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Synergistic antitumor efficacy of combined DNA vaccines targeting tumor cells and angiogenesis

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ABSTRACT

To further enhance the antitumor efficacy of DNA vaccine, we proposed a synergistic strategy that targeted tumor cells and angiogenesis simultaneously. In this study, a Semliki Forest Virus (SFV) replicon DNA vaccine expressing 1–4 domains of murine VEGFR2 and IL12 was constructed, and was named pSVK-VEGFR2-GFc-IL12 (CAVE). The expression of VEGFR2 antigen and IL12 adjuvant molecule in 293T cells in vitro were verified by western blot and enzyme-linked immune sorbent assay (ELISA). Then CAVE was co-immunized with CAVA, a SFV replicon DNA vaccine targeting survivin and β-hCG antigens constructed previously. The antitumor efficacy of our combined replicon vaccines was evaluated in mice model and the possible mechanism was further investigated. The combined vaccines could elicit efficient humoral and cellular immune responses against survivin, β-hCG and VEGFR2 simultaneously. Compared with CAVE or CAVA vaccine alone, the combined vaccines inhibited the tumor growth and improved the survival rate in B16 melanoma mice model more effectively. Furthermore, the intratumoral microvessel density was lowest in combined vaccines group than CAVE or CAVA alone group. Therefore, this synergistic strategy of DNA vaccines for tumor treatment results in an increased antitumor efficacy, and may be more suitable for translation to future research and clinic.

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1. Introduction

Despite of the development of medicine and the improvement of lifespan of people, cancer is still the most serious medical problem, which is a leading cause of death [1]. Many malignant tumors are diagnosed at the advanced stage, and patients don't have the opportunity to receive radical treatment. The standard therapies such as chemotherapy and radiotherapy have limited efficacy for advanced malignant tumors but with severe side effects. So the development of novel treatment approach is required.

Over the past few years, tumor associated antigen (TAA) specific DNA vaccine has become a promising immunotherapy for cancer [2,3]. It has the potency to induce specifically humoral and cellular immune response in vivo [4], which could eradicate systemic tumor cells and control the potential metastases. Besides, DNA vaccines also have the advantages of safety, stability, low-costs, and ease of preparation. Therefore DNA vaccine is an ideal approach for cancer therapy.

However, many DNA vaccines targeting TAA that exhibit efficient antitumor activity in mice, don't exhibit correlated antitumor efficacy in large animals or humans [3]. Some tumor cells may avoid immune surveillance through malignant transformation or immune editing [5,6]. So single DNA vaccine targeting TAA alone may be not enough to eradicate the tumor cells. This challenge may be overcome by combining another antitumor approach.

The angiogenesis plays an important role in tumor growth, invasion and metastasis [7]. Without angiogenesis, tumor would not growth more than 2 mm. As a result, anti-angiogenesis has been already a well-recognized strategy for tumor therapy. Vascular endothelial growth factor (VEGF) is the most potent angiogenic cytokine, which is induced in tumor cells due to hypoxia [8]. And vascular endothelial growth factor receptor 2 (VEGFR2), which is a

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major tyrosine kinase receptor for VEGF, mediates most angiogenic effects of VEGF. Meanwhile, it is mainly expressed in newborn vascular endothelial cells in tumor tissue, which makes VEGFR2 be an ideal antitumor target [9].

Recently we developed a Semliki Forest Virus (SFV) replicon DNA vaccine that targeted surviving and β-hCG antigens [10], and we named it CAVA. This DNA vaccine exhibited a promising antitumor effect in mice model. However, in our subsequent experiment, the antitumor efficacy was found to be unsatisfactory.

To further improve the antitumor efficacy of DNA vaccine, we proposed a synergistic antitumor strategy, which consists of targeting tumor cells and anti-angiogenesis simultaneously. Therefore in this study, we constructed a recombinant SFV replicon DNA vaccine expressing 1–4 domains of murine VEGFR2, which was intended to be co-immunized with CAVA. Then the antitumor efficacy of our combined replicon vaccines was evaluated in mice model and the possible mechanism was further investigated.

2. Material and methods

2.1. Mice and cell lines

Female C57BL/6 mice (6–8 weeks old) were purchased from Beijing Experimental Animal Center (Beijing, China) and were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (National Institute of Health publication no.85-23, Revised 1996). Animal experiment procedure in this study were approved by the Animal Ethics Committee of Beijing Institute of Basic Medical Sciences.

B16F10-β-hCG melanoma cell line (C57BL/6), which stably expressed human β-hCG antigen, was established previously in our laboratory [10]. 293T cells were obtained from ATCC (Rockville, MD). All cells were cultured in RPMI 1640 medium (Gibco-BRL, Gaitherburg, MD, USA) supplemented with 10% fetal calf serum, penicillin (100 U/mL) and streptomycin (100 U/mL), and they were maintained at 37 °C, 5% CO_2 in a humidified incubator.

