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Cross-priming by temozolomide enhances antitumor immunity of dendritic cell vaccination in murine brain tumor model

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Abstract

Although chemotherapy remains among the best treatment options for most cancers, adjuvant therapies such as dendritic cell (DC)-based immunotherapy have been added to treatment protocols to destroy residual tumor cells. IFN-γ secreting T cells specific for survivin was found after temozolomide (TMZ) treatment in C57BL/6 mice intracranial (i.c.) inoculated with GL26 cells. Furthermore, combination treatment with low-dose TMZ (2.5 mg/kg/day, i.p.) chemotherapy followed by vaccination with survivin RNA-transfected DCs (1×10^6 cells/mouse, s.c.) enhanced T cells responses specific for survivin and improved survival rate compared with DC vaccination alone or TMZ treatment alone in tumor inoculated mice. However, these enhancements of T cells responses by TMZ treatment were not observed in mice without tumor inoculation. These results suggested that cross-priming by TMZ may enhance antitumor immunity of DC vaccination in murine brain tumor model.

Keywords: Temozolomide chemotherapy; Dendritic cell-based immunotherapy; Cross-priming

1. Introduction

Malignant glioma is the most common and aggressive primary brain tumor of the central nervous system; it is associated with a very poor prognosis despite progress in conventional therapies such as surgical removal, radiotherapy, and chemotherapy [1]. Therefore, further development and implementation of novel therapeutic strategies for treatment of these tumors are needed. As an alternative therapeutic strategy, dendritic cell (DC)-based immunotherapy, for the treatment of a variety of cancers including malignant glioma, has been observed to stimulate antitumor immune responses [2–4]. However, further development of such immunotherapies is required to improve on consistent tumor destruction and safety while extending the life of cancer patients.

Survivin, a member of the inhibitor of apoptosis protein family, is overexpressed in gliomas [5] and many other tumors [6,7]. Survivin is present during normal fetal development but absent in normal adult tissues with only a few exceptions [8,9]. Therefore, the survivin protein represents a tumor-specific target for the application of vaccination therapies. Recently, DCs pulsed with survivin peptides have been used to induce survivin-specific cytotoxic T lymphocytes (CTLs) in both healthy donor [10], and cancer patients [11]. In other studies, survivin-specific CTLs generated from mice and human individuals have been shown to induce potent cytotoxic activity and antitumor immunity against primary hematological tumor cell lines [12].

Temozolomide (TMZ) is a new generation of alkylating agent shown to be effective for the treatment of malignant

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glioma patients [13,14]. Combination of other therapeutic modality with TMZ chemotherapy has been shown to lead to significant prolongation of life in malignant glioma patients [14] and an animal model with intracranial (i.c.) glioma [15]. Therefore, further investigation of additional therapeutic strategies that combine TMZ chemotherapy and DC-based immunotherapy against malignant glioma may further enhance the survival. Recently, it has been reported that intergrating tumor vaccine with standard cytotoxic chemotherapy could have a profound pharmacodynamic influence on the vaccine-induced antitumor response [16]. These interactions could influence the magnitude, quality, and efficacy of the tumor-specific T cell response, as well as other variables of the immune response.

In the present study, we evaluated the in vivo effects of combined low-dose TMZ chemotherapy and immunotherapy with survivin RNA-transfected DCs, and found that this approach enhanced antitumor immunity by increasing tumor-specific immune responses in an i.c. GL26 glioma model.

2. Materials and methods

2.1. Animals and cell lines

Six to eight-week-old female C57BL/6 (H-2^b) mice were purchased from SLC (Shizuoka, Japan). The murine glioma cell line GL26 (H-2^b) was kindly provided by Dr. John S. Yu (Cedars Sinai Medical Center, Los Angeles, CA, USA). The GL26 cells were cultured in DMEM (Gibco BRL Co., Grand Island, NY, USA) supplemented with 10% heat-inactivated FBS (Gibco BRL), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin. The overexpression of survivin in GL26 cells was confirmed by Western blot analysis (Kim et al., in preparation and unpublished data).

