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Development and evaluation of a novel anti-colorectal cancer monoclonal antibody, WL5

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ABSTRACT

The WL5 antibody is an anti-colorectal cancer antibody secreted by the WL5 hybridoma clone. Flow cytometric analysis showed that WL5 specifically binds to the HT29 and LS180 colorectal cancer cell lines. Immunohistochemical analysis performed on a tissue microarray demonstrated that the WL5 antibody can be used for the specific and sensitive diagnosis of colorectal carcinoma. Furthermore, WL5 mediated antibody dependent cell-mediated cytotoxicity (ADCC) of tumor cells and exhibited similar antitumor activity to adriamycin (ADM) but avoided the cardiomyopathy and decrease in peripheral white blood cell counts associated with prolonged ADM treatment. The glycoprotein, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), was identified as the target antigen of WL5 through immunoprecipitation and mass spectrometric analyses, which might provide a potential biomarker and therapeutic target for colorectal cancer.

1. Introduction

Cancer is one of the leading causes of death worldwide [1]. Colorectal cancer (CRC) is the third most common cancer in males and the second in females, according to data published in 2011 based on the GLOBOCAN 2008 estimates [2–4]. There are two general options for CRC screening currently: stool tests and structural examinations. Stool tests, such as immunochemical fecal occult blood tests (iFOBT), are most appropriate for the diagnosis of CRC, while structural examinations, including colonoscopy (CSPY), flexible sigmoidoscopy (FSIG), double-contrast barium enema (DCBE), and computed tomographic colonography (CTC), detect not only adenocarcinoma but also identify adenomatous polyps [5,6]. Serum tests are also widely used for CRC screening because this method allows ease of sample collection, is non-invasive and low cost [6]. The carcinoembryonic antigen (CEA) is commonly used as a serum biomarker of CRC although the sensitivity and specificity of this approach is not high [6,7]. CRC is commonly treated by surgical removal of the cancer and nearby lymph nodes in the early stages (stage I and II) and with chemotherapy (alone or incorporated with radiation therapy) for late-stage disease, before or after surgery [2].

A monoclonal antibody (mAb) is an immunoglobulin secreted by a hybridoma clone that has been generated by fusing a myeloma cell with a B lymphocyte from a donor or from an immunized animal [8,9]. In 1975, Köhler and Milstein fused myeloma cells with plasma cells to establish hybridomas, thus generating the first mAb [10]. The administration of tumor-targeting monoclonal antibodies is considered to be a successful form of immune therapy for cancer. The infused antibodies bind to their targets and function through steric inhibition and neutralization, complement activation, or activation of cell-mediated cytotoxicity [11]. The first mAb approved for use in humans was OKT3 (muromonab-CD3) [12]. To date, the US Food and Drug Administration (FDA) has approved three monoclonal antibodies targeted against metastatic colorectal cancer: bevacizumab, betuximab, and panitumumab [2].

In addition to the use of monoclonal antibodies alone, antibody-drug conjugates have also been developed to achieve high specificity and therapeutic efficacy with lower toxicity. The first conjugate approved by the FDA for clinical use was mylotarg, which is

Abbreviations: CEA, carcinoembryonic antigen; CEACAM1, carcinoembryonic antigen-related cell adhesion molecule 1; CRC, colorectal cancer; FBS, fetal bovine serum; GFP, green fluorescent protein; MS, mass spectrum; PBS, phosphate-buffered saline; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TBST, tris buffered saline with Tween-20.

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composed of a humanized anti-CD33 mAb and the DNA-alkylating agent, calicheamicin [13,14].

The WL5 antibody is an anti-colorectal cancer mAb that was produced in our laboratory. In this study, the specificity of WL5 was evaluated using different tumor cells and tumor tissues. Furthermore, the antitumor potential of WL5 was investigated both *in vitro* and *invivo*. The WL5 antigen was also identified and implicated as a potential biomarker of CRC.

2. Materials and methods

2.1. Animals

Female and male BALB/c mice (aged 6–8 weeks) were purchased from the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China). Animals received humane care and all experimental procedures involving animals were carried out with the approval of the Animal Use and Care Committee of Beijing Institute of Radiation Medicine.

2.2. Antibodies and reagents

WL5 antibody was purified from mouse peritoneal fluid. Mouse anti-CEACAM1 antibody was from R&D (Minneapolis, MN, USA). Coomassie brilliant blue R-250 (0.25%) was prepared in a solution containing methanol (45%) and acetic acid (10%). Tissue microarrays were purchased from Biochip Co. Ltd. (Shanghai, China). Adriamycin (ADM) was purchased from Huafeng United Technology Company Ltd. (Beijing, China).

2.3. The generation of WL5 antibody by hybridoma technology

BAL B/c mice were immunized with human CRC cells obtained from freshly isolated human CRC tissues. B cells from the immunized mice were fused with the mouse myeloma SP2/0 using polyethylene glycol-1500 (PEG-1500) to establish hybridomas. After screening and culturing, one hybridoma clone with excellent stability and specificity to colorectal cancer tissues was generated and named WL5.

