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Characterization of DNA vaccines encoding the domains of calreticulin for their ability to elicit tumor-specific immunity and antiangiogenesis

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Abstract

Antigen-specific cancer immunotherapy and antiangiogenesis are feasible strategies for cancer therapy because they can potentially treat systemic tumors at multiple sites in the body while discriminating between neoplastic and non-neoplastic cells. We have previously developed a DNA vaccine encoding calreticulin (CRT) linked to human papillomavirus-16 E7 and have found that this vaccine generates strong E7-specific antitumor immunity and antiangiogenic effects in vaccinated mice. In this study, we characterized the domains of CRT to produce E7-specific antitumor immunity and antiangiogenic effects by generating DNA vaccines encoding each of the three domains of CRT (N, P, and C domains) linked to the HPV-16 E7 antigen. We found that C57BL/6 mice vaccinated intradermally with DNA encoding the N domain of CRT (NCRT), the P domain of CRT (PCRT), or the C domain of CRT (CCRT) linked with E7 exhibited significant increases in E7-specific $CD8^+$ T cell precursors and impressive antitumor effects against E7-expressing tumors compared to mice vaccinated with wild-type E7 DNA. In addition, the N domain of CRT also showed antiangiogenic properties that might have contributed to the antitumor effect of NCRT/E7. Thus, the N domain of CRT can be linked to a tumor antigen in a DNA vaccine to generate both antigen-specific immunity and antiangiogenic effects for cancer therapy.

Keywords: Calreticulin; DNA vaccine; NCRT; CCRT; PCRT; HPV-16; E7 antigen

1. Introduction

Antigen-specific cancer immunotherapy and antiangiogenesis are feasible strategies for cancer therapy because they are able to treat systemic tumors at multiple sites in the body while discriminating between neoplastic and non-neoplastic cells. Activation of antigen-specific T cell-mediated immune responses allows for killing of tumors associated with a specific antigen [1,2] while inhibition of angiogenesis controls neoplastic growth by sequestering neoplastic cells from an adequate blood supply [3,4]. Therefore, an innovative approach that combines both mechanisms will likely generate the most potent antitumor effect.

We have previously combined tumor-specific immunity and antiangiogenesis in an innovative DNA vaccine strategy

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encoding calreticulin (CRT) linked to model antigen HPV-16 E7 [5]. CRT is an abundant 46 kDa Ca^{2+}-binding protein located in the endoplasmic reticulum (ER) [6]. The protein has been shown to associate with peptides delivered into the ER by transporters associated with antigen processing (TAP-1 and TAP-2) [7] and with MHC class I-β2 microglobulin molecules to aid in antigen presentation [8]. Previous studies have shown that CRT can be complexed with peptides in vitro to elicit peptide-specific $CD8^+$ T cell responses through exogenous administration [9]. Recently, full-length calreticulin has been reported to be an endothelial cell inhibitor and exerts antitumor effects in vivo via antiangiogenesis [10–12]).

The CRT protein is composed of three domains, the N domain, P domain and C domain. The N domain (residues 1–180), also known as vasostatin, is extremely conserved among calreticulins from different species [13]. The N domain interacts with the DNA-binding domain of the glucocorticoid receptor in vitro [14], with rubella virus RNA [15], with α-integrin [16], and with protein disulphide-isomerase (PDI) and ER protein 57 (ERp57) [17]. The N domain of calreticulin also inhibits proliferation of endothelial cells and suppresses angiogenesis [10]. The P domain (residues 181–280) is rich in proline and contains two sets of three sequence repeats. This region of the protein binds Ca^{2+} with high affinity [18]. The P domain is thought to be critical for the lectin-like chaperone activity of calreticulin [19]. The P domain of calreticulin also interacts with PDI [17] and perforin [20,21]. The C-terminal region of the protein is highly acidic and terminates with the KDEL ER retrieval sequence [22]. This C domain of CRT binds to calcium [18] and to blood-clotting factors [23] and inhibits injury-induced restenosis [24].

