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ORIGINAL ARTICLE

Prognostic value of MAGE-A9 expression in patients with colorectal cancer

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Summary MAGE-A9 is a novel member of the melanoma-associated antigen (MAGE) family and is expressed in testicular cancer. The present study investigated MAGE-A9 expression as a potential biomarker in colorectal cancer (CRC). Immunohistochemical analysis was used to determine the expression of MAGE-A9 in 201 cases CRC tissues. We used quantitative real-time polymerase chain reaction (RT-PCR) and western blot analysis to further verify the results. The correlation between MAGE-A9 expression, clinicopathological features and prognosis of CRC patients was analyzed. The results showed that MAGE-A9 was predominantly localized in the cytoplasm of cancer cells and stromal cells. Compared to normal adjacent tissues, the high expression rate of MAGE-A9 in CRC tissues was significantly increased ($P < 0.001$). High MAGE-A9 expression was significantly associated with venous invasion ($P = 0.008$) and lymph node metastasis ($P < 0.001$). The survival rate of the CRC patients who were positive for MAGE-A9 expression was significantly lower than that of CRC patients with negative MAGE-A9 expression. Moreover, univariate and multivariate analyses showed that high MAGE-A9 expression was a poor prognostic factor for CRC patients. Hence, MAGE-A9 is expected to become a new target for CRC treatment.

Introduction

Colorectal cancer (CRC) is a major health problem that accounts for a large proportion of all human malignancies; overall, approximately 3.25 million people are diagnosed with the disease each year worldwide [1,2]. Despite recent advances in the diagnosis and treatment of this disease,

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most patients with CRC present with advanced disease and experience poor prognoses and low survival rates. Surgery is the primary treatment modality for CRC, whereas lymph node metastasis is the main factor for tumor recurrence. Therefore, metastasis to regional lymph nodes plays a critical role in CRC tumor progression, and lymph node involvement often promotes further hematogenous metastasis [3,4]. Although some proteins and genes related to the recurrence and lymph node metastasis of CRC have been described, the molecular mechanism is unclear. Therefore, further elucidation of the molecular mechanism of lymph node metastasis in CRC will benefit clinical treatment.

The melanoma-associated antigen (MAGE) family is a large gene family that includes more than 60 members, some of which encode tumor-specific antigens that have broad expression in various tumors such as lung cancer, melanoma, pancreatic cancer, breast cancer, and some urological malignancies [5—9]. MAGE-A9 is a novel member of the MAGE family that is expressed in testicular cancer. It was previously found that MAGE-A9 is abnormally expressed in bladder carcinoma, whereas it is expressed at low levels in normal tissues [10]. Past studies illustrated that high expression of MAGE-A9 is closely related to the development of tumors such as testicular, bladder, liver, and throat cancers [10—13]. However, there are few data about the relationship between MAGE-A9 and CRC. This study aimed to evaluate the expression of MAGE-A9 in CRC and correlate the expression of MAGE-A9 with the clinicopathological parameters and prognosis of the disease.

Materials and methods

Patients and samples

Tumor tissues and matched adjacent noncancerous tissues were obtained from 201 patients who were diagnosed with CRC and who had undergone a complete surgical resection at the People's Hospital of Gansu Province (Lanzhou, People's Republic of China) between January 2005 and October 2008. All patients were diagnosed with CRC by pathology, and none received any preoperative treatment, including radiotherapy or chemotherapy in particular. The study included 99 women and 102 men between the ages of 32 and 75 years (median, 61.66 ± 10.43 years). Patients were selected for this study only if follow-up examination and clinical data were available. Participants signed informed consent forms. All clinicopathological data including age, sex, tumor size, differentiation status, venous invasion, lymph node invasion, venous invasion, tumor depth, and lymph node metastasis were obtained from the patients' medical records. This study was approved by the Medical Ethics Committee of the People's Hospital of Gansu Province.

Immunohistochemistry and staining analysis

Tissue sections were deparaffinized in xylene and rehydrated through graded alcohol. The slides were processed in citrate buffer (0.01 mmol/L, pH 6.0) and heated to 121 °C in an autoclave for 20 min to retrieve the antigen. After rinsing with phosphate-buffered saline (PBS), the sections were blocked with 10% goat serum to block nonspecific background staining. Tissue sections were incubated with rabbit polyclonal anti-MAGE-A9 antibody (1:200; Abgent, San Diego, CA, USA) for 2 h at room temperature, and then the sections were washed with PBS. Samples incubated with PBS instead of the primary antibody served as negative controls. After being rinsed in PBS, the sections were visualized using diaminobenzidine solution. The sections were then rinsed in water, counterstained with hematoxylin, dehydrated, and covered with cover slips. The immunohistochemical staining was evaluated on the basis of the staining intensity and the extent of staining by two pathologists. For the statistical analysis of MAGE-A9 staining, five high-power fields from each slide were randomly selected, and nuclear staining was examined. Using a semiquantitative scoring system, each slide was evaluated for both the intensity of the stain and the percentage of positive malignant cells. The staining intensity was scored using the following scale: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The percentage of positively stained tumor cells was scored as follows: 0 (0—25%), 1 (26—50%), 2 (51—75%), and 3 (76—100%). The two scores were multiplied and used to classify the patients into low expression (0—4.5) and high expression (4.5—9) groups.

RNA extraction and quantitative real-time PCR assays

MAGE-A9 mRNA expression was determined by reverse transcription-polymerase chain reaction (RT-PCR). Total RNA (5 μg) was isolated from tissue using TRIZOL reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol. A 25-μL system was used for PCR amplification of the target gene, and the cycling conditions were as follows: pre-denaturation at 94 °C for 5 min, denaturation at 94 °C for 30 s, annealing at 55 °C for 26 s, and extension at 72 °C for 40 s. After 30 cycles, there was a final elongation step at 72 °C for 10 min. The expression of β-actin was used as the internal control. The MAGE-A9 gene- and β-actin gene-specific primers for PCR amplification were as follows: MAGE-A9, 5′-CCCCAGAGCAGCACTGACG-3′ (sense) and 5′-CAGCTG AGCTGGGTCGACC -3′ (antisense); β-actin, 5′-TCATCACCATTGGCAATGAG-3′ (sense) and 5′-GATGT CCACGTCACACTTC-3′ (antisense).

Western blot analysis

Tumor tissues and corresponding adjacent tissues were lysed in 2× sodium dodecyl sulfate lysis buffer, and UV spectrometry was employed to determine the protein concentration. Aliquoted protein was used to perform sodium dodecyl sulfate—polyacrylamide gel electrophoresis. The protein from the gel was then transferred onto a polyvinylidene fluoride membrane by the wet transfer method. The membrane was then incubated overnight at 4 °C with rabbit polyclonal anti-MAGE-A9 antibody. β-actin was used as the loading control. Bio-Rad Quantity software (version 7.3.0; Bio-Rad Laboratories, Inc., USA) was used to analyze and measure zone absorbance. The ratio of the absorbance of the target protein to that of β-actin was used to evaluate the protein expression level.