Journal of Controlled Release 207 (2015) 70–76

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Generation of a safe and effective live viral vaccine by virus self-attenuation using species-specific artificial microRNA

Junwei Li, Maria T. Arévalo, Diana Diaz-Arévalo, Yanping Chen, Jang-Gi Choi, Mingtao Zeng *

Center of Excellence for Infectious Diseases, Department of Biomedical Sciences, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center, 5001 El Paso Drive, El Paso, TX 79905, USA

ARTICLE INFO

Article history:
Received 21 August 2014
Received in revised form 12 February 2015
Accepted 2 April 2015
Available online 7 April 2015

Keywords:
Live attenuated vaccine
Artificial microRNA
Influenza virus
Safety
Cross-protection

ABSTRACT

Vaccination with live attenuated vaccines (LAVs) is an effective way for prevention of infectious disease. While several methods are employed to create them, efficacy and safety are still a challenge. In this study, we evaluated the feasibility of creating a self-attenuated RNA virus expressing a functional species-specific artificial microRNA. Using influenza virus as a model, we produced an attenuated virus carrying a mammalian-specific miR-93 expression cassette that expresses a viral nucleoprotein (NP)-specific artificial microRNA from an insertion site within the non-structural (NS) gene segment. The resulting engineered live-attenuated influenza virus, PR8-amiR-93NP, produced mature and functional artificial microRNA against NP in mammalian cells, but not in avian cells. Furthermore, PR8-amiR-93NP was attenuated by 10^4 fold in mice compared with its wild-type counterpart. Importantly, intranasal immunization with PR8-amiR-93NP conferred cross-protective immunity against heterologous influenza virus strains. In short, this method provides a safe and effective platform for creation of live attenuated RNA viral vaccines.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Classical LAVs were produced experimentally by repeated passaging of a virus in cultured cells, but this method is not always reliable, and safety issues occurred in some cases, for example, when there was a reversion to wild-type virulence. With advances in molecular virology, several novel methods, such as altering replication fidelity [1] and deoptimizing codons [2], have been employed in the creation of live attenuated vaccines with better-controlled replication and pathogenesis. MicroRNAs (miRNAs) are non-coding endogenous RNAs that direct post-translational regulation of gene expression by interacting with messenger RNAs and targeting them for degradation. miRNA-based gene silencing is also a promising approach to controlling viral replication and may be used to improve the safety of attenuated live vaccine. Recent studies showed that many miRNAs are species-and tissue-specific [3–5]. These characters of miRNAs can be used to modify the replicative tropism of RNA and DNA viruses [6–9]. A number of studies have inserted miRNA target sequence into some viral genomes for successful RNA inhibition (RNAi) [5,10,11]. Although miRNA targeting is a promising approach to the rational design of LAVs, the risk of accumulating mutations in the miRNA target sequence that cause virulence reversion should be kept in mind.

Previously, the design of artificial miRNAs (amiRNAs) that produce functional short interfering RNAs was only limited to DNA viruses and retroviruses [11–14]. miRNAs were hypothesized to be problematic in RNA viruses because of the potential degradation of the viral RNA genome during the excision of virus-encoded pre-miRNA. However, a very intriguing study by Varble et al. showed that a miRNA cassette can be successfully inserted into the non-structural (NS) segment of influenza virus [15], and the rescued influenza virus produced functional miRNAs in vitro and in vivo. Results published by Schmid et al. showed that replication-incompetent influenza virus could be developed as an RNA viral vector for delivery of amiRNAs [16]. Recent published results by other groups also showed that tick-borne encephalitis virus (TBEV), Sindbis virus (SV), and vesicular stomatitis virus (VSV) can produce functional amiRNAs [17–19]. These results suggest that it is possible to create live attenuated RNA virus vaccine by incorporating an amiRNA cassette into the RNA virus genome.

In this study, we took influenza virus as a test case and designed an artificial miR-93 cassette for insertion into NS gene segment of influenza viral genome, which produces a specific amiRNA for NP gene that would result in a virus that is attenuated in mammalian cells, but could be propagated in chicken eggs at reasonable titers. In animal experiments,

* Corresponding author at: Center of Excellence for Infectious Diseases, Department of Biomedical Sciences, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center, El Paso, TX 79905, USA.
E-mail address: mt.zeng@ttuhsc.edu (M. Zeng).

http://dx.doi.org/10.1016/j.jconrel.2015.04.001
0168-3659/© 2015 Elsevier B.V. All rights reserved.

