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Genetically engineered Newcastle disease virus expressing interleukin 2 is a potential drug candidate for cancer immunotherapy

Fuliang Bai [1], Zeshan Niu [1], Hui Tian, Siming Li, Zheng Lv, Tianyuan Zhang, Guiping Ren, Deshan Li *

Biopharmaceutical Teaching and Research Section, College of Life Science, Northeast Agricultural University, Harbin 150030, China

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ABSTRACT

Newcastle disease virus (NDV) is an intrinsically tumor-specific virus, several clinical trials have reported that mesogenic NDV is a safe and effective agent for human cancer therapy. Interleukin 2 (IL2) is a cytokine that stimulates T cell propagation to trigger innate and adaptive immunity. IL2 has been used for cancer therapy and has achieved curative effects. In this study, a recombinant NDV LaSota strain expressing human interleukin 2 (rLaSota/IL2) was generated. The ability of rLaSota/IL2 to express human IL2 was detected in the infected tumor cells. In addition, the activity of IL2 was analyzed. The antitumor potential of rLaSota/IL2 was studied by xenograph mice carrying H22 and B16-F10 cells. Tumor-specific $CD4^+$ and $CD8^+$ T cells and MHC II were also analyzed in the two tumor-bearing models. Our study showed that rLaSota/IL2 significantly stimulated tumor-specific cytotoxic T-lymphocyte (CTL) responses and increased regulatory $CD4^+$ and cytotoxic $CD8^+$ T cells proliferation. The treatment with rLaSota/IL2 led to tumor regression in tumor-bearing mice and prolonged the survival of tumor-bearing mice. Furthermore, tumor challenging experiments demonstrated that rLaSota/IL2 invoked mice a unique capacity to remember a pathogen through the generation of memory T cells, which protect the host in the event of reinfection and form adaptive immune system. The result indicates that tumor-infiltrating $CD4^+$ T regulatory cells may denote the effective regression of tumors. Taken together, rLaSota/IL2 has potential for immunotherapy and oncolytic therapy of cancers and may be an ideal candidate for clinical application in future cancer therapy.

1. Introduction

Hepatocellular Carcinoma (HCC) and melanoma are major worldwide health concern, ranking among the top ten most prevalent malignancies [1,2]. The incidence of hepatoma has more than doubled over the past two decades, and epidemiological trends suggest that the rate of hepatoma diagnoses will continue to rise, presumably as a consequence of an ever-increasing prevalence of hepatitis B and C virus infection and increased alcohol consumption in most industrialized countries [3–5]. Melanoma is less common than other skin cancers, however, it is much more dangerous and causes the majority (75%) of deaths related to skin cancer [6]. According to a WHO report, about 48,000 melanoma related deaths occur worldwide every year [7], and malignant melanoma is not sensitive to chemotherapy, so in the traditional treatment, is incurable disease. Although incremental advances in treatment, the outcome for hepatoma and malignant melanoma patients has not changed significantly. Although surgical resection and liver transplantation are considered to be curative, the majority of hepatoma cases are detected at an advanced stage, at which time the treatment options are extremely limited [5,8].

Currently, cancer therapy by using oncolytic viruses has attracted attention of clinicians and researchers [9–13]. Oncolytic viruses provide an attractive new horizon for cancer therapy. NDV is a member of oncolytic viruses [14–16], recently, many studies have been carried out to investigate the oncolytic effects of NDV because it replicates in human tumor cells but not in normal human cells [13,17–21].

At present, malignant melanoma and hepatoma are diseases with only few effective treatments. Due to the predominant failure, limited efficacy and more side effects of the cytotoxic chemotherapy, a number of alternative immunologic approaches have been devised. According to this, a fundamental problem in treatment

* Corresponding author at: Biopharmaceutical Teaching and Research Section, College of Life Science, Northeast Agricultural University, 59 Mucai Street, Box 180, Harbin 150030, China. Tel.: +86 451 59190645; fax: +86 451 59190645.
E-mail address: desganli@163.com (D. Li).
[1] These two authors contribution equally to this study.

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of malignant melanoma and hepatoma lie in the inadequate host immune response to the tumors antigens [22]. The mechanism of this immune suppression is not completely clear, but immune suppression in cancer has been shown to un-regulate expression of antigen presenting cell (APC), tumor cells loss of expression of tumor-specific antigens, and lack of appropriate costimulatory signals for T-cell activation [23–25]. Indeed, treatments with immunocytokines have shown promising results in immunosuppressive tumor growth and clinical benefits against malignancy. Also, different approaches for anti-malignancy immune enhancement have been approved for treatment of melanoma and hepatoma on the basis of the previous study [26–33].

Naturally occurring NDVs are oncolytic agents against a variety of experimental cancers [34–37]. Recently, employing reverse genetics has provided a way of generating recombinant NDV (rNDV) strains with improved oncolytic and immune regulatory properties [38]. Promising results with recombinant NDV for oncolytic therapy of cancers have been reported by many researchers [18,19,35,39]. However, most of these NDV strains are mesogenic, and such highly infectious strains are problematic in clinical use due to possible unintentional release of highly infectious virus into the environment, which affects almost all species of domestic and wild birds [40]. Mesogenic strains of NDV may cause losses to poultry industries. Eggs production of chicken without maternal antibody will drop when infected with mesogenic NDV [41]. In addition, mesogenic NDVs have been forbidden in many countries for its high pathogenicity for chicken without maternal antibody. Therefore, lentogenic NDV strains may be better alternatives for future clinical use [42]. Notably, Robert J Walter et al. reported that mesogenic strain was 1555 times stronger than lentogenic strain in killing normal cells. These innately targeted lentogenic viruses may have meaningful potential in treating cancer [43]. Therefore, application of mesogenic NDV is limited in the world, and the safety of mesogenic NDV makes people worried [44]. As a result, avirulent lentogenic strains may be a better alternative as cancer therapy vector. The lentogenic strain LaSota is safe to humans and animals and has been recommended by World Health Organization (WHO) as a NDV vaccine. The advantage of LaSota is that it has no geographical constraints for widely used in the world.