To produce antibody, female BAL B/c mice were injected intraperitoneally with WL5 hybridoma cells and the WL5 antibody was purified from the peritoneal fluid of the mice (Supplemental Experimental Procedures).

2.4. Flow cytometric assays

Cells were digested and resuspended in culture medium, and then washed with PBS containing 2% FBS. Then cells were incubated with 200 μL WL5 or mouse anti-CEACAM1 antibody solution (10 μg/mL) at 4 °C for 1 h. After being washed with PBS containing 2% FBS three times, cells were incubated with 200 μL fluorescein isothiocyanate (FITC) conjugated goat anti-mouse antibody (1:500) at 4 °C for 45 min. Another wash was carried out and specific antibody binding was then detected by flow cytometry.

2.5. Immunoprecipitation

A total cell lysate of HT29 was incubated with 10 μg WL5 at 4 °C overnight to allow WL5 antibody/antigen complex formation. Complexes were then isolated by the addition of protein A/G (Santa Cruz, CA, USA) plus-agarose followed by incubation at 4 °C for 8 h. Agarose beads were then washed three times with PBS and boiled with 2× loading buffer for 5 min. The supernatant was harvested and proteins were separated by SDS–PAGE.

2.6. Antibody dependent cell-mediated cytotoxicity (ADCC) assays

To determine cytotoxicity, the CytoTox 96 Nonradioactive Cytotoxicity Assay (Promega, WI, USA) based on the colorimetric detection of the released enzyme LDH was used. HT29 cells were harvested as target cells and plated in a 96-well plate (5×10^3 cells/well). Peripheral blood mononuclear cells (PBMCs) were generated from a healthy BALB/c mouse by Ficoll purification and were used as effector cells. The ratio of effector to target cells was 5:1. All of the conditions were tested in quadruplicate and the experiments were performed three times. HepG2 cells were used as control target cells. The assay was carried out following the manufacturer's protocol.

The background absorbance of the culture medium and the lysis solution was subtracted and the percentage cytotoxicity was calculated as follows:

$$\text{Cytotoxicity}\ (\%) = \frac{\text{Experimental} - \text{Effector spontaneous} - \text{Target spontaneous}}{\text{Target maximum} - \text{Target spontaneous}} \quad (1)$$

2.7. In vitro cytotoxicity assays of ADM conjugated WL5

To investigate the cytotoxic activities of the WL5-ADM conjugate and free ADM, HT29 cells were seeded into a 96-well plate (1.5×10^4 cells/well) and incubated with different concentrations of WL5-ADM or ADM (at equivalent concentrations of ADM) for 48 h. Each concentration was tested in quadruplicate. HCT116 cells were used as a negative control. Cell viabilities were evaluated using Cell Counting Kit-8 (CCK-8, Solar bio, Beijing, China) following the manufacturer's instruction. The 50% inhibitory concentration (IC_{50}) values were determined separately for each group using Graph Pad Prism 5.0 software.

2.8. In vivo antitumor activity of WL5

The antitumor activity of WL5 was evaluated in the HT29 tumor implantation model using male BALB/c mice. ADM was used as a positive control. On day 0, HT29 cells (5×10^6 cells in 0.1 mL PBS) were subcutaneously injected into each mouse to establish solid tumors. On day 3, ADM and WL5 were injected intravenously (0.2 mg/kg body weight with respect to ADM combined with a molar equivalent dose of WL5) every 4 days. Control animals received physiological saline. The tumors were measured every 4 days from day 7 and the volumes were calculated as: volume = length × $(\text{width})^2/2$.

On day 19, a 20 μL blood sample was drawn from the tail-vein of each mouse into an ethylenediamine tetraacetic acid (EDTA)-coated tube. Each sample was immediately added to 1 mL dilution buffer. Peripheral white blood cells (WBC) were counted using a hemocytometer. Mice were then humanely sacrificed and subcutaneous tumors were collected, weighed and fixed in 4% paraformaldehyde. Heart and lung samples were also harvested and fixed. Fixed tissue samples were embedded in 5 μm thick paraffin sections. After being dewaxed in xylene and rehydrated in a graded series of alcohols, the slides were stained with hematoxylin and eosin (H&E) for histopathological visualization. ApopTag Peroxidase *In Situ* Apoptosis Detection Kit (Chemicon International, Temecula, CA) was used for terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) immunohistochemical staining of heart specimens.

3. Results

3.1. The generation of WL5 antibody

After 61 rounds of fusion experiments mentioned in the Methods section, 3450 hybridoma clones were obtained, 14 of which could secret antibodies that react specifically with CRC cancer tissue samples (Supplementary Fig. 1). These hybridoma clones were clone-cultured three times and a single stable antibody-secreting clone was selected. This clone was designated WL5.

