Biomedicine & Pharmacotherapy 90 (2017) 147–152

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Original article

Enhance the anti-renca carcinoma effect of a DNA vaccine targeting G250 gene by co-expression with cytotoxic T-lymphocyte associated antigen-4(CTLA-4)

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ARTICLE INFO

Article history:
Received 31 October 2016

Keywords:
Renal carcinoma
DNA vaccine
G250
CTLA-4

ABSTRACT

Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) is a negative regulator of T cell activation, which competes with CD28 for B7.1/B7.2 binding with a greater affinity. Co-expression specific antigens and extracellular domain of CTLA4 represents a promising approach to increase the immunogenicity of DNA vaccines. In this study, we evaluated this interesting approach for its enhancement on G250/MN/CA IX (G250)-specific immune responses and its anti-tumor effects in renal carcinoma mice model. Consequently, we constructed a DNA vaccine containing the G250 and the CTLA-4 gene. Vaccination with the co-expression DNA not only induced much higher level of anti-CTLA4 and anti-G250 antibody, but also increased G250-specific T cell response in mice. To evaluate the anti-tumor efficacy of the plasmids, murine models with G250-expressing tumors were generated. After injection into the tumor-bearing mouse model, the plasmid carrying the co-expression gene of CTLA4 and G250 showed stronger inhibition of tumor growth than the plasmid expressing CTLA4 or G250 alone. These observations emphasize the potential of the CTLA4 and G250 co-expression DNA vaccine, which could represent a promising approach for tumor immunotherapy.

1. Introduction

Kidney cancer accounts for 2.5% of all cancers, with an estimated 62,700 new cases and 14,240 estimated deaths in 2016 [1]. The major histologic subtype (80% of cases) is clear cell renal cell carcinoma (ccRCC) followed by papillary subtype 1 and 2, Bellini tumor and chromophobe carcinoma. ccRCC is a chemo-resistant tumor probably because the cells derive from the luminal cells of proximal tubules that express a high intrinsic level of multidrug resistance (MDR-1) protein [2]. RCC is not sensitive to radiotherapy and chemotherapy [3]. Since RCC is a highly immunogenic tumor, advances in molecular and immune biology allow for the potential use of tumor vaccines as immune therapy.

The research showed that the expression of tumor specific antigen G250 reached 90% in renal carcinoma, and in renal clear cell carcinoma is to reach more than 95%. Most of the other normal renal tissues and normal tissues were not detected the expression of G250 (only a small amount of expression in gastric mucosa and bile duct epithelial cells). G250 antigen is highly expressed in renal cell carcinoma, and its expression in normal tissue is very little, which shows an apparent renal cell carcinoma specificity [4]. Human G250 protein, composed of 459 amino acids, in which 38–414 amino acid (AA) constitute extracellular domain, provides the ideal targets for immune therapy of renal cell carcinoma, with high efficiency and low toxicity of dual advantages [5]. So far, there have been many kinds of research on renal cell carcinoma vaccine and immunotherapy targeted G250, including anti G250 antibodies, peptide vaccine, G250 antigen loading DC cells and DNA vaccine [6].

DNA vaccine constructs have been evaluated and tested in numerous human clinical trials for generating an immune response to various diseases, including infectious, allergic and autoimmune diseases, as well as cancers [7,8]. Unfortunately, the immunogenicity of plasmid DNA in humans has proven to be modest as compared with the immunogenicity observed in other species treated using microbial expression vectors. A number of approaches have been tested to overcome this limitation and to

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http://dx.doi.org/10.1016/j.biopha.2017.03.015
0753-3322/

enhance the immunogenicity and efficacy of DNA vaccines [9,10]. Some of these approaches include improved plasmid design, immunomodulatory and immune enhancing molecules, prime-boost administration, and strategies to break the immunosuppressive networks.

Co-expression of antigens with extracellular domain of CTLA-4 has been proven to be a potential approach to enhance the immunogenicity of DNA vaccine, especially in large animals [11–13]. Boyle et al. demonstrated that a fusion DNA vaccine containing the extracellular domain of CTLA-4 was able to induce both robust antibody response and T cell response [13].

In the present study, we evaluated this interesting approach for its enhancement on G250/MN/CA IX (G250)-specific immune responses and its anti-tumor effects in renal carcinoma mice model [6,14]

2. Materials and methods

2.1. Cell lines and mice

The renca carcinoma cell line (RenCa, from BALB/c mice) was purchased from the Shanghai Cell Institute (Shanghai, China). To generate a cell line that stably expressed human G250 (NCBI Reference Sequence: NM_001216.2), renca cells were transfected with a plasmid carrying G250 (pIRES-neo-G250), and then the transfectants were subjected to selection by treatment with G418. This cell line is hereafter referred to as renca-G250, and western blot was performed to confirm the expression of G250 in the renca-G250 by using the anti-G250 antibody (Abcam, ab107257)

BALB/c female mice, aged between 6 and 8 weeks. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee of the Chengdu Military General Hospital.