IL2 is a pleiotropic cytokine with important effects on cells of the innate and adaptive immune systems. IL2 plays a pivotal role in T-cell activation and effector functions, including T cell proliferation, interferon-γ production and cytotoxicity [45]. IL2 can stimulate the propagation of lymphocytes and induce the cytotoxic T lymphocytes and lymphokine-activated killer cells for tumor cells. IL2 also influences memory T cell homeostasis through the regulation of memory T cell numbers and enhances major histocompatibility complex II (MHC II) expression on tumor cells [46]. IL2 drives the generation of antigen-specific T cells, promotes the survival of memory $CD8^+$ T cells, and stimulates the homeostatic proliferation of memory phenotype $CD8^+$ T cells. Therefore, it has been used for cancer immunotherapy [47]. Recently, expressing therapeutic IL2 gene by rNDV as new strategy for cancer therapy has been reported and the results demonstrate that IL2 enhances therapeutic effects of NDV though stimulating body's immune system to produce tumor-specific cells [23,27,48]. Expressing the human IL2 protein generated by the recombinant LaSota virus shows that IL2 is capable to express by the recombinant virus, but they did not evaluate the efficacy of the virus treatment of the tumor-bearing animal models [48]. Another study expressing the murine IL2 protein by the recombinant mesogenic virus shows that murine IL2 increases the number of $CD4^+$ and $CD8^+$ T cells and survival rate of the tumor-bearing animals [23]. Recombinant Newcastle disease virus producing IL-2 as ATV-NDV tumor also has been reported [27]. In this study, our results confirmed previous therapeutic effects of rNDV/IL2. However, human IL2 instead of murine IL2 was inserted into NDV genomes, which is closer to clinical experiment and avoid side effects for species differences. In addition, increasing MHC II expression on tumors was detected after treatment with NDV. At last, immunological memory were identified by tumor challenge experiment in this study. These results indicate that rLaSota/IL2 represents an enhanced oncolytic virus, which could be potentially applied in clinic for HCC and melanoma patients.

2. Materials and methods

2.1. Ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by Chinese Association For Laboratory Animal Sciences (CALAS), Animal Health Products, Committee on the Ethics of Animal Experiments Defence Research and Development China and Animal Experiments of the University of Northeast Agricultural (approval number: SCXK-2012-0002). All surgery and euthanasia were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.2. Cell lines, virus, and other reagents

NDV strain LaSota, rLaSota, rNDV/EGFP and rLaSota/IL2 viruses were grown in embryonated specific-pathogen-free (SPF) eggs. All viruses were propagated in DF1 chicken fibroblasts cells (ATCC, Manassas, VA) with Dulbecco's Modified Eagle's Medium (DMEM; BioWhittaker Inc., Walkersville, MD) containing 10% fetal bovine serum (FBS; Sigma, St. Louis, MO) and 1% chicken allantoic fluid. Mouse malignant melanoma cell line (B16-F10 cell line, ATCC, Rockville, MD) is a kind gift from Dr. Jiahuai Han (XiaMen University, China). H22 cells (ATCC, Rockville, MD) were provided by the Technology Center, Harbin Pharmaceutical Group, and primary chicken embryo fibroblasts were prepared from 9 to 11 day-old SPF embryos. H22, A549, U251 and BHK-21 cells were purchased from ATCC (Rockville, MD) and were maintained in DMEM supplemented with 10% FBS. B16-F10 cells were maintained in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% FBS. DF1 and chicken embryo fibroblast cells were maintained in DMEM supplemented with 10% FBS. All cell lines were grown at 37 °C under 5% CO_2. All cell cultures and cells were regularly tested for mycoplasma contamination.

2.3. Construction of the recombinant NDV containing the transcription cassettes of IL2 and EGFP genes

The recombinant Newcastle disease viruses (rNDVs) were derived from LaSota. RT-PCR was performed using viral RNA as template. Ten individual fragments of the viral genome (F1–F10) were ligated into pBluescript, which was named pBl-rLaSota. The IL2 gene with gene end and gene star was cloned into pMD18-T vector (TaKaRa) by PCR using the sense primer (5′-cgacgcgtttaagaaaaaatgtacgggtagaacccgccaccatgtacaggatgcaactcctgt-3′) and antisense primer (5′-cgacgcgttaatcaagtcagtgttgagatgatgct-3′). The resulting plasmid was named pMD-IL2. The EGFP gene was cloned in to pMD18-T vector by the method using the sense primer (5′-aggcgcgccttaagaaaaaatacgggtagaaccgccaccatggtgagcaagggcgaggag-3′) and the antisense primer (5′-aggcgcgcctaacttgtacagctcgtccatgccgag-3′). The resulting plasmid was named pMD-EGFP. The IL2 and EGFP genes were subcloned into the HN-L junction of rLaSota at the *Mlu*I or *Asc*I site, to form the recombinant plasmids pBl-rLaSota/EGFP and pBl-rLaSota/IL2, respectively. All PCR products were sequenced for fidelity.