The WL5 antibody was purified from the peritoneal fluid of the mice that were intraperitoneally injected with WL5 hybridoma cells. The purified antibody was analyzed by SDS–PAGE and the gel was stained by Coomassie brilliant blue. As shown in Supplementary Fig. 2, the antibody appeared two bands at 55 kD and 30 kD which represented the heavy chain and the light chain of immunoglobulin, respectively. Thus, the WL5 antibody could be used in the following experiments.

3.2. The specificity of WL5 antibody to tumor cells

Using flow cytometry, we analyzed the specificity of WL5 using various tumor cell lines: colorectal cancer (HT29, LS180, SW620, HCT116 and LOVO), lung cancer (A549), prostatic carcinoma (PC3), breast cancer (MDA-MB-435) and hepatoma ($HepG_2$ and SMMC7721). The results (Fig. 1A–C) indicated that WL5 recognized HT29 (99.81%), LS180 (97.69%) and SW620 (51.0%). Low levels of WL5 bound to HCT116 cells (6.58%) and no binding of WL5 antibody to LOVO cells was observed (Fig. 1D and E). Tumor cells from other organs were either not recognized or minimally detected by WL5 (Fig. 1F–J). The binding rate of WL5 to different tumor cell types is shown in Fig. 1K.

3.3. WL5 tumor tissue specificity

Immunohistochemical analysis of WL5 antibody tissue specificity was performed using a tissue microarray containing tumor tissue samples derived from 31 patients with different types of carcinoma; esophageal (5 patients), gastric (5 patients), colon (5 patients), rectal carcinoma (5 patients), hepatocarcinoma (5 patients) and pancreatic (6 patients). Paired tumor-adjacent non-cancerous tissues were taken from each patient.

As shown in Fig. 2A, the WL5 antibody primarily detected colorectal carcinoma, with 9 of 10 (90%) samples exhibiting strong or moderate positive staining (staining in colon and rectal tissues is shown in Fig. 2B), while all tumor-adjacent tissues were negative for WL5 staining. No obvious binding of the WL5 antibody to esophageal, gastric and hepatocarcinoma tissues was observed. The tests were carried out three times and the differences in staining between colorectal carcinoma tissues and the other three types of carcinoma tissues were significant ($P < 0.001$, data not shown). Interestingly, 6 of 6 (100%) pancreatic carcinoma specimens were positively stained, while none of the tumor-adjacent tissues were positively stained with WL5 antibody.

3.4. WL5 antibody exerts antitumor activity through ADCC

The capacity of the WL5 antibody to mediate ADCC against colorectal cancer cells was investigated using a WL5 antigen-positive cell line, HT29 (Fig. 1). The WL5 antigen-negative cell line, HepG2, was used as a control. The WL5 antibody did not mediate cytotoxicity of HepG2 cells. In contrast, 86% cytotoxicity of HT29 cells was detected (Fig. 2C), demonstrating that WL5 mediated antigen-specific ADCC *in vitro*. WL5 did not mediate complement-dependent cytotoxicity (CDC) of either HT29 cells or HepG2 cells (data not shown).

3.5. The WL5-ADM conjugate possesses a low IC_{50} value in vitro

WL5 was then conjugated with the chemotherapeutic agent, ADM. The cytotoxic effects of WL5-ADM were compared with those of free ADM. HT29 cells and HCT116 cells were selected as target cells based on WL5 antigen expression levels (Fig. 1). The IC_{50} values are shown in Table 1. For HT29 cells which overexpress the WL5-antigen, the IC_{50} value of the WL5-ADM conjugate (49.08 ng/mL) was lower (29% reduction) than that of free ADM (69.02 ng/mL). In contrast, for HCT116 cells, which did not bind the WL5 antibody, there was no difference in the IC_{50} values calculated for ADM and WL5-ADM. These results indicate that the WL5-ADM conjugate more effectively mediated cytotoxicity against HT29 cells than free ADM. This is likely to be due to the ability of the WL5 antibody to target delivery of ADM to HT29 cells.

3.6. In vivo therapeutic efficacy of WL5

The therapeutic efficacy of WL5 was investigated in mice bearing xenograft colorectal tumors, using ADM as a positive control. The mice were divided randomly into three groups ($n = 8$ per group).

As shown in Fig. 3A, tumor growth was slower in the ADM and WL5 treated groups than in the control group. At the end of the experiment (day 19), the mean tumor volumes in the WL5 antibody (3580 mm^3) and ADM (3715 mm^3) treated groups were both significantly smaller than the mean tumor volume in the control group (4798 mm^3). These values represented reductions in tumor volume of approximately 25% ($P < 0.01$) and 22% ($P < 0.01$) in the WL5 antibody and ADM-treated groups, respectively. Furthermore, the tumor burden in the WL5 (0.521 g) and ADM (0.551 g) treated groups was significantly lower than that in the control group (0.840 g). These values represent reductions in tumor burden of approximately 38% ($P < 0.001$) and 34% ($P < 0.01$) in the WL5 and ADM treated groups, respectively (Fig. 3B). These data demonstrated that the WL5 antibody had comparative therapeutic efficacy with ADM in terms of tumor growth inhibition.

