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Induction of immune response and anti-tumor activities in mice with a DNA vaccine encoding human mucin 1 variable-number tandem repeats

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Summary Mucin 1 (MUC1) is a tumor-associated antigen that carries the important variable-number tandem repeat (VNTR) epitopes for inducing cytotoxic T lymphocytes. Such a property makes MUC1 VNTR potentially attractive for immunotherapy. This study explored the possibility of developing an efficient anti-tumor vaccine strategy using the specific antitumor immunity induced by the MUC1 VNTR DNA vaccine combined with the adjuvant effect of a plasmid expressing murine interleukin-2 (IL-2). The results showed that the MUC1 VNTR DNA vaccine successfully induced both humoral and cellular immune responses against MUC1 VNTR in mice. The effect could be obviously enhanced by increasing the number of tandem repeats, the number of immunizations, and by co-administration of the cytokine plasmid. The growth of MUC1-expressing ($MUC1^+$) tumors was significantly inhibited in mice immunized with the MUC1 VNTR DNA vaccine combined with the IL-2 plasmid, both before and after tumor challenge. A much larger percentage of the immunized mice survived tumor challenge than the non-immunized mice. The combination of the MUC1 VNTR DNA vaccine and the IL-2 adjuvant plasmid provides an attractive alternative for prophylactic and therapeutic vaccinations against $MUC1^+$ tumors.

Introduction

Mucin 1 (MUC1) is a suggested target for the development of vaccines in cancer research because of the differences between normal and tumor-associated MUC1 [1,2]. MUC1 is a type I transmembrane protein containing a variable number of tandem repeats (VNTR; 20–120 repeats) of a 20-amino acid sequence in its extracellular domain. The repetitive sequence of the VNTR is VTSAPDTRPAPGSTAPPAHG. In normal cells, VNTR is heavily glycosylated at the threonine and serine residues, with up to 70% carbohydrates by weight. However malignant cells contain underglycosylated VNTR domains that are overexpressed in 90% of all adenocarcinomas, including breast, lung, pancreatic, prostate, stomach, colon, and ovarian cancers. The overexpression of MUC1 and underglycosylation of its VNTR make hypoglycosylated and non-glycosylated VNTR-derived epitopes potential targets for immunodiagnostics and immune interventions [3–8].

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ABBREVIATIONS

A9G	APDTRPAPG
CTL	cytotoxic T lymphocyte
H20A	HGVTSAPDTRPAPGSTAPPA
IL	interleukin
LDH	lactate dehydrogenase
LLC	murine Lewis lung carcinoma
MHC	major histocompatibility complex
MUC1	mucin 1
$MUC1^+$	LLC MUC1 VNTR- expressing LLC
nm	multirepeats of MUC1 VNTR (n = 2 and 33)
VNTR	variable-number tandem repeats

The analysis of immune responses in cancer patients with various adenocarcinomas suggested that the presence of a low titer of anti-MUC1 antibodies [9–11] and a low frequency of MUC1-specific CTLs [12–14] are not sufficient to eradicate growing tumors. Immunotherapy targeting the tandem repeat would be useful to enhance pre-existing weak immune responses against MUC1 or to induce anti-MUC1 immunity. The induction of MUC1-specific immune responses has already been previously reported in mice and human beings. Reports include vaccination with peptides corresponding to the MUC1 tandem repeat [15–17], proteins/fusion proteins [18], MUC1 recombinant viruses [19,20], dendritic cells, and dendritic cell/tumor cell fusions [21–23]. Many of the approaches targeting MUC1 showed that MUC1-specific immunodominant B- and T-cell epitopes are derived from the VNTR region [12,13,16,24,25]. Some of the MUC1 VNTR-based vaccines such as the peptide vaccines have been used in clinical trials [16,26,27]. However, although MUC1-specific antibodies and/or CTLs were detected in some patients, it was clear that the induction of clinically effective anti-tumor immune responses remained to be achieved. Clinical trials with MUC1 showed that MUC1 is a relatively poor immunogen in human beings. Consequently, the development of new vaccination protocols that can reproducibly induce strong anti-tumor immune responses in human beings is a vital concern.

