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LL-37 enhances adaptive antitumor immune response in a murine model when genetically fused with M-CSFR$_{J6-1}$ DNA vaccine

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Abstract

DNA vaccine against M-CSFR$_{J6-1}$ (macrophage colony-stimulating factor receptor cloned from the J6-1 leukemic cell line) has shown both protective and therapeutic effects. In this study, to explore the adjuvant effects of LL-37 to M-CSFR$_{J6-1}$ DNA vaccines, we constructed genetically fused vaccines encoding M-CSFR$_{J6-1}$ and LL-37(pF). After immunizing BALB/c mice, specific humoral and cellular immune responses were detected. Compared with pR (encoding the extracellular region of M-CSFR$_{J6-1}$), pF was more effective in inducing humoral and cytotoxic immune response, prolonging survival of mice challenged with SP2/0-CSFR$_{J6-1}$ tumor cells, and inducing IFN-γ and IL-4 release by splenocytes. In this study, we also constructed pLL37 (encoding the mature LL-37) and coadministrated pLL37 and pR to see whether the genetic fusion was necessary. We found that compared with pR alone, pLL37 + pR could not prolong survival of mice challenged with SP2/0-CSFR$_{J6-1}$ tumor cells. Our results suggest that when genetically fused with M-CSFR$_{J6-1}$, LL-37 could enhance adaptive immune response against M-CSFR$_{J6-1}$ in a murine model challenged with tumor cells bearing M-CSFR$_{J6-1}$.

Keywords: LL-37; Macrophage colony-stimulating factor receptor; Antitumor activities; Adaptive immunity

1. Introduction

hCAP-18 is the unique cathelicidin antimicrobial protein found in humans so far. It is widely presented in the blood, seminal plasma and body surface. Cleavage of hCAP-18 by proteinase 3 liberates its C-terminal, active biologic domain (LL-37) from the conserved cathelin-like prosequence [1]. LL-37 has the capacity to kill a broad spectrum of microorganisms including those which have developed resistance to conventional antibiotics [2]. It is important in host defense by direct antimicrobial activity.

Involvement of LL-37 in pathological processes has been reported in the inflammatory skin diseases (psoriasis, atopic dermatitis, dermatitis herpetiformis, etc.) [3,4], the infections of respiratory [5] and gastrointestinal tracts [6]. Deficiency of LL-37 in neutrophils, plasma and saliva correlates with occurrence of infection and periodontal disease in patients with morbus Kostmann (a severe congenital neutropenia) [7]. Mice with disrupted *Cnlp* (mouse cathelicidin antimicrobial gene) showed increased susceptibility to skin infections with group A streptococcus [8]. Over-expression of hCAP-18/LL-37 results in augmented protection against bacterial infection [9]. Furthermore, LL-37 could bind lipopolysaccharide (LPS) and neutralize its biological activity [10].

Abbreviations: hCAP-18, human cationic antimicrobial protein 18; LL-37the C-terminal, active biologic domain of hCAP-18; M-CSFR$_{J6-1}$, macrophage colony-stimulating factor receptor (M-CSFR) cloned from the J6-1 leukemic cell line; mM-CSF, membrane-bound macrophage colony-stimulating factor; FCS, fetal calf serum; PVDF, polyvinylidene difluoride; IL, interleukin; BSA, bovine serum albumin; IFN, interferon; AML, acute myeloid leukemia; PCR, polymerase chain reactions; ABC complex, avidin–biotin–peroxidase complex; NS, normal saline; CTL, cytotoxic T lymphocyte; E:T ratio, the ratio of effectors and target cells; DCs, dendritic cells; PTK, protein tyrosine kinase; APCs, antigen-presenting cells; mDF2β, murine β-defensin2; TLR-4, toll-like receptor 4

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Recently, other effects of LL-37 related to immune response have been gradually unveiled. LL-37 is chemotactic for human monocytes, neutrophils and T lymphocytes and acts as a potent modifier of DC differentiation [11–13]. It is also a potent chemotactic factor for mast cells and stimulates the degranulation of mast cells [14]. Defensins are also multifunctional antimicrobial peptides with chemotactic responses [15]. It has been reported that defensins act as potent adjuvants to enhance the adaptive immunity induced by ovalbumin (OVA) or keyhole limpet hemocyanin (KLH) in vivo [16,17]. Biragyn et al. reported that murine β-defensin2 (mDF2β) could enhance the immunogenicity of the tumor antigen or viral antigen when fused with these antigens in DNA vaccination [18,19]. Whether LL-37 has similar effects in antitumor immunity remains unclear.

