Biochemical and Biophysical Research Communications xxx (2016) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Improved transduction efficiencies of adeno-associated virus vectors by synthetic cell-permeable peptides

Kitako Tabata [a], Eriko Sugano [a, b], Fumika Murakami [a], Tetsuro Yamashita [b, c], Taku Ozaki [b], Hiroshi Tomita [a, b, d, *]

[a] Laboratory of Visual Neuroscience, Graduate Course in Biological Sciences, Iwate University Division of Science and Engineering, 4-3-5 Ueda, Morioka, Iwate 020-8551, Japan
[b] Soft-Path Engineering Research Center (SPERC), Faculty of Engineering, Iwate University, Morioka, 020-8551, Japan
[c] Department of Biological Chemistry, Iwate University Faculty of Agriculture, Morioka, Japan
[d] Clinical Research, Innovation and Education Center, Tohoku University Hospital, 1-1 Seiryo, Aoba, Sendai, Miyagi 980-8574, Japan

ARTICLE INFO

Article history:
Received 30 August 2016
Accepted 2 September 2016
Available online xxx

Keywords:
Adeno-associated virus vector
Cell-permeable peptide
Transduction efficiency
Epidermal growth factor receptor

ABSTRACT

Various serotypes of adeno-associated virus (AAV) vectors have been used for gene therapy and as research tools. Among these serotypes, the AAV type 2 vector has been used successfully in human gene therapies. However, the transduction efficiency of AAV2 depends on the cell type, and this poses a problem in the efficacy of gene therapy. To improve the transduction efficiency of AAV2, we designed a small peptide consisting of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor peptide and the HIV-Tat sequence Tat-Y1068. Pre- or co-treatment of CYNOM-K1 cells from cynomolgus monkey embryo skin with Tat-Y1068 increased the transduction efficiencies in a dose-dependent manner and caused p38 phosphorylation. The transduction efficiency of AAV2 into the rat fibroblast cell line RAT-1 highly expressing EGFR was less than the transduction efficiency of AAV2 into CYNOM-K1 cells. Tat-Y1068 increased the transduction efficiency in RAT-1 cells in the same manner as in CYNOM-K1 cells. In conclusion, cell-permeable peptides possessing the EGFR tyrosine kinase inhibitor function might serve as a useful ingredient of AAV2 vector solution for increasing the transduction efficiency of gene therapies.

1. Introduction

The adeno-associated virus (AAV), a non-pathogenic human parvovirus, is widely used as a promising vector for gene therapies. A characteristic feature of AAVs is that they can transduce a gene even into non-dividing cells like neurons. Furthermore, transgene expression mediated by an AAV vector in the central nervous system enables long-term expression. Thus far, AAV vectors have been applied in gene therapy for cystic fibrosis [1], α-1 anti-trypsin deficiency [2], haemophilia B [3], Batten's disease, and muscular dystrophy, and in addition, as a tool for molecular biology [4–7].

Retinitis pigmentosa (RP) is an inherited degenerative disease of the eye, the symptoms of which include night blindness, the loss of the peripheral visual field and central vision [8], and finally, complete blindness. We have been researching gene therapy using AAV for blind patients with RP using optogenetic technology. Gene therapy using optogenetic technology has been put forth as a new method for restoring vision. Bi et al. [9] and our group [10–12] have reported that transduction of the channelrhodopsin-2 (ChR2) gene, derived from the green alga *Chlamydomonas*, restores vision in blind mice and rats. Recently, we succeeded in developing a new type of optogenetic gene, *mVChR1* [13], which has a different wavelength sensitivity from that of ChR2, and safety studies have also been performed for this gene [14,15]. Visual function restored by gene therapy depends on transduction efficiency. Therefore, it is important to improve the transduction efficiency of AAV-mediated gene therapy.

It is well-known that transduction efficiency depends on the cell type. Various serotypes of AAVs have been developed and improved for use as vectors for gene therapies and research. Wild-type AAV consists of the Rep and capsid (Cap) ORFs and 2 inverted terminal

* Corresponding author. Graduate Course in Biological Sciences, Iwate University, 4-3-5 Ueda, Morioka, Iwate 020-8551, Japan. Tel./fax: +81 19 621 6427.
E-mail addresses: ktabata@iwate-u.ac.jp (K. Tabata), sseriko@iwate-u.ac.jp (E. Sugano), t0113073@iwate-u.ac.jp (F. Murakami), yamashit@iwate-u.ac.jp (T. Yamashita), tozaki@iwate-u.ac.jp (T. Ozaki), htomita@iwate-u.ac.jp (H. Tomita).

http://dx.doi.org/10.1016/j.bbrc.2016.09.014
0006-291X/

Table 1
PCR primer pairs for EGFR and β-actin.