It has been reported that prolonged ADM-therapy results in the development of cardiomyopathy. As shown in Fig. 3C, the myofibrils of mice in the ADM treated group were thin and sparse with massive cardiomyocyte apoptosis. However, the WL5 antibody protected cardiocytes against apoptosis and maintained the ventricular wall thickness. Histopathological visualization revealed metastasis in the lungs (Fig. 3C). Tumor metastases were commonly detected in all the mice (8 of 8) in the control group and fewer metastases (3 of 8) were found in the ADM-treated group, whereas no metastases were observed in the WL5 treated group. Moreover, the diminution of peripheral white blood cells (WBC) induced by ADM treatment was abolished in the WL5-treated group (Fig. 3D). These data indicate that the side-effects of myocardial necrosis and leucopenia associated with ADM treatment were avoided by WL5 antibody treatment and also, demonstrated the metastases-inhibition potential of WL5.

3.7. Identification of the target antigen of WL5 antibody

In Western blot analyses, two HT29 lysate proteins were detected specifically by the WL5 antibody, while none were detected in HepG2 lysates (Fig. 4A), thus demonstrating that the WL5 antigen is overexpressed in HT29 cells. Therefore, HT29 cell lysates were used in immunoprecipitation studies with WL5 in order to identify the target antigen. $HepG_2$ cells were used as a negative control. Mouse IgG was used as a negative control antibody. The

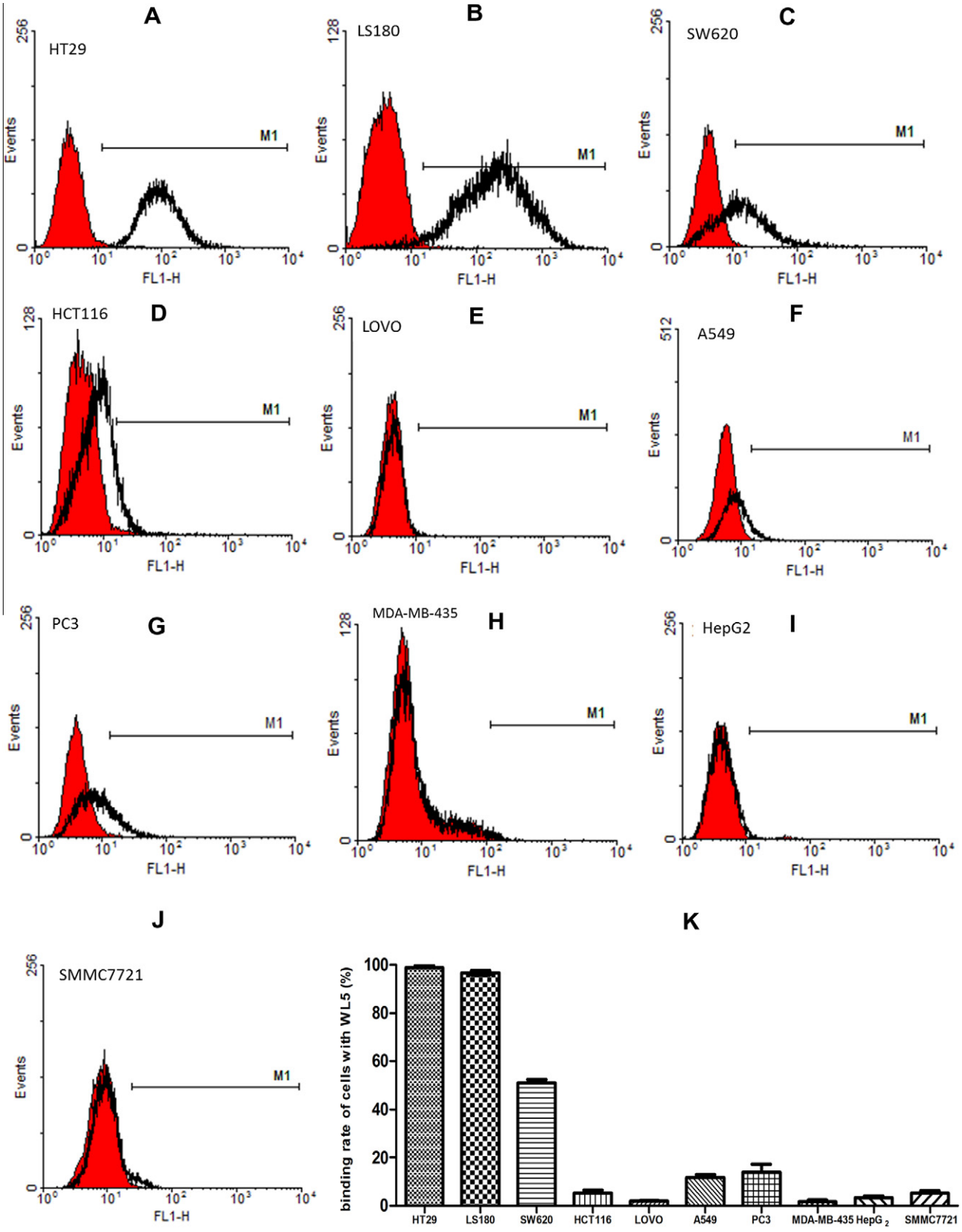

Fig. 1. Flow cytometric analysis of WL5 specificity. (A–J) Results of flow cytometric assays performed on various tumor cell lines using WL5 as the detection antibody. (K) The binding rate of WL5 to different tumor cells based on flow cytometry data.

