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ARTICLE

Targeted gene therapy into a safe harbor site in human hematopoietic progenitor cells

Fatima Rodriguez-Fornes[1] · Oscar Quintana-Bustamante[1] · M. Luz Lozano[1] · Jose C. Segovia[1] · Juan A. Bueren[1] · Guillermo Guenechea[1]

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Abstract

Directed gene therapy mediated by nucleases has become a new alternative to lead targeted integration of therapeutic genes in specific regions in the genome. In this work, we have compared the efficiency of two nuclease types, TALEN and meganucleases (MN), to introduce an EGFP reporter gene in a specific site in a safe harbor locus on chromosome 21 in an intergenic region, named here SH6. The efficiency of targeted integration mediated by SH6v5-MN and SH6-TALEN in HEK-293H cells was up to 16.3 and 15.0%. A stable expression was observed both in the pool of transfected cells and in established pseudoclones, with no detection of off-target integrations by Southern blot. In human hematopoietic stem and progenitor $CD34^+$ cells, the nucleofection process preserved the viability and clonogenic capacity of nucleofected cells, reaching up to 3.1% of specific integration of the transgene in colony forming cells when the SH6-TALEN was used, although no expression of the transgene could be found in these cells. Our results show the possibility to specifically integrate genes at the SH6 locus in $CD34^+$ progenitor cells, although further improvements in the efficacy of the procedure are required before this approach could be used for the gene editing of hematopoietic stem cells in patients with hematopoietic diseases.

Introduction

Although lentiviral vector (LV)-mediated gene therapy (GT) is offering clinical data demonstrating the safety and efficacy of this approach in monogenic diseases [1, 2], targeted GT approaches offer further improvements in the safety of GT [3]. The main approach used for targeted GT is based on homologous recombination (HR) [4, 5] promoted by the generation of double strand breaks (DSB) generated by engineered nucleases, such as Zinc-finger nucleases (ZFN), CRISPR/Cas system, meganucleases (MN) and transcription activator-like effector nucleases (TALEN) [6, 7]. Three different strategies of gene editing have been considered for the integration of a donor template in the target cells: gene correction [8, 9], targeted Knock-In [10–13], and gene targeting in a Safe Harbor (SH) [9