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Direct vaccination with pseudotype baculovirus expressing murine telomerase induces anti-tumor immunity comparable with RNA-electroporated dendritic cells in a murine glioma model

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Abstract

Baculovirus pseudotyped with vesicular stomatitis virus G protein (Bac-VSV-G) was found to efficiently transduce and express transgenes on mammalian cells. In this study, this recombinant virus was used for induction of anti-tumor immunity against murine telomerase reverse transcriptase (mTERT) and was compared with RNA-electroporated dendritic cells (DCs) in a murine glioma model. Splenocytes from the mice vaccinated with Bac-VSV-G expressing mTERT (Bac-VSVG-mTERT) showed significantly increased numbers of mTERT-specific IFN-γ-secreting T cells using an ELISPOT technique, and also showed increased NK cell activity. In addition, the TERT-specific T cells activated by Bac-VSVG-mTERT and mTERT RNA-electroporated DCs were predominantly $CD4^+$ T cells and $CD8^+$ T cells, respectively. The protective anti-tumor effect of Bac-VSVG-mTERT was similar to that of mTERT RNA-electroporated DCs. These results suggest that the pseudotype baculovirus expressing TERT may be a good candidate for a genetic vaccine for use in the treatment of malignant gliomas.

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Keywords: Pseudotype baculovirus; VSV G; Gene therapy; Glioma; Cytotoxic T cells; Anti-tumor immunity

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1. Introduction

Malignant gliomas are the most common primary brain tumors found in the adult central nervous system [1]. The prognosis for patients who are diagnosed with a high-grade glioma remains very poor regardless of the treatments used, which include surgical removal, radiotherapy, and chemotherapy [2]. As an alternative therapeutic strategy, immunotherapy is used to induce an anti-tumor immune response for a variety of cancers, including malignant glioma [3,4]. However, finding a successful treatment using immunotherapy requires further investigation and development to improve levels of consistent tumor destruction, extend life span, and provide an effective and safe alternative treatment option for cancer patients.

Several studies have reported that tumor antigens such as MART-1, gp 100, and proteins from the MAGE family could be used as targets for cancer immunotherapy in a murine glioma model [5,6]. TERT protein has recently been described as a "universal" tumor antigen for cancer immunotherapy because approximately 90% of malignant tumors of various origins express TERT, whereas most normal tissues do not [7]. Immunization with hTERT mRNA-transfected DCs has been shown to induce tumor-specific cytotoxic T lymphocytes (CTL) responses, and inhibited the growth of unrelated tumors in a tumor-bearing mouse model [8] and in cancer patients with advanced disease [9]. Recently, hTERT gene-modified DCs using recombinant adenovirus were reported to be capable of generating a specific CTL response against a variety of human TERT-expressing cancer cell lines [10].

As an alternative system of gene delivery, pseudotype baculovirus has been shown to be capable of transferring and expressing foreign genes in mammalian cells [11] as well as in animal models [12]. The potential advantages for this vector in mammalian gene transfer include: easy manipulation, high recombinant viral titers, simple scale-up, a large DNA insertion capacity, lack of replication in mammalian cells, and lack of toxicity [13].

In the present study, anti-tumor immunity was examined in mice vaccinated with pseudotype baculovirus expressing mTERT and compared with TERT RNA-transfected DCs.

2. Materials and methods

2.1. Animals and cell culture

Female C57BL/6(H-2K^b) mice aged 6–8 weeks were purchased from Japan SLC (Shizuoka, Japan). A murine (C57BL/6) glioma cell line, GL26, was obtained from Dr. John S. Yu (Cedars Sinai Medical Center, Los Angeles, CA). The YAC-1 lymphoma cell line was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cell lines were cultured in complete RPMI 1640 medium supplemented with 10% heated-inactivated fetal bovine serum (FBS), 10 mM Hepes, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 5×10^{-5} M^2. The insect cell line, Sf9, was grown in Grace's medium (Invitrogen, USA) supplemented with 10% FBS (HyClone, USA).

2.2. Pseudotype baculovirus

Pseudotype baculoviruses were constructed as described previously [14]. The baculovirus plasmid, Bac-GFP, was generated by inserting an expression cassette into the standard baculovirus transfer vector, pBlueBac4.5 (Invitrogen). First, the VSV-G gene was excised as a 1665-bp *Xho*I/*Eco*RI fragment from the full-length VSV gene cDNA. This fragment was inserted into the *Xho*I/*Eco*RI site of pBlueBac4.5 in direct orientation with respect to the polyhedrin promoter to create pBlueBacG. In order to place the mTERT gene and GFP genes under the control of the CMV promoter, EGFP and mTERT genes were subsequently inserted at the *Sal*I/*Xho*I and *Eco*RI sites of pCEP4 (Invitrogen). *Sal*I fragments (CMV promoter cassette) from pCEP4-VSVG-EGFP and pCEP4-VSVG-mTERT were cloned into *Sna*BI-digested pBlueBacG, generating Bac-VSVG-EGFP and Bac-VSVG-mTERT (Fig. 1A). The recombinant viruses were generated using a Bac-To-Bac Baculovirus Expression System (Gibco-BRL). The virus was also generated using the Bac-to-Bac system (Invitrogen). The virus was further amplified by propagation in Sf9 (*Spodoptera frugiperda*) cells grown in a suspension of Grace's supplemented insect media containing 10% (vol/vol) FBS, 0.1% (vol/vol) pluronic F-68, and 25 mg/ml gentamycin, according to standard protocols. Stocks of virus were concentrated by centrifugation at 35,000*g* for 60 min, and the pelleted virus was resuspended in Dulbecco's PBS supplemented with 1% (vol/vol) FBS. The virus titers were determined by a plaque assay on Sf9 cells.

2.3. RT-PCR analysis of the expression of survivin in GL 26 cells

RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Genomic DNA was checked by PCR without reverse transcriptase as

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