immunoprecipitation products were separated by SDS–PAGE and stained with Coomassie brilliant blue solution. Immunoblotting was also carried out on the immunoprecipitation products and two bands which reflected the putative antigen were detected by WL5 in the presence of HT29 cells (Fig. 4B and C). The two specific bands corresponding to proteins of 130 kD and 95 kD were

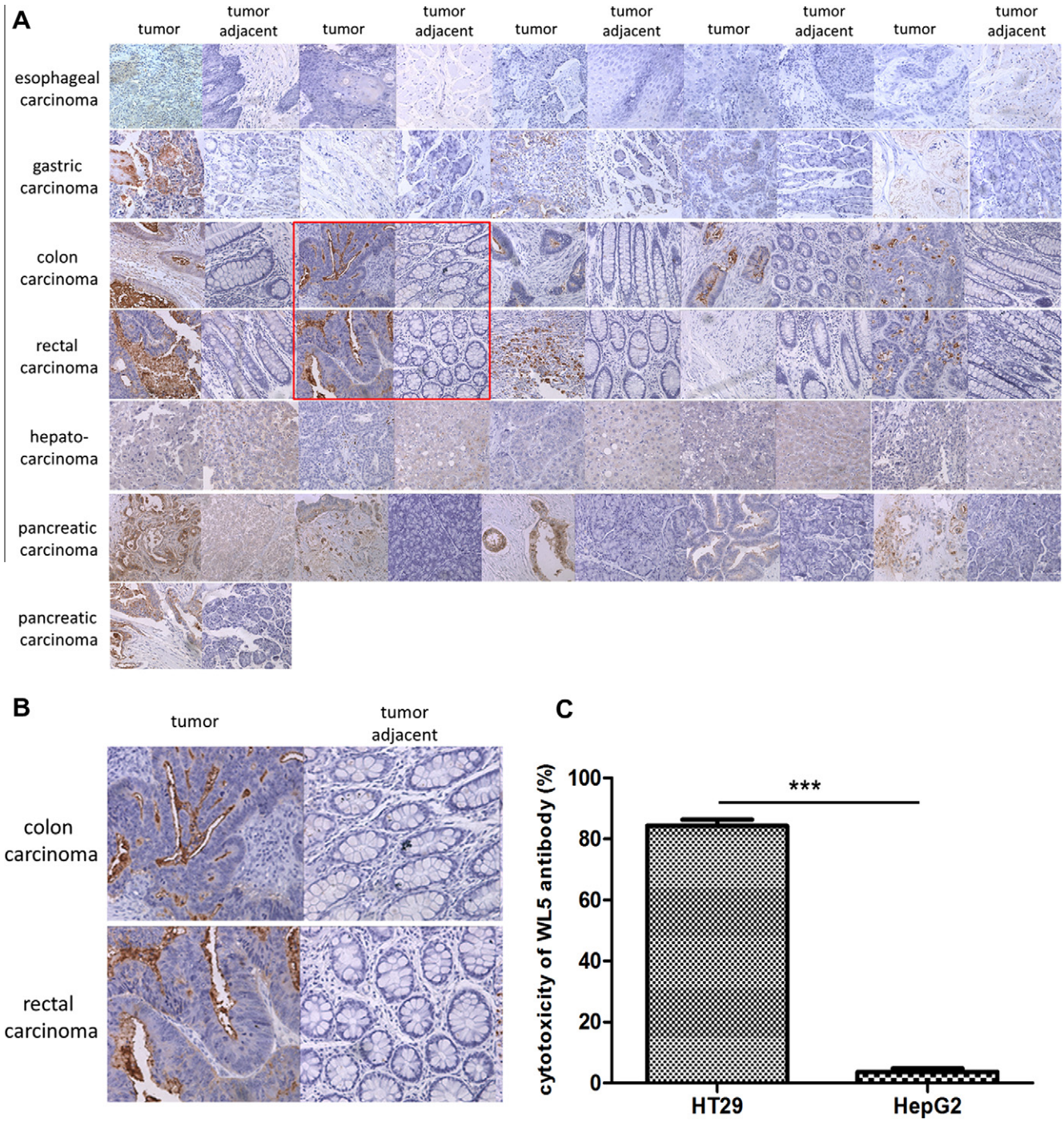

Fig. 2. Immunohistochemical analysis of WL5 tissue specificity using a tissue microarray and ADCC efficacy of the WL5 antibody. (A) Immunohistochemical analysis of the WL5 antibody using a tissue microarray. (B) Amplification of the staining results in colon and rectal tissues. (C) Analysis ADCC mediated by the WL5 antibody on HT29 CRC cells and hepatocarcinoma HepG2 cells.