2.2. Construction and expression of DNA vaccines

Based on the coding regions of G250 (NCBI Reference Sequence: NM_001216.2) and CTLA-4 (GenBank: BC074893.2), we designed a G250 (coding gene 43-1422) and CTLA-4 (coding gene 32-703) co-expression gene. The G250 gene was connected to the CTLA-4 gene by Furin-2A (F2A). Synthesis of the co-expression gene was carried out by Invitrogen. The co-expression gene was cloned into the expression vector pVAX1. This resulted in the eukaryotic expression plasmid pVAX1-G250-F2A-CTLA-4, which was transiently transfected into 293T cells, and flow cytometry and confocal microscopy was performed to confirm the validity of these constructs by using the FITC labeled anti-G250(R&D, FAB2188F) and PE labeled anti-CTLA-4 antibody(GeneTex, GTX75284), respectively.

2.3. DNA vaccination and in vivo tumor treatment experiments

The experimental mice were randomly divided into 5 groups ($n = 8$): the blank control group, the empty vector pVAX1 group, the pVAX1-G250 group, pVAX1-CTLA-4 and the pVAX1-G250-F2A-CTLA-4 group. 1 week before the first vaccination, the mice were subcutaneously inoculated with renca-G250 cells (1×10^5) in the right flank. Each mouse ($n = 5$–8/group) was injected with 50 μg of plasmid and subjected to electroporation. On the 10th day and the 20th day after the first immunization, the mice were given an immune boost. Tumor development was monitored every two days in individual mice. The parameters of tumor growth

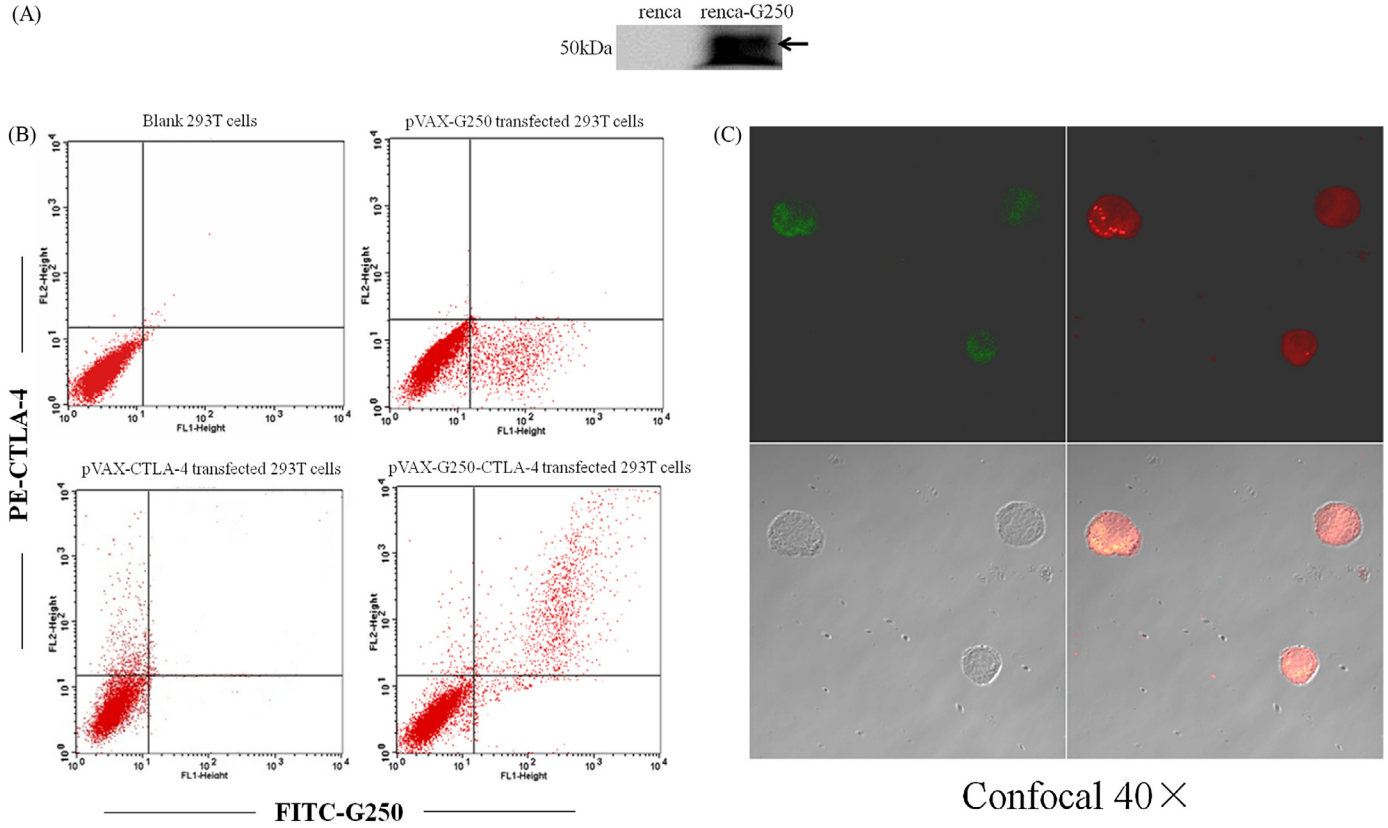

Fig. 1. The expression of G250 in renca-G250 cells and the expression of plasmid DNA vaccine in 293T cells. Western bolt, flow cytometry and confocal microscopy was used to examine the expression. (A) The expression of G250 in renca-G250 cells. (B) The expression of plasmid was studied by flow cytometry. (C) The co-expression of G250 and CTLA-4 was studied by confocal microscopy.