Biochemical and Biophysical Research Communications xxx (xxxx) xxx

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

DNA repair protein RAD51 enhances the CRISPR/Cas9-mediated knock-in efficiency in brain neurons

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ARTICLE INFO

Article history:
Received 21 January 2020
Accepted 22 January 2020
Available online xxx

Keywords:
CRISPR/Cas9
Homology directed repair
RAD51
In utero electroporation
β-Actin
camk2a

ABSTRACT

Gene knock-in using the CRISPR/Cas9 system can be achieved in a specific population of neurons in the mouse brain, by using *in utero* electroporation to introduce DNA fragments into neural progenitor cells. Using this strategy, we previously knocked-in the EGFP coding sequence into the N-terminal region of the β-actin gene specifically in the pyramidal neurons in layer 2/3 of the somatosensory cortex. However, the knock-in efficiency was less than 2% of the transfected neurons. In this study, we sought to improve the knock-in efficiency using this system. First, we varied the length of the homology arms of the β-actin donor template DNA, and found that the knock-in efficiency was increased to ~14% by extending the length of the 5′ and 3' homology arms to 1.6 kb and 2.0 kb, respectively. We then tested the effect of the DNA repair protein RAD51 and the knock-in efficiency was increased up to 2.5-fold when co-transfecting with two different β-actin and a camk2a targeting EGFP knock-in modules. The RAD51 overexpression did not alter the migration of developing neurons, density or morphology of the dendritic spines compared to those in neurons not transfected with RAD51. RAD51 expression will be useful for increasing the knock-in efficiency in neurons *in vivo* by CRISPR/Cas9-mediated homology directed repair (HDR).

1. Introduction

In utero electroporation is a powerful technique that enables genes to be introduced into neurons in specific cortical layers, by selecting the timing of electroporation into the neural progenitor cells residing in the ventricular zone [1–3]. This technique has been used to visualize the structures of neurons and to examine the function of genes in specific cortical layers, by introducing plasmid constructs expressing fluorescent marker proteins, wild-type or mutant proteins, or RNAi. Despite the many advantages of this technique, however, the possibility of artificial effects cannot be ruled out, because it involves the introduction of multiple copies of gene-expression constructs.

To overcome this problem, we previously developed a technique for knocking-in genes using the homology directed repair (HDR) mechanism of CRISPR/Cas9-mediated genome editing, combined with *in utero* electroporation [4]. We successfully applied this strategy to knock-in EGFP in-frame into the N-terminal region of the β-actin gene, specifically in a small population of pyramidal neurons in layer 2/3 of the somatosensory cortex in mice. The endogenous β-actin distribution pattern revealed strong EGFP signals in the dendritic spines of these neurons. Moreover, the density and electrophysiological properties were unaltered in the neurons overexpressing the EGFP-tagged β-actin expression construct. Other laboratories have performed similar studies [5,6]. However, the limitation of this technique is that the knock-in efficiency is low, which restricts its application. For example, extensive single-cell genotyping will be required to look for a few knock-in neurons from all transfected cells when introducing a point mutation of gene which does not have visible knock-in markers.

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https://doi.org/10.1016/j.bbrc.2020.01.132
0006-291X/