Gene		Length	Sense	Antisense
EGFR	CYNOM-1	109	5′-ACGGGGACCAGACAACTGTA-3′	5′-CTTCCAGACCAGGGTGTTGT-3′
	RAT-1	117	5′-ACAACACCCTGGTCTGGAAG-3′	5′-GCCCTTCTGGTTGTTGACAT-3′
β-actin	CYNOM-1	142	5′-CTGGAACGGTGAAGGTGACA-3′	5′-AAGGGACTTCCTGTAACAATGCA-3′
	RAT-1	75	5′-GGGAAATCGTGCGTGACATT-3′	5′-GCGGCAGTGGCCATCTC-3′

repeats (ITRs). Among these, the Cap protein plays a key role in the specificity of the AAV infection of cells, and receptors associated with AAV binding have been identified [16–20]. The appropriate serotype corresponding to the target cells needs to be selected in this approach. Following viral binding and entry into cells, second-strand DNA synthesis affects transduction efficiency [21–27]. The single-stranded D-sequence–binding protein (ssD-BP) phosphorylated at tyrosine residues interacts with the single-stranded D

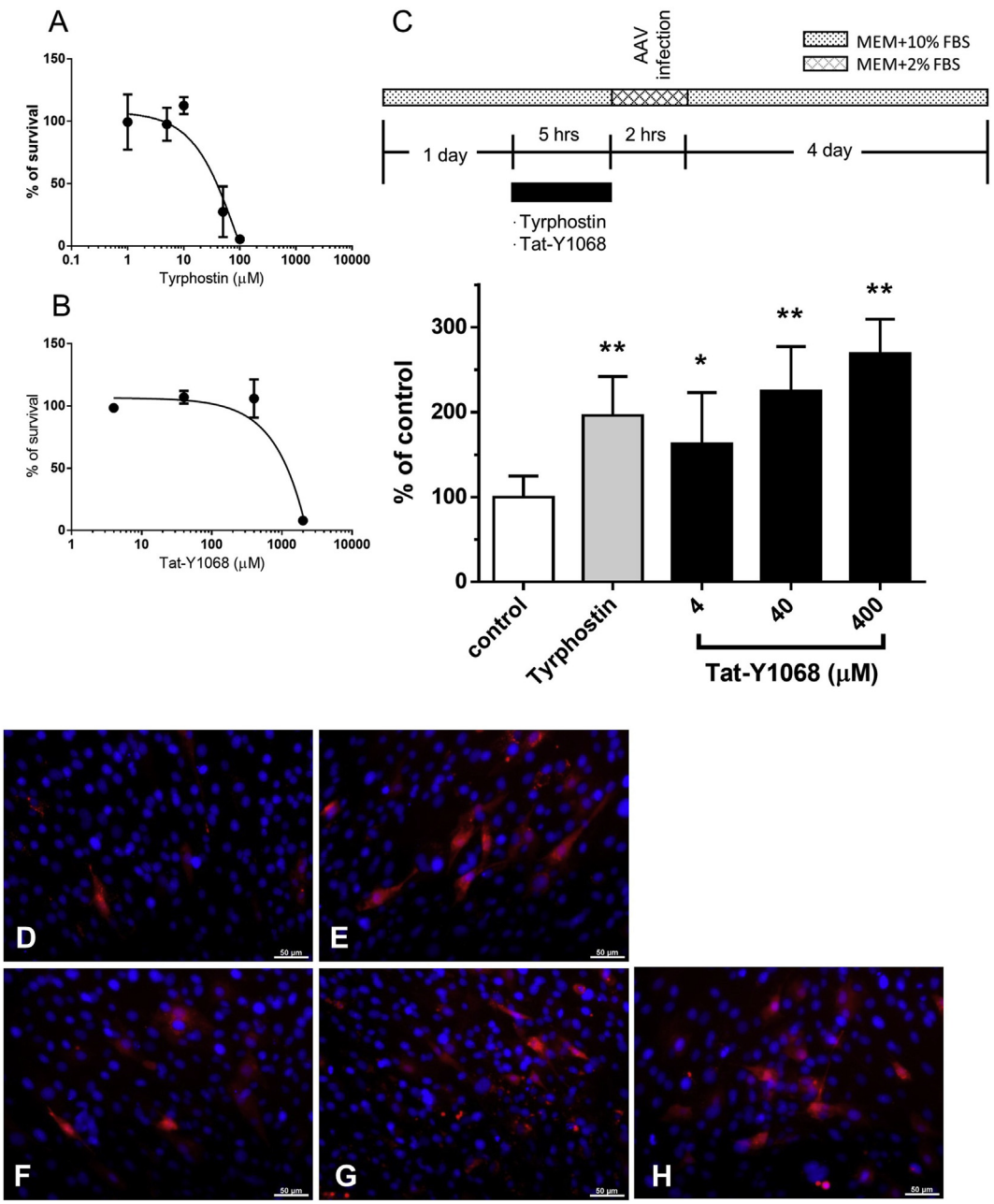

Fig. 1. Effects of the pre-treatment of a cell-permeable peptide on the transduction efficiencies of the AAV vector. Cytotoxicities were evaluated by the pre-treatment of tyrphostin (A) or Tat-Y1068 (B) in cultured CYNOM-K1 cells. Data are shown as mean ± SD (n = 5). Pre-treatment with tyrphostin or Tat-Y1068 improved the transduction efficiencies (C). Data are shown as mean ± SD (n = 9 for control and tyrphostin, n = 6 for Tat-Y1068. *, **p < 0.05, 0.001). Fluorescence micrographs of CYNOM-K1 cells transduced with the pmCherry gene by AAV without (D) or with the pre-treatment with tyrphostin (E) or Tat-Y1068 (F: 4 μM, G: 40 μM, H: 400 μM).

Please cite this article in press as: K. Tabata, et al., Improved transduction efficiencies of adeno-associated virus vectors by synthetic cell-permeable peptides, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.09.014