2.2. Generation of survivin RNA in vitro transcription

The pET15b-moSur vector was linearized with *Hin*dIII and in vitro transcription was performed with the mMessage mMacine Ultra T7 Kit (Ambion, Austin, TX, USA) according to the manufacture's instructions. The mRNA concentration and quality were assessed by spectrophotometry and agarose gel electrophoresis. RNA samples were routinely evaluated by formaldehyde/agarose gel electrophoresis for size and integrity and stored at −70 °C.

2.3. Generation of bone marrow-derived DCs

The DCs were prepared from bone marrow as described previously, with minor modifications [17]. In brief, bone marrow cells were harvested from tibias and femurs of normal C57BL/6 mice. The cells were washed twice in serum-free RPMI 1640 (Gibco BRL) medium and cultured in six-well culture plates at 5×10^6 cells/well in complete RPMI 1640 medium supplemented with 10% heat-inactivated FBS, recombinant murine GM-CSF (20 ng/ml) (R&D Systems, Minneapolis, MN, USA) and recombinant murine IL-4 (20 ng/ml) (R&D Systems). On day 2, non-adherent granulocytes were gently removed and fresh medium with GM-CSF and IL-4 was added. On day 7, non-adherent and loosely adherent cells obtained from these cultures were considered to be immature bone marrow-derived DCs.

2.4. Preparation of DCs and electroporation

Prior to electroporation on day 6, immature DCs were washed twice with serum-free medium and resuspended to a final concentration of 1×10^7 cells/ml. The cell suspension (250 μl) was preincubated in a 0.2 cm gap electroporation cuvette for 5 min on ice. Next, 20 μg of RNA was added and cells were pulsed with ECM 830 electroporator (BTX, San Diego, CA, USA). The physical parameters were voltage of 300 V and pulsed time of 50 ms. After electroporation, the cells were immediately transferred into mature medium and incubated for 24 h at 37 °C. The viability of the cells was more than 80% after electroporation. The transfection efficacy of electroporation on DCs was determined with EGFP RNA by FACS analysis [32].

2.5. In vivo antitumor effect of TMZ

An intracranial tumor model was used as described previously [18]. In brief, the mice were anesthetized by intraperitoneal (i.p.) injection of xylazine (Rompun; Cutter Laboratories, Shawnee, KS, USA) 12 mg/kg and ketamine (Ketalar; Parke-Davis & Co., Morris Plains, NJ, USA) 30 mg/kg. The mice were placed in a stereotactic frame with ear bars. Then, 1×10^4 GL26 cells suspended in 3 μl of PBS were stereotactically injected through an entry site at the bregma 2 mm to the right of the sagittal suture and 3 mm below the surface of the skull of anesthetized C57BL/6 mice with a sterile Hamilton syringe fitted with a 26 gauge needle. Mice were treated i.p. with TMZ (2.5 mg/kg/day, 5 mg/kg/day, and 10 mg/kg/day) from 2 to 6 days after the i.c. GL26 cell inoculation. Five mice in each group were used for the experiments.

2.6. In vivo antitumor effect of combined treatment

The GL26 i.c. model was used for experiments as described previously [17]. Mice were treated i.p. with TMZ (2.5 mg/kg/day) from 2 to 6 days and subcutaneously (s.c.) with DCs (1×10^6 cells/mouse in 200 μl of PBS) or survivin RNA-transfected DCs (1×10^6 cells/mouse in 200 μl of PBS) on day 13, 20, and 27 after i.c. GL26 cell inoculation. Six groups were studied (control PBS, DCs alone, TMZ alone, survivin RNA-transfected DCs [Surv.RNA/DCs], combined TMZ and unpulsed DCs injection [TMZ + DCs], and combined TMZ and survivin