Table 1
Cytotoxic activity of free ADM and WL5-ADM on tumor cells.[a]

Cells	IC50 (ng/mL) of ADM equivalent	
	Free ADM	WL5-ADM
HT29	69.02	49.08
HCT116	210.5	210.2

[a] Cells were seeded in 96-well plates at a density of 1.5×10^4 cells/well and exposed to free ADM or WL5-ADM for 48 h. Cell viability was assayed by CCK-8 and the cytotoxicity was showed as IC_{50}.

purified, digested, and then analyzed by MALDI-TOF MS at the National Center of Biomedical Analysis (Academy of Military Medical Sciences, Beijing, China). The proteins identified successfully by peptide mass fingerprinting of both bands were shown to be identical (Fig. 4D and E), corresponding to carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1, also known as biliary glycoprotein [15]). Thus, human CEACAM1 was identified as the probable WL5 antigen. This was further confirmed by Western blot analysis, which showed that the specific protein immunoprecipitated by WL5 was also detected by anti-CEACAM1 antibody

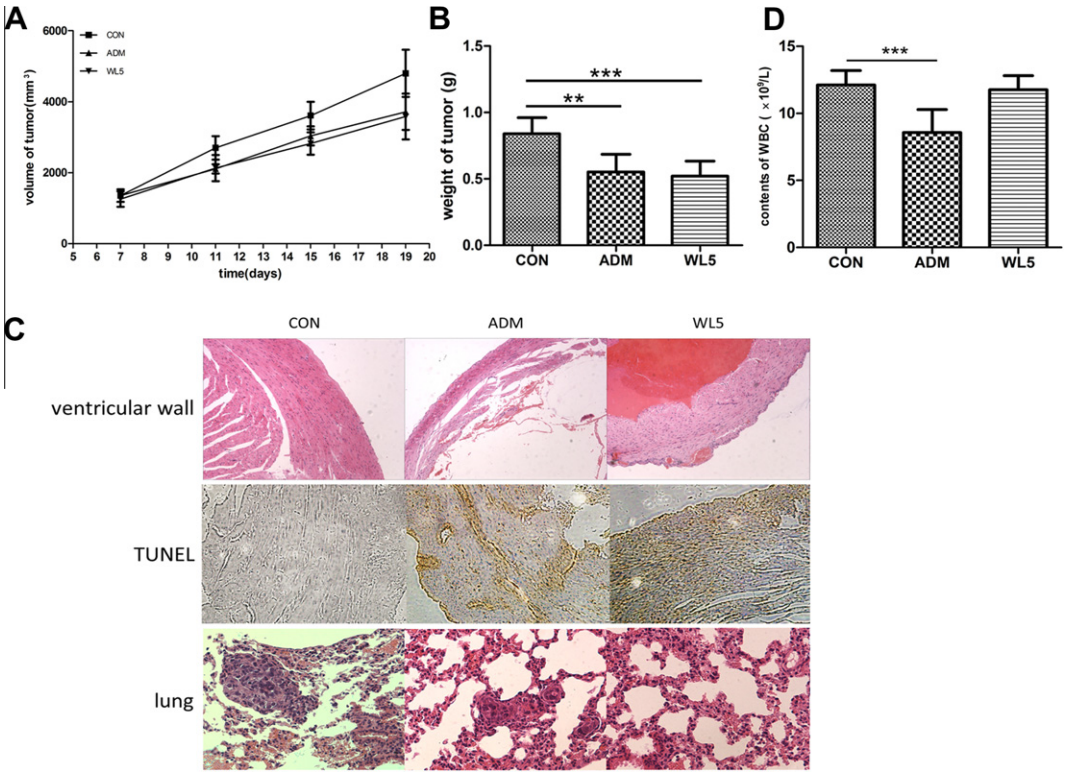

Fig. 3. *In vivo* therapeutic efficacy of the WL5 antibody. Investigation of parameters of the CRC tumor xenograft model in mice administered physiological saline, ADM or the WL5 antibody. (A) Development of tumor volumes in the three groups. (B) Tumor burdens of the three groups on the final day of therapy. (C) White blood cell counts in mouse peripheral blood. (D) Histopathological analysis of ventricular walls and lungs and TUNEL immunohistochemical staining for myocardial necrosis.

(Fig. 4F). We transfected the plasmid containing the human CEACAM1 gene (refseq ID: BC014473) into HEK293 cells using Lipofectamine 2000 (Invitrogen CA, USA) according to the manufacturer's instructions. Forty-eight hours after transfection, total proteins of the transfected HEK293 were analyzed by Western blot. A specific 130 kD protein was detected by WL5 (Fig. 4G), suggesting that CEACAM1 is the specific antigen recognized by the WL5 antibody and a potential biomarker of colorectal cancer.