In the present study, we investigated DNA vaccines encoding each of the N, P, and C domains of calreticulin chimerically linked to HPV-16 E7 for their abilities to elicit antigen-specific $CD8^+$ T cell responses and antitumor immunity in vaccinated mice. We found that C57BL/6 mice vaccinated intradermally with NCRT/E7, PCRT/E7 or CCRT/E7 DNA exhibited significant increases in E7-specific $CD8^+$ T cell precursors and impressive antitumor effects against E7-expressing tumors compared with mice vaccinated with wild-type E7 DNA. We also determined that the N domain of calreticulin (NCRT) resulted in antiangiogenic antitumor effects. Thus, cancer therapy using NCRT linked to a tumor antigen holds promise for treating tumors through a combination of antigen-specific immunotherapy and antiangiogenesis.

2. Materials and methods

2.1. Plasmid DNA constructs and preparation

The generation of pcDNA3-E7 has been described previously [5,25]. The generation of pcDNA3-CRT has also been described previously [5]. There is more than 90% homology between rabbit, human, mouse, and rat CRT [26]. For the generation of pcDNA3-NCRT, DNA encoding the N domain of CRT, NCRT was first amplified with PCR by using rabbit CRT cDNA as the template [27] and a set of primers, 5′-CCGG-TCTAGAATGCTGCTCCCTGTGCCGCT-3′ and 5′-CCC-GAATTCGTTGTCCGGCCGCACGATCA-3′. The amplified product was further cloned into the *Xba*1/*Eco*RI site of pcDNA3 (Invitrogen Corp., Carlsbad, CA, USA). For the generation of pcDNA3-PCRT, DNA encoding the P domain of CRT was first amplified with PCR using rabbit CRT cDNA as the template and a set of primers, 5′-TGCTCTAGAAC-GTATGAGGTGAAGATTGA-3′ and 5′-CCGGAATTCGG-GGTTCTGAATCACCGGC-3′. The amplified product was further cloned into the *Xba*I/*Eco*RI site of pcDNA3. For the generation of pcDNA3-CCRT, DNA encoding the C domain of CRT was first amplified with PCR using rabbit CRT cDNA as the template and a set of primers, 5′-TGCTCTAGAGA-GTACAAGGGTGAGTGGAAGC-3′ and 5′-CCGGAATTC-CAGCTCGTCCTTGGCCTGGC-3′. The completed product was then cloned into the *Xba*I/*Eco*RI site of pcDNA3. For the generation of pcDNA3-NCRT/E7, PCRT/E7, and CCRT/E7, E7 was first amplified with pcDNA3-E7 as a template and a set of primers, 5′-GGGGAATTCATGGAGATACACCTA-3′ and 5′-GGTGGATCCTTGAGAACAGATGG-3′, and then cloned it into the *Eco*RI/*Bam*HI sites of pcDNA3-NCRT, pcDNA3-PCRT, or pcDNA3-CCRT to generate pcDNA3-NCRT/E7, pcDNA3-PCRT/E7, or pcDNA3-CCRT/E7.

2.2. Cell line

The production and maintenance of TC-1 cells have been described previously [28]. In brief, HPV-16 E6, E7 and *ras* oncogene were used to transform primary C57BL/6 mice lung epithelial cells to generate TC-1.

2.3. DNA vaccination

Preparation of DNA-coated gold particles and gene gun particle-mediated DNA vaccinations were performed using a helium-driven gene gun according to a protocol described previously with some modifications [25]. Gene gun particle-mediated DNA vaccinations were performed using a Low Pressure-accelerated Gene Gun (BioWare Technologies Co. Ltd., Taipei, Taiwan). The gold particles (Bio-Rad 1652263) were weighted and suspended in 70% ethanol. This suspension was vortexed vigorously and then centrifuged to collect the particles. After washing by distilled water three times, the collected particles were resuspended in DNA solution (1 μg DNA per mg gold particles), vortexed and sonicated for a few seconds, and then added 2.5 M $CaCl_2$ and 0.05 M spermidine solution with vortex. This solution was kept on ice for 10 min and the DNA-coated gold particles were collected and washed by 100% ethanol three times. Finally, the particles were resuspended in 100% ethanol with appropriate concentration and used to make bullets. Control plasmid (no insert), E7, NCRT, NCRT/E7, PCRT/E7, CCRT/E7, or CRT/E7 DNA-coated gold particles were delivered to the shaved