Gene delivery, Immunotherapy, Vaccine

Development of a novel LCMV vector capable of controlling foreign gene expression

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Abstract

Lymphocytic choriomeningitis virus (LCMV) generates mRNA with a 5′-capped untranslated region (UTR) and a non-polyadenylated 3′-UTR (vmRNA). We previously reported that the non-polyadenylated 3′-UTR, which is derived from a non-coding intergenic region (IGR) in the LCMV RNA genome, regulates viral protein levels at the posttranscriptional level. In this study, we found that a short 3′-UTR sequence located at the 5′ side of the vmRNA (proximal region, PR) was a major determinant of translation efficiency. Furthermore, the reporter protein level was altered in cells infected with recombinant LCMV in which the IGR sequence that corresponded to the PR was modified.

Background & Results

Several mammalian arenaviruses (mammarenavirus) cause hemorrhagic fever in humans and are a major public health concern in endemic regions. Lassa virus is the most significant mammarenavirus, being estimated to infect approximately 900,000 individuals in West Africa each year, resulting in high numbers of Lassa fever cases. Case fatality rates among patients hospitalized with Lassa fever are reported to be approximately 15%. The worldwide-distributed prototypic mammarenavirus, LCMV, is genetically closely related to Lassa virus and can be handled in a biosafety level 2 facility, offering surrogate infection models for Lassa virus. On the basis of our previous finding, we developed live-attenuated vaccines against human pathogenic mammarenaviruses by manipulating the IGR sequence. In this study, we further investigated the contribution of the 3′-UTR sequence of LCMV mRNA (vmRNA) to translation regulation employing a reporter system with *in vitro* transcribed mRNA (vlmRNA) that contained the ZsGreen open reading frame flanked by 5′-capped and 3′-non-polyadenylated UTRs derived from the vmRNA (Fig. 1). The analysis using vlmRNAs that contained chimeric 3′-UTR sequences swapped between nucleoprotein (NP, high translation efficiency) and glycoprotein precursor (GPC, low translation efficiency) mRNAs showed that a 10-nucleotide sequence proximal to the nucleoprotein open reading frame and its predicted secondary structure were essential for promoting translation. Modification of this 10-nucleotide sequence in recombinant LCMV (Fig. 2) also impacted expression of the reporter gene (Fig. 3), suggesting that the expression of a foreign gene was controlled by modifying the PR sequence.

Significance of the research and Future perspective

Because LCMV can induce robust long-term CD8+ cytotoxic T lymphocyte responses against virus antigens, attenuated recombinant LCMV was proposed as a tumor immunotherapy platform to deliver tumor-associated antigens. Our finding provides the opportunities to improve recombinant LCMV-based gene delivery systems to fine-tune both the expression of a foreign gene and the degree of attenuation by altering the PR sequence. In addition to the noncytolytic nature and broad tissue tropism, this flexibility to manage foreign gene expression levels expands the utility of a recombinant LCMV-based gene delivery system to more demanding applications, such as the generation of induced pluripotent stem cells, in which the levels of transduced reprogramming factors must be precisely controlled for effective derivation.

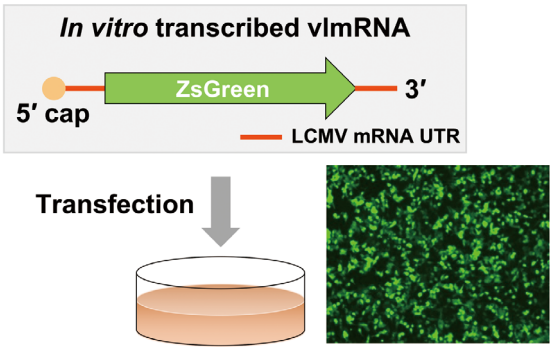

Fig. 1

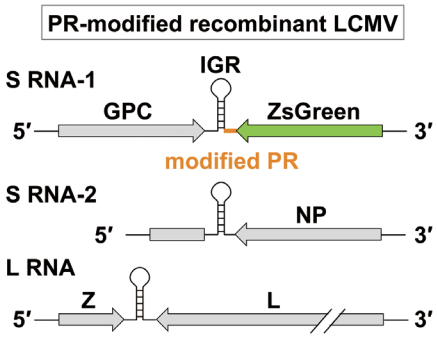

Fig. 2

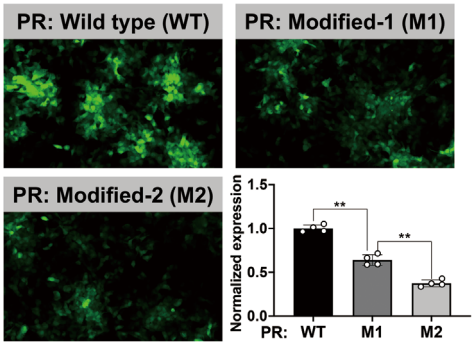

Fig. 3

Patent

Treatise Hashizume, Mei; Takashima, Ayako; Iwasaki, Masaharu. A small stem-loop-forming region within the 3′-UTR of a non-polyadenylated LCMV mRNA promotes translation. Journal of Biological Chemistry, 2022, 298 (2): 101576, doi: 10.1016/j.jbc.2022.101576

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Keyword virus vector, untranslated region, translation regulation