tumor cell growth both in vitro and in vivo. Moreover, hCAb might be used in radioimmunotherapeutic approach, and the radioimmunotherapy (RIT) studies are ongoing.

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REFERENCES

1. Prang N, Preithner S, Brischwein K, *et al.* (2005) Cellular and complement-dependent cytotoxicity of Ep-CAM specific monoclonal antibody MT201 against breast cancer cell lines. *Br. J. Cancer*. 92: 342-9.

2. Grillo-Lopez AJ, White CA, Varns C, Shen D, *et al.* (1999) Overview of the clinical development of rituximab: first monoclonal antibody approved for the treatment of lymphoma. *Semin. Oncol.* 26: 66-73.
3. Smith MR. (2003) Rituximab (monoclonal anti-CD20 antibody): mechanisms of action and resistance. *Oncogene*. 22: 7359-68.
4. Vogel C, Cobleigh MA, Tripathy D, *et al.* (2001) First-line, single-agent Herceptin (trastuzumab) in metastatic breast cancer: a preliminary report. *Eur. J. Cancer*. 37: Suppl. 1: S25-9.
5. Hale G, Zhang MJ, Bunjes D, *et al.* (1998) Improving the outcome of bone marrow transplantation by using CD52 monoclonal antibodies to prevent graft-versus-host disease and graft rejection. *Blood*. 92: 4581-90.
6. Kottaridis PD, Milligan DW, Chopra R, *et al.* (2000) In vivo CAMPATH-1H prevents graft-versus-host disease following nonmyeloablative stem cell transplantation. *Blood.* 96: 2419-25.
7. Faulkner RD, Craddock C, Byrne JL, *et al.* (2004) BEAM-alemtuzumab reduced-intensity allogeneic stem cell transplantation for lymphoproliferative diseases: GVHD, toxicity, and survival in 65 patients. *Blood*. 103: 428 -34.
8. Liu MP, Ma SY, Zou YL, *et al.* (1992) Characteristics and relativities of a panel of monoclonal antibodies (McAb) recognizing human colon cancer. *Chin. J. Tumor* 14(2): 91-3 (*in Chinese*)
9. Ma SY, Liu MP, Chen XL, *et al.* (1991) Measurements of the relative affinity constants of a panel of monoclonal antibodies against tumor antigens by sandwich ABC-ELISA. *Chin. J. Immunol.* 7(4): 265-7 (*in Chinese*)
10. Liu MP, Ma YS, Chen W, *et al.* (1993) The study and application of SC serial anti-colorectal cancer associated antigen MAb. *J. China Tumor*. 2, 11: 27.
11. Veronese ML, O'Dwyer PJ. (2004) Monoclonal antibodies in the treatment of colorectal cancer. *Eur. J. Cancer.* 40: 1292-1301.
12. Waldmann TA. (1991) Monoclonal antibodies in diagnosis and therapy. *Science*. 252, 1657-62.
13. Yang XM, Xing JL, Yao XY, *et al.* (2004) The cloning and expression of Fab gene of MAb CAb-1 against human colorectal cancer. *J. Fourth Military Medical University*. 25: 1768-71.
14. Xiong H, Ran YL, Xing JL, *et al.* (2005) Expression vectors for human-mouse chimeric antibodies. *J. Biochem. Mol. Bio*. 38, 4: 414-9.
15. Bleeker WK, van Bueren L, van Ojik HH, *et al.* (2004) Dual mode of action of a human anti-epidermal growth factor receptor monoclonal antibody for cancer therapy. *J. Immunol.* 173: 4699-4707.
16. Piloto O, Levis M, Huso D, *et al.* (2005) Inhibitory anti-FLT3 antibodies are capable of mediating antibody-dependent cell-mediated cytotoxicity and reducing engraftment of acute myelogenous leukemia blasts in nonobese diabetic/severe combined immunodeficient mice. *Cancer Res*. 65, 4:1514-22.
17. Niwa R, Sakurada M, Kobayashi Y, *et al.* (2005) Enhanced natural killer cell binding and activation by low-fucose IgG1 antibody results in potent antibody-dependent cellular cytotoxicity induction at lower antigen density. *Clin. Cancer Res*. 11:2327-36.
18. Lorenzo CD, Tedesco A, Terrazzano G, *et al.* (2004) A human, compact, fully functional anti-ErbB2 antibody as a novel antitumour agent. *Br. J. Cancer*. 91: 1200-4.
19. Harris M. (2004) Monoclonal antibodies as therapeutic agents for cancer. *Lancet Oncol.* 5: 292-302.
20. Xiang W, Wimberger P, Dreier T, *et al.* (2003) Cytotoxic activity of novel human monoclonal antibody MT201 against primary ovarian tumor cells. *J. Cancer Res. Clin. Oncol*. 129: 341-8.
21. Wu X, Rubin M, Fan Z. (1996) Involvement of p27KIP1 in G1 arrest mediated by an anti-epidermal growth factor receptor monoclonal antibody. *Oncogene*. 12: 1397-1403.
22. Mendelsohn J. (2000) Blockade of receptors for growth factors: An anticancer therapy. *Clin. Cancer Res*. 6: 747-53.
23. Liu B, Fang M, Schmidt M. (2000) Induction of apoptosis and activation of the caspase cascade by anti-EGF receptor monoclonal antibodies in DiFi human colon cancer cell lines do not involve the c-jun N-terminal kinase activity. *Br. J. Cancer*. 82: 1991-9.
24. Kopple MJ, Bleichrodt RP, Oyen WJG, *et al.* (2005) Radioimmunotherapy and colorectal cancer. *Br. J. Surg*. 92: 264-76.
25. Mao WG, Luis E, Ross S, *et al.* (2004) $EphB_2$ as a therapeutic antibody drug target for the treatment of colorectal cancer. *Cancer Res*. 64: 781-8.
26. Goodwin RA, Tuttle SE, Bucci DM. (1987) Tumor-associated antigen expression of primary and metastatic colorectal carcinomas detected by monoclonal antibody 17-1A. *Am. J. Clin. Pathol.* 88: 462-77.
27. Shetye J, Frodin JE, Christensson B, *et al.* (1988) Immunohistochemical monitoring of metastatic colorectal carcinoma in patients treated with monoclonal antibodies (Mab 17-1A). *Cancer Immunol. Immunother.* 27: 154-62.
28. Balzar M, Winter J, de Boer CJ, *et al.* (1999) The biology of the 17-1A antigen (Ep-CAM). *J. Mol. Med.* 17: 699-712.
29. Lenz HJ, Mayer R, Gold PJ, *et al.* (2004) Activity of cemximab in patients with colorectal cancer refractory to both irinorecan and oxaliplatin. *Proceedings (Post-Meeting Edition) of the 2004 ASCO Annum eeting: June 5-8, 2004; New Orleans, LA. J. Clin. Oncol.* 22. Abstract 3510.
30. Garambois V, Glaussel F, Foulquier E, *et al.* (2004) Fully human IgG and IgM antibodies directed against the carcinoembryonic antigen (CEA) gold 4 epitope and designed for radioimmunotherapy (RIT) of colorectal cancers. *BMC cancer.* 4:75.
31. Norderhaug L, Olafsen T, Michaelsen TE, *et al.* (1997) Versatile vectors for transient and stable expression of recombinant antibody molecules in mammalian cells. *J Immunol Methods*. 204, 77-87.