4. Discussion

WL5, secreted by the hybridoma clone WL5, is an anti-colorectal cancer antibody produced in our laboratory. The antibody was purified from the peritoneal fluid of mice which had been injected intraperitoneally with WL5 hybridoma cells.

Flow cytometric analysis was carried out on various tumor cells using WL5 antibody (Fig. 1). Among the investigated cells, the WL5-antigen was expressed at high levels on the membrane of three colorectal cancer cell lines; HT29, LS180 and SW620. The WL5-binding rates of these were 99.81%, 97.69% and 51%, respectively. Although two colorectal cancer cell lines, HCT116 and LOVO, were negative for WL5-antigen expression, we showed that the WL5 antibody possesses specificity for CRC cells, as other types of cancer cell lines expressed the antigen at low levels. To investigate the tissue specificity of the WL5 antibody, immunohistochemical analysis was performed using a tissue microarray. The results indicated that the WL5 antibody primarily detected colorectal carcinoma with high specificity and sensitivity (Fig. 2A and B).

Furthermore, a WL5-ADM conjugate was synthesized chemically with the aim of using WL5 specificity to target delivery to the HT29 CRC cell line and to enhance the availability of ADM. The resulting conjugate exhibited a 29% reduction in IC_{50} compared with free ADM (Table 1).

Monoclonal antibodies, including trastuzumab, cetuximab, panitumumab, and bevacizumab, have been licensed as effective clinical drugs for the treatment of tumors with lower toxicity than traditional cytotoxic cancer chemotherapy. By binding to their targets, monoclonal antibodies function through several effector mechanisms, including steric inhibition and neutralization, ADCC and complement-dependent cytotoxicity (CDC) [9]. In our study, we found that the WL5 antibody mediated antitumor activity through ADCC (Fig. 2C) but not CDC. The WL5 antibody mediated 86% cytotoxicity against HT29 cells expressing WL5-antigen at high levels. In the CRC tumor xenograft model, WL5 antibody treatment resulted in a significant reduction in solid tumor volume (25% reduction, $P < 0.01$) and weight (38% reduction, $P < 0.001$) compared with the control group, indicating that the WL5 antibody is as effective as ADM in mediating tumor growth inhibition (Fig. 3A and B). Furthermore, our observations indicated that WL5 therapy avoids myocardial necrosis and leucopenia which are side-effects of ADM treatment (Fig. 3C) and also appeared to be more effective in tumor metastasis inhibition (Fig. 3D).

Serum tests are a convenient method for CRC screening. The carcinoembryonic antigen (CEA) is commonly used as a serum biomarker of CRC because it is the most cost-effective method available [16]. However, the sensitivity (36%) and specificity (87%) of CEA detection are not optimal for CRC screening [16,17]. Monoclonal antibodies, which are generated by hybridoma technology have been used to identify cell surface antigens and to develop biomarkers to differentiate cancerous cells from normal cells [8,18]. In this study, we aimed to identify the antigen recognized by the WL5 antibody for evaluation as a potential biomarker of CRC cells. This marker could then be used in combination with CEA as a multimarker in serum tests for CRC screening. Immunoprecipitation and MS analysis results indicated that the target antigen of the WL5 antibody was the glycoprotein, CEACAM1 (also known as