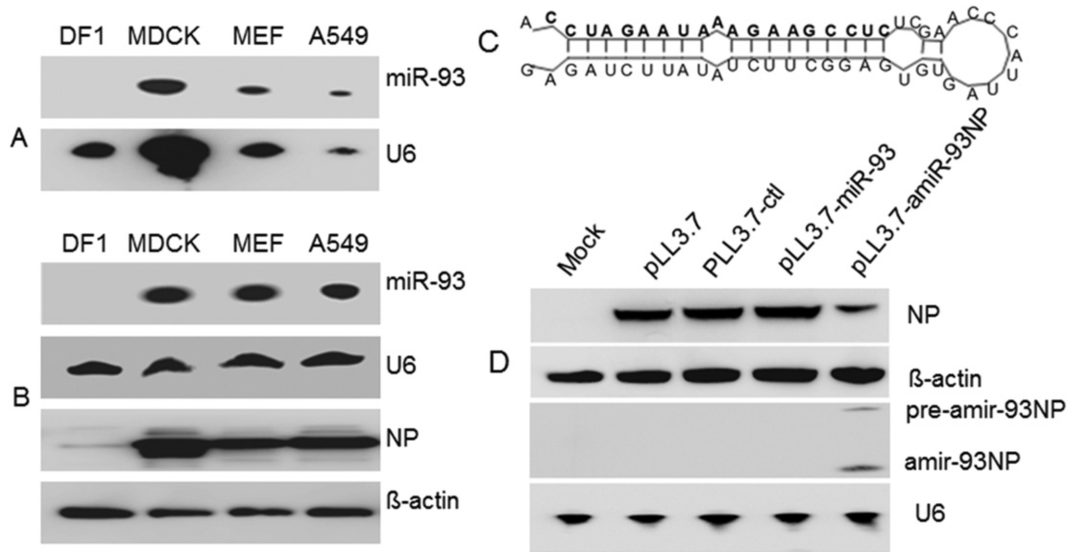

Fig. 1. Detection miR-93 in non-infected cells and PR8-infected cells and design of artificial miRNA based on miR-93 backbone. (A) Evaluation of natural miR-93 expression in DF1, MDCK, MEF, and A549 cell lines. U6 was used as a control RNA probe. (B) Evaluation of miR-93 expression in cells infected with PR8 influenza virus. NP protein expression was used to verify viral infection, and β-actin was used as loading control. (C) Sequence and secondary structure of amiR-93NP. Mature artificial miR93-NP sequence is highlighted in bold. (D) pcDNA-NP with pLL3.7, pLL3.7-ctl, pLL3.7-miR93, or pLL3.7-amiR93NP was transfected into 293T cells. Cells were harvested at 24 h post-transfection. Cell lysates were prepared for Western-blot analysis to detect NP or β-acting proteins. RNA was prepared for Northern blot analyses to detect amiR-93NP expression.

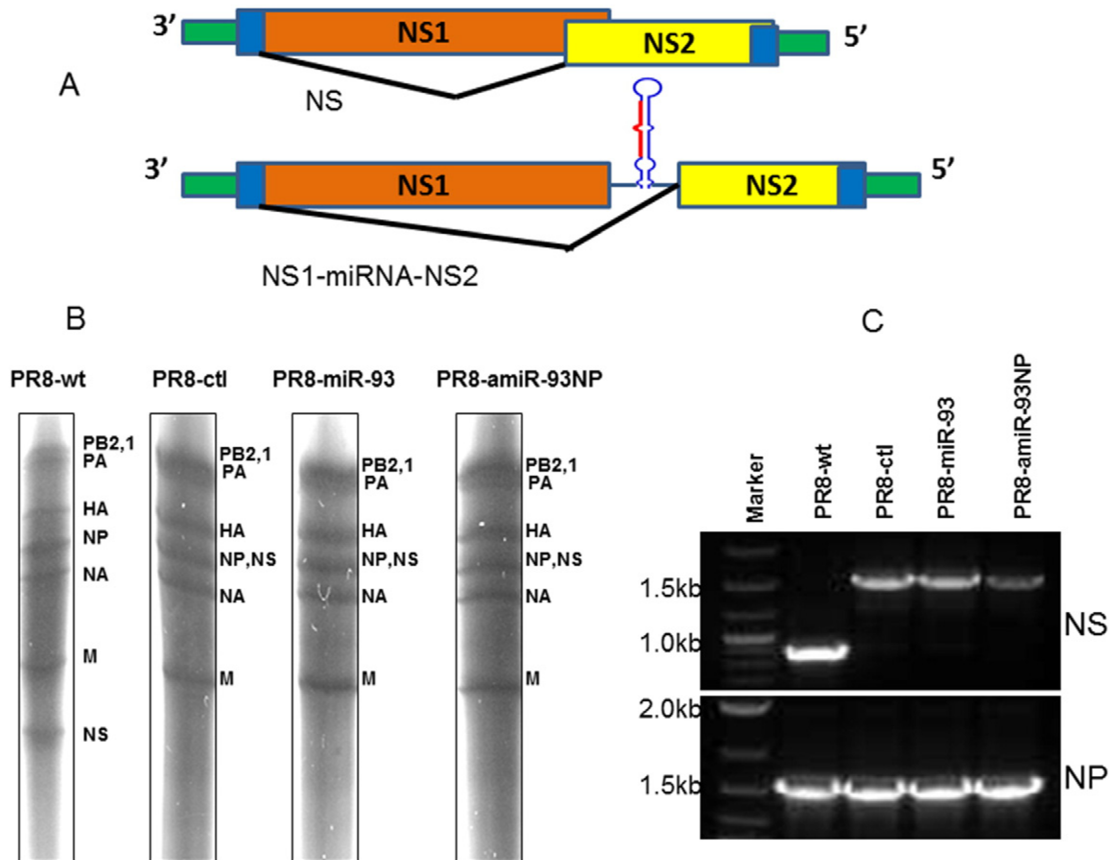

Fig. 2. Engineering of NS gene segment and verification of rescued influenza viruses. (A) Diagram of engineered and original NS gene segments. Green represents 5′ and 3′ noncoding regions; blue represents packaging signal within open reading frame; orange with blue to the left represents NS1 coding sequence; yellow with blue to the right represents NS2 coding sequence. (Top) Organization of original NS gene segment. (Bottom) Organization of modified NS gene segment engineered with ctl, miR-93 or amiR-93NP expression cassettes. (B) RNA was isolated from purified PR8 wild type, PR8-ctl, PR8-miR-93 and PR8-amiR93-NP influenza viruses, and 1 μg was separated on a 4% acrylamide TBE urea gel. Bands were visualized by silver staining. Each RNA segment is labeled to the right of the gel. (C) NS and NP gene segments were amplified by RT-PCR and separated by electrophoresis on agarose gel.

Download English Version:

https://daneshyari.com/en/article/1423716

Download Persian Version:

https://daneshyari.com/article/1423716

Daneshyari.com