2.2. Construction of DNA replicon vaccine

The plasmid DNA replicon vaccine CAVA was constructed and researched in our previous study [10]. In this study we constructed the DNA replicon vaccine pSVK-VEGFR2-GFc-IL12, which was named CAVE. The schematic representation of CAVE vaccine was shown in Fig. 1C. Briefly, the VEGFR2-GFc-IL12 gene fragment was obtained by double enzyme digestion from plasmid pVAX1-VEGFR2-GFc-IL12, which was constructed in previous study. Then the DNA fragment was inserted into the replicon vector pSVK to create pSVK-VEGFR2-GFc-IL12 (CAVE). In this recombinant plasmid, the gene sequences of Fc fragment of human IgG1 and glycosyl phosphatidyl inositol (GPI) signal peptide were fused to VEGFR2. And internal ribosome entry site (IRES) and IL-12 were then fused to the end of GPI. Both CAVA and CAVE plasmids were prepared by Endotoxin free Giga kit (Qiagen, Shanghai, China). 293T cells were transfected with CAVE plasmids, and the expression of mVEGFR and IL-12 were identified by western blot and ELISA respectively.

2.3. Plasmids DNA vaccines immunization in C57BL/6 mice

Female C57BL/6 mice aged 6–8 weeks were randomly divided into 5 groups (5 mice per group). Plasmids dosage used for each vaccination was explored and optimized by preliminary experiments. The five groups of mice were vaccinated three times at 10-day intervals with 100 μL PBS, 100 μL (0.1 μg/μL) pSVK-vector, 100 μL (0.1 μg/μL) CAVA, 100 μL (0.1 μg/μL) CAVE and 100 μL (0.1 μg/μL CAVA plus 0.1 μg/μL CAVE) combined vaccines

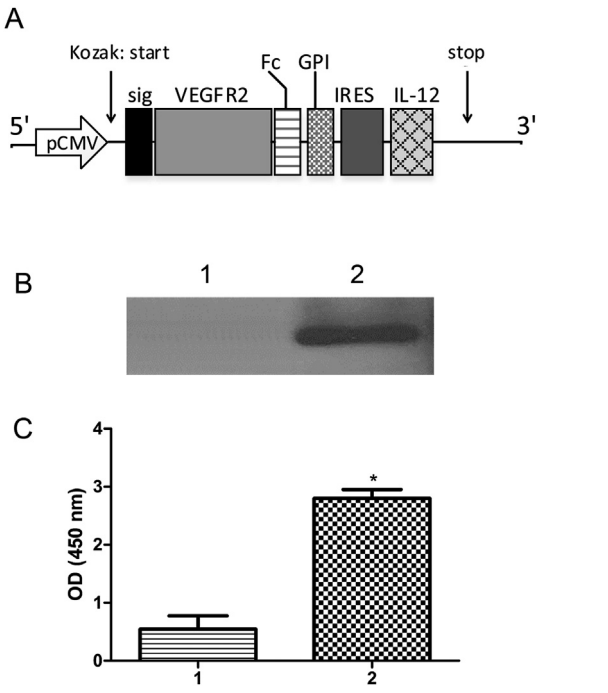

Fig. 1. Construction and expression of DNA-based replicon vaccine pSVK-VEGFR2-GFc-IL12 (CAVE). (A) Schematic illustration of the vaccine CAVE. (B) The expression of mVEGFR2 gene in 293T cells transfected with CAVE plasmids was detected by western blot with anti-mVEGFR2 antibody; 1: 293T cells transfected with pSVK-vector, 2: 293T cells transfected with CAVE plasmids. (C) The expression of IL-12 gene in 293T cells transfected with CAVE plasmids was detected by ELISA assay; 1: the supernatant of 293T cells transfected with pSVK-vector, 2: the supernatant of 293T cells transfected with CAVE plasmids.

respectively via intramuscular injection followed by electroporation stimulation. Two weeks after the last vaccination, bloods and spleens of each group of mice were collected for the evaluation of humoral and cellular immune activity.

2.4. Antibody detection

Anti-suvivin, anti-β-hCG and anti-VEGFR2 antibodies were detected by enzyme-linked immune sorbent assay (ELISA). 96-well plates were coated with purified survivin, β-hCG and VEGFR2 protein (0.25 μg/well) respectively overnight at 4 °C. The blood was collected at 2nd and 4th weeks after the last vaccination. The isolated serum was diluted at 1:100 and incubated in the precoated plates for 2 h at 37 °C. Then the plates were washed with PBST (0.05% Tween 20 in PBS) and blocked with 1% bovine serum albumin for 1 h at 37 °C. HRP-conjugated goat anti-mouse IgG (dilution 1:5000) was added into the plates (100 μL/well), and the plates were incubated for 1 h at 37 °C. Finally, the antibody titers were detected using TMB system and the OD values were measured at 492 nm by a Bio-Rad plate reader.

2.5. IFN-γ ELISPOT assay

To evaluate cellular immune responses to specific antigen in vaccinated mice, splenic lymphocytes were tested for IFN-γ secretion using enzyme-linked immunospot assay (ELISPOT). First, spleen cells were harvested two weeks after the last immunization. Then RBC were lysed and the spleenic lymphocytes were isolated by density gradient centrifugation method using lymphocyte isolation liquid (TIAN JIN HAO YANG Biological Manufactory Co. Ltd, China). The isolated lymphocytes were suspended in 10% FCS/RPMI

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