Robbins et al. transduced the MC-38 mouse colon adenocarcinoma cell line with human carcinoma expressing antigen (CEA) and injected these cells in syngeneic C57BL/6 mice. This model could be used in the design of regents and protocols to study adaptive immunotherapy directed against a human CEA [20]. Our previous work suggested that M-CSFR was a potential target for tumor immunotherapy [21–25]. Using the strategy mentioned above, our lab developed an SP2/0-BALB/c murine model instead of the MC-38-C57BL/6 murine model. First, we constructed the M-CSFR$_{J6\text{-}1}$ (M-CSFR cloned from the J6-1 leukemic cell line with three point mutations at amino acid level) DNA vaccine and found that it could induce specific humoral and cellular immune response and have protective and therapeutic effect [26]. We further proved that co-immunization with M-CSFR$_{J6\text{-}1}$ and mM-CSF DNA vaccines were better than M-CSFR$_{J6\text{-}1}$–mM-CSF fusion DNA vaccine [27]. In this study, we constructed genetic fusion vaccines encoding M-CSFR$_{J6\text{-}1}$ and LL-37, and our results showed when genetically fused with M-CSFR$_{J6\text{-}1}$, LL-37 could enhance the specific humoral and cellular immune response against M-CSFR$_{J6\text{-}1}$ in the murine model.

2. Materials and methods

2.1. Murine model

The SP2/0-BALB/c murine model used to evaluate the effects of the DNA vaccines was established in our lab previously [26,27]. Six to 8-week old, specific pathogen-free, female BALB/c (H-2d) mice were purchased from Center for experimental animals, Chinese Academy of Medical Sciences and were housed in a specific pathogen-free animal facility under isothermal conditions and allowed access to food and water ad libitum. The housing, experiments, and all other conditions were approved by the Ethics Committee at the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College. Mouse myeloma cell line SP2/0 stably expressing the M-CSFR$_{J6\text{-}1}$ fragment (designated as SP2/0-CSFR$_{J6\text{-}1}$) was established in our lab previously and was maintained in RPMI-1640 supplemented with 10% heat-inactivated FCS at 37 °C and 5% CO_2. Intraperitoneal administration of SP2/0 cells could induce ascetic fluid in mice. In the model established in our lab, SP2/0 cells (wild-type or transfected with relative plasmid) were subcutaneous injected into the right lateral flank of the mice and the solid tumor developed.

2.2. Fusion gene cloning and plasmid constructions

In this study, we constructed four plasmids (pR, pF, pF′ and pLL37) and the primers used in plasmid construction were synthesized by Sangon Co. (Shanghai, China) and were listed below (5′–3′):

DVP1: CTATGCGGATCCATGGGCCCAGGAGTTC
DVP1′: GGAATCCCAGTGATACTGCTGGGTGATTTCTTC
DVP2: GCCTCCAGAGCCACCCTCAGAGCTCAAGTTC
DVP2′: GTACGTCGACTACTCAGAGCTCAAGTTC
DVP3: GGCTCTGGAGGCTCGCTGCTGGGTGATTTCTTC
DVP3′: GGCTCTGGAGGCTCGCCCTGCTGGGTGATTTCTTC
DVP4: GTACGTCGACTAGGACTCTGTCCTGG
DVP4′: GTACGTCGACTAAGGACTCTGTCCTGG

The plasmids pCSFR (encoding M-CSFR$_{J6\text{-}1}$) [26] and phCAP-18 (encoding hCAP-18) [28] have been constructed previously. The plasmid pR was constructed by inserting the fragment encoding the signal peptide and extracellular domain of M-CSFR$_{J6\text{-}1}$, which was amplified from pCSFR using DVP1 and DVP2′ into pTARGET vector. The M-CSFR$_{J6\text{-}1}$–LL-37 fusion DNA fragment was constructed by overlap PCR. Briefly, two fragments, either encoding signal peptide and extracellular domain of M-CSFR$_{J6\text{-}1}$ or encoding mature LL-37, were amplified from pR and phCAP-18 using DVP1 and DVP2 or DVP3 and DVP4, respectively. Then a second PCR was operated by DVP1 and DVP4 using the first-round PCR products as templates and the fusion fragment was generated. Finally the PCR product was digested and inserted into pTARGET and named pF. Similarly, the control plasmid pF′ containing M-CSFR$_{J6\text{-}1}$ fused with mutant LL-37 (frame-shift mutation) was constructed using the primers DVP1–DVP2, DVP3′–DVP4′ and DVP1–DVP4′. The plasmid pLL37 containing the mature LL-37 and the signal peptide of M-CSFR$_{J6\text{-}1}$ (synthesized by AUGCT Co, Pekin, China) was also constructed using the primers DVP1′–DVP4 and DVP1–DVP4. And this did not change the reading frame of LL-37 and the cleavage site of the signal peptide (analyzed by SignalP 3.0 Server) [29]. The *E. coli* DH5α which transformed by pR, pF, pF′ or pLL37 were screened by PCR and restriction endonuclease digestion to find the positive clones. Finally, the successful construction of these plasmids was confirmed by sequencing. The plasmids used to immu-