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Dendritic cells transduced with CPEB4 induced antitumor immune response

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ABSTRACT

Much evidence leads to the exploration of immunologic approaches for eliminating tumor cells. Cytoplasmic polyadenylation element binding protein 4 (CPEB4) is considered to be a novel therapeutical target for glioblastoma. In this study, we transduced DCs with CPEB4 to explore the immune response in vivo. We found that DCs transduced with recombinant adenovirus encoding CPEB4 could induce specific cytotoxic T lymphocytes (CTLs) to lyse glioma cells and augment the number of IFN-γ secreting T-cells in mice. In addition, the modified DCs could effectively protect mice from lethal challenges against glioma cells, reduce tumor growth and increase the mice life span. These results suggest that the DC transduced with CPEB4 may induce anti-tumor immunity against glioma cells and might be used as an efficient tumor vaccine in clinical applications.

Introduction

Glioblastoma multiforme is the most common glioma arising in adults and accounts for approximately 75% of all newly diagnosed glioma cases in adults Scott et al., 2011; Salvati et al.; 2009, Brandes et al.; 2009. Considerable interest has arisen in finding alternate and/or complementary approaches to treating these patients. One such approach has been the development of immunologic therapies, ultimately designed to enhance recognition of these tumors by the patient's immune system and to increase the activity of tumor-infiltrating lymphocytes (TILs) Pellegatta et al., 2010; Xiong and Ohlfest, 2011; Ginzkey et al., 2010.

Cytoplasmic polyadenylation element binding protein 4 (CPEB4), an RNA binding protein that mediates meiotic mRNA cytoplasmic polyadenylation and translation, is overexpressed in pancreatic ductal adenocarcinomas and glioblastomas, where it supports tumor growth, vascularization and invasion Ortiz-Zapater et al., 2012; D'Ambrogio et al., 2013; Novoa et al., 2013.

Dendritic cells (DC) can process tumor-associated antigens (TAAs) correctly and display them on their surface Simon et al., 2013; Van Lint et al., 2012; Smits et al., 2009. In previous studies, evidence showed that DC loading of tumor lysate, tumor RNA, or cell fusion hybrids could be selected as a vaccine for explorative clinical trial Cao et al., 2009; Javorovic et al., 2008; Wang et al., 2006. Therefore, in the present study, we elucidate whether DC transduced with CPEB4 could induce anti-tumor immunity in vivo, which might be applied in forthcoming glioma vaccination studies.

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Materials and methods

Mice and cell line

C57BL/6 (8-week-old) mice were obtained from Experimental Animal Center of Chinese Academy of Medical Science (Beijing, China). All animals were housed under specific pathogen-free conditions. Experiments were conducted in accordance with animal care guidelines approved by the Animal Ethics Committee of the Fujian Military Medical University. All animal studies were approved by the Institutional Animal Care and Use Committee of the Fujian Military Medical University. The syngeneic murine glioma cell line GL261 and murine lung carcinoma Lewis were purchased from ATCC (American Type Culture Collection). Cells were grown in RPMI 1640 supplemented with 10% fetal calf serum, 1×10^5 IU/L penicillin, 100 mg/L streptomycin, 2-mmol/L glutamine in the presence of 5% CO_2.

Construction of recombinant adenovirus encoding CPEB4

The recombinant adenovirus vector encoding CPEB4 was constructed using the Adeno-XTM Expression System (Clontech, Palo Alto, CA, USA) according to the manufacturer's instructions. Briefly, the CPEB4 cDNA was cloned into the shuttle vector pDC315 and sequenced. The desired replication-deficient adenovirus containing the full length cDNA of CPEB4 was generated by homologous recombination through co-transfection of plasmids pDC315-CPEB4 and pBHG1oXE1, 3Cre in HEK 293 cells using the DOTAP liposome reagent (Roch, Germany). After several rounds of plaque purification, the adenovirus containing the CPEB4 gene was amplified and purified from cell lysates by banding twice in CsCl density gradients. Viral products were desalted and stored at −80 °C in phosphate buffered saline (PBS) containing 10% glycerol (v/v). The infectious titer was determined by a standard plaque assay. A second recombinant, E1- and E3-deleted adenovirus carrying the LacZ protein under the control of CMV promoter (Ad-LacZ), was used as a control vector.

DC generation from mouse bone marrow

In brief, bone marrow was flushed from the tibias and femurs of C57BL/6 mice and depleted of erythrocytes with commercial lysis buffer (Sigma, St. Louis, MO, USA). The cells were washed twice in serum-free RPMI-1640 medium and cultured in a six-well plate at 5×10^6 cells per well with RPMI-1640 medium supplemented with 10% (v/v) FBS containing 10 ng/mL recombinant murine GMCSF (R&D System, Inc., USA) and 10 ng/mL recombinant murine IL-4 (R&D System, Inc.). On days 3 and 5, half of the media were refreshed without discarding any cells and fresh cytokine containing (mGM-CSF and mIL-4) media were added. On days 7 and 8 of culture, mTNF-α (R&D System, Inc.) was added to the media. On day 10, non-adherent cells obtained from these cultures were considered mature bone marrow-derived DCs. FACScan confirmed the phenotypic markers of DCs.