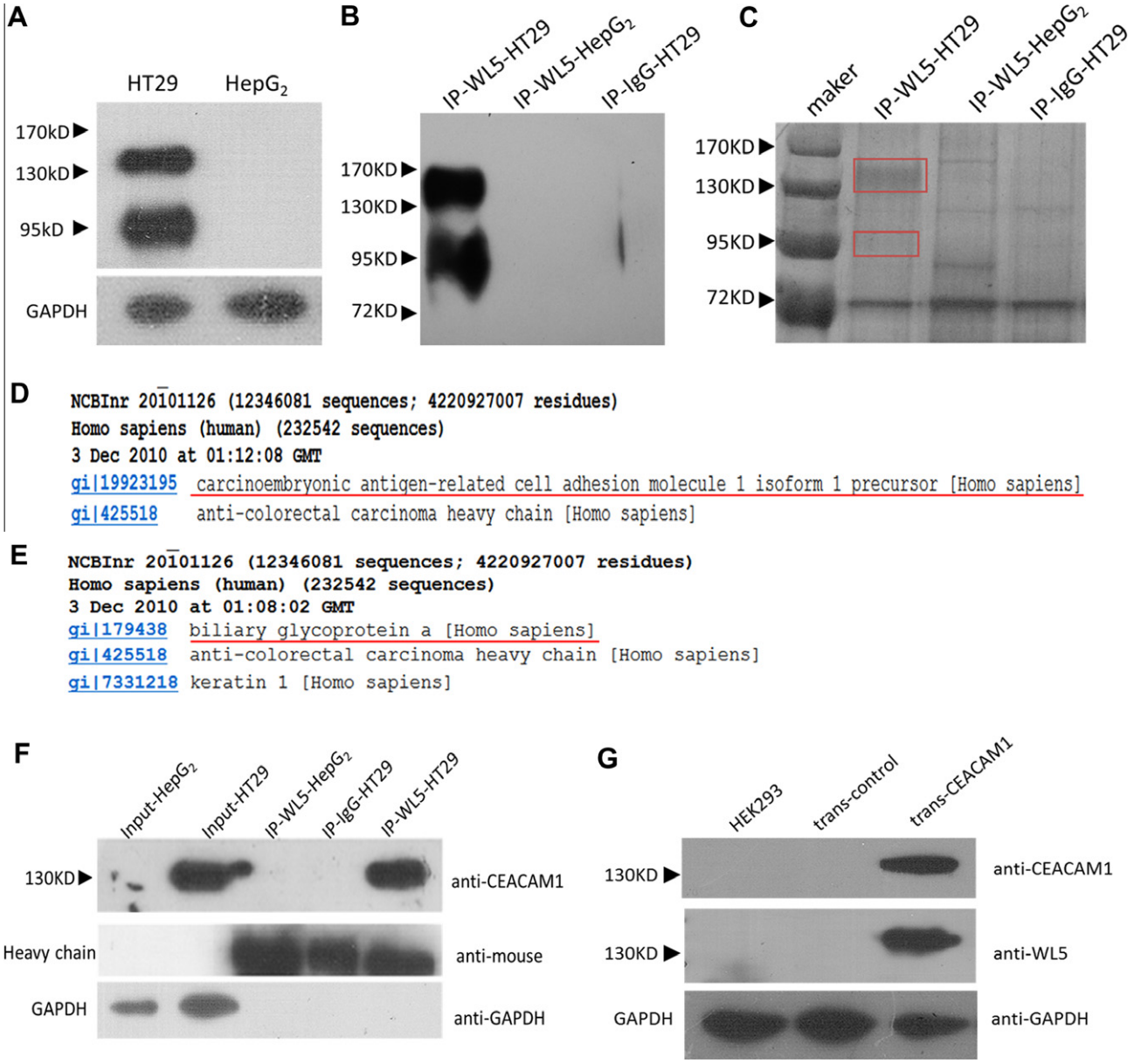

Fig. 4. WL5 antigen identification. (A) Western blot assay using WL5 as a detection antibody. (B) WL5 immunoprecipitation products were analyzed by Western blot using WL5 as the primary antibody. (C) WL5 immunoprecipitation products were separated by SDS–PAGE and stained with Coomassie brilliant blue solution. The two specific bands (red boxes) at the corresponding position of the Western blots were purified and analyzed by MS: (D) 130 kD protein; (E) 95 kD protein. (F) Western blot analysis of WL5 immunoprecipitation products using anti-CEACAM1 antibody as the primary antibody. (G) Plasmids containing human CEACAM1 genes were transfected into HEK293 cells and lysates of the transfected cells were analyzed by Western blot. A 130 kD protein was detected by the WL5 antibody or anti-CEACAM1 antibody. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

biliary glycoprotein, Fig. 4D and E). Subsequent immunoprecipitation and DNA transfection studies further confirmed CEACAM1 as the target antigen of the WL5 antibody (Fig. 4F and G). Our results showed that, in addition to the 130 kD protein, WL5 also binds to another 95 kD protein, which was identified as CEACAM1 by MS analysis. However, the 95 kD protein was not be detected by an anti-CEACAM1 antibody. This might be because the two antibodies recognize different isoforms of CEACAM1, which has been reported in humans to exist in 11 isoforms that differ in the numbers of extracellular immunoglobulin-like domains and the length of the cytoplasmic tail [15].

High expression of the CEACAM1 isoform with a long cytoplasmic tail (CEACAM1-L) has been shown to be positively correlated with tumor invasion, migration and stage [19–21]. Results of immunohistochemical analyses performed on clinical samples from 164 colorectal cancer patients have shown that CEACAM1-L is overexpressed at the invasion front, suggesting that CEACAM1-L contributes to colorectal cancer invasion [22]. Similarly, immunohistochemical studies have shown a positive correlation between the CEACAM-1 expression level and tumor metastases in 96 patients with metastatic pulmonary adenocarcinoma [23]. It had also been reported CEACAM1$^{-/-}$ mice exhibit a reduced metastatic burden when injected with metastatic mouse colorectal cancer cells [24]. These data implicate CEACAM1 as a novel therapeutic target of metastatic colorectal carcinoma.

In summary, the anti-colorectal cancer antibody, WL5, has a high specificity for CRC cells and tissues. This mAb inhibits tumor growth effectively via ADCC and minimizes the side-effects of

myocardial necrosis and leucopenia produced by chemotherapeutic interventions. The antigen recognized by the WL5 antibody was identified as CEACAM1, which has been reported to promote tumor migration and might be an important novel biomarker of CRC.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.01.089.

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