DNA vaccines provide prolonged antigen expression, leading to amplification of immune responses, and induction of memory responses against weakly immunogenic tumor-associated antigens (TAA). DNA vaccination represents an exceptionally potent strategy, which can activate both cellular and humoral immune responses as the encoded antigen is processed through both endogenous and exogenous pathways, and its peptide epitopes generated by proteolysis in antigen presenting cells are presented by major histocompatibility complex (MHC) classes I and II [28,29]. Studies with animal models [30–35] and clinical trials [36,37] showed that DNA vaccination can induce specific protective immunity against viral or neoplastic cell challenge. Reports of MUC1 DNA vaccines are relatively less available, and most vaccines have been constructed with the full-length MUC1 cDNA [30–33]. One report showed that MUC1-specific CTLs were undetectable after MUC1 DNA immunization but became evident only after tumor challenge. In another report, the CTL response induced by the co-administration of MUC1 DNA with a plasmid encoding murine interleukin-18 (IL-18) was not detected, which might be below the detection limit, though it was sufficient to generate MUC1-tumor protection, and prolong survival after tumor challenge. The nature of MUC1-specific cellular immune responses remains unclear, and whether there are some unfavorable sequences in the full-length MUC1 sequence that likely inhibit the potency of the DNA vaccine remains controversial. The VNTR region of MUC1 was selected in our test to explore the immune efficacy of the MUC1 VNTR DNA vaccine.

Here, we constructed recombinant vectors encoding the multiple repeats of human MUC1 to investigate the application of the DNA vaccine in inducing cellular and humoral immune responses, and test its anti-tumor activities using a tumor challenge model. We report the development of the protocols for DNA immunization using different immunization protocols including different numbers of tandem repeats, different numbers of immunizations, and co-injection of a murine IL-2 expression plasmid. We found that the co-administration of VNTR with IL-2-expressing plasmids resulted in a greater vaccination effect than the VNTR DNA vaccine alone, and reduced antitumor responses against murine Lewis lung carcinoma in a subcutaneous tumor model.

Subjects and methods

Vectors, cell lines, mice, and antibodies

The VR-1012 (VR) empty vector was purchased from Vical Inc. (San Diego CA). Mouse IL-2-encoding plasmid VR1012-IL-2 (VR-IL2), 2 or 33 repeats of human MUC1 VNTR- (2m or 33m) containing recombinant VR1012-2m (VR-2m) or VR1012-33m (VR-33m), and the 33m stably expressing LLC (murine Lewis lung carcinoma, H-2b) cell line ($MUC1^+$ LLC) were constructed in our laboratory. The P815 (H-2d) murine mastocytoma cell line was maintained in our laboratory, and cultured in RPMI-1640 medium with 10% FBS (Gibco-BRL, Grand Island, NY). Female BALB/c (H-2d) mice and C57BL/6 (H-2b) mice (age, 6–8 weeks; weight, 18–22 g) were purchased from the Shanghai laboratory animal center, and hosted in appropriate animal care facilities. They were handled in accordance with the Institutional Animal Care and Use Committee guidelines.

Mouse monoclonal antibodies against human MUC1 VNTR or IFN-r were purchased from BD pharmingen. The CellTiter 96® Aqueous Non-Radioactive Cell Proliferation and Cyto Tox 96® non-radioactive cytotoxicity assay kits were purchased from Promega Co. (Madison, WI). The MUC1 VNTR epitope H-2d-binding 9-mer peptide (APDTRPAPG, A9G) and the MUC1 VNTR 20-mer peptide (HGVTSAPDTRPAPGSTAPPA, H20A) were synthesized by an Applied Biosystems Model 430A machine, in our laboratory.

Plasmid construction and preparation

The sequence of the multirepeats of MUC1 VNTR (nm) is shown in Figure 1. The sequence of one repeat of MUC1 VNTR is "CACGGCGTCACCTCTGCCCCAGACACCAGGCCGGCCCCGGGTTCCACTGCTCCTCCAGCT." The VR-2m or VR-33m plasmid was created by cloning 2m (n = 1) or 33m (n = 33) fragment into the expression

Figure 1. Schematic representation of the domain structure of mucin 1 (MUC1) variable number of tandem repeats (VNTR) vaccines. Letter "m" represents the sequence of one repeat of MUC1 VNTR; "n" denotes the repeat number (n = 1 or n = 32).