Flow cytometric analysis of cell populations

DCs were collected and resuspended in cold FACS buffer (1× PBS, 5% FBS, and 0.1% sodium azide). Cells were immunostained with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse CD11c, H-2Kb, and CD86 antibodies (eBioscience, USA). Corresponding FITC immunoglobulin G (IgG) isotype control antibody (eBioscience, USA) was used. A total of 1×10^6 cells were incubated at 4 °C with antibodies for 2 h. The cells were then washed once with FACS buffer, resuspended, and tested on a FACScan (Becton-Dickinson, USA). The results showed that these mature DCs expressed high level of CD11c, H-2Kb, CD86, MHC II, CD80 and CD40.

Adenovirus-mediated gene transfer

Transduction of mouse mature DCs with Ad vector was conducted in six-well plates with 1×10^6 DCs per well in a 3 mL volume of RPMI-1640 medium containing 10% FBS. Viruses were added to the wells at a multiplicity of infection (MOI) of 100 and the DCs were harvested after 24 h of incubation.

Reverse transcription PCR analysis

Total RNA was extracted using TRIZOL reagent (Invitrogen, Carlsbad, CA) according to the protocol of the manufacturer. Two micrograms of RNA was subjected to reverse transcription. The polymerase chain reaction (PCR) primers used were as follows: for CPEB4, 5′-CCAA CC ATCA AGGATAAACCA-3′ (forward), 5′-AGCCATCCAT CACAA A GTCA-3′ (reverse) and for β-actin, 5′-ATG ATATCG CCG CGC TCG TC-3′ (forward), 5′-CGC TCG GTG AGG ATC TTCA-3′ (reverse). PCR products were separated on a 1% agarose gel, visualized and photographed under ultraviolet light. Quantification of cDNA product was completed using a Kodak 1D image quantification software (Kodak Co, NY), and target PCR products were compared by normalizing each sample to the production of β-actin.

Western blot assay

For Western blot assay, proteins of the cell extracts were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto a nitrocellulose membrane. The membrane was incubated with 5% non-fat milk in PBS and then with anti-CPEB4 antibody (Santa Cruz Biotechnology, USA) for 2 h at room temperature. After washing, the membranes were incubated with an alkaline phosphatase-conjugated goat antimouse IgG antibody (Amersham Biosciences, UK) for 1 h at room temperature. Immunoreactive bands were detected using the ECL Western blotting analysis system (Amersham Biosciences).

Induction of specific CTLs in mice

For induction of specific CTLs in vivo, 1×10^6 Ad-CPEB4 transduced DCs were resuspended in a 100 μL volume of PBS and injected subcutaneously into C57BL/6 mice. Control groups were injected subcutaneously with either 1×10^6 Ad-LacZ transduced DCs or 1×10^6 DCs alone. At days 7 and 14, the mice were given booster vaccinations using the same protocol as described above. On day 21, spleens were removed and homogenized. Single-cell suspensions of splenocytes were prepared as effector cells using stainless steel mesh screens and red blood cell lysing solution. CD8 + T-cells were purified from splenocytes with MACS and were assayed as effector cells in a 4-h 51Cr-release release assay and enzyme-linked immunospot assay.

Chromium release assays

To evaluate levels of CTL activity, a standard 4-h 51Cr-release assay was used. GL261 cells were used as target cells. Briefly, target cells were incubated with 51Cr (100 u Ci per 1×10^6 cells; Amersham Biosciences Corp.) for 2 h in a 37 °C water bath. After incubation with 51Cr, target cells were washed three times with PBS, resuspended in RPMI-1640, and mixed with effector cells at effector-to-target (E/T) ratios of 25:1, 50:1, or 100:1. Assays were performed in triplicate for each sample at each ratio in a 96-well round-bottomed plate. After 4 h incubation, the supernatants were harvested, and the amount of 51Cr released was measured with a γ-counter. The percentage of specific lysate was calculated as 100 × (experimental release − release) / (maximum release − spontaneous release). Maximum release was determined from supernatants of cells that were lysed by the addition of 2% Triton X-100.

Enzyme-linked immunospot assay

Nitrocellulose-bottomed 96-well plates (MultiScreen MAIP N45; Millipore) were coated with an anti-IFN-γ antibody and nonspecific binding was blocked. 1×10^5 effector cells were added to each well and incubated overnight at 37 °C. The plates were washed two times and biotinylated detection antibody was added. Specific binding was visualized using alkaline phosphatase–avidin together with the respective substrate. The reaction was terminated upon the appearance of dark purple spots, which were quantitated using the AlphaImager System (Alpha Innotech, San Leandro, CA).

Tumor cell challenge assay

DCs transduced with Ad-CPEB4 were resuspended in a 100 μL volume of PBS and injected subcutaneously into C57BL/6 mice. Control groups were injected subcutaneously with either Ad-LacZ transduced DCs (1×10^6) or 1×10^6 DCs alone. At days 7 and 14, the mice were given booster vaccinations using the same protocol as described above. 7 days before the first immunization, C57BL/6 mice were challenged with s.c. injection of 1×10^5 GL261 cells into the left flank to induce primary tumor model. The tumor size and mean lifespan of C57BL/6 mice were observed. The tumor size was measured in two dimensions and calculated as follows: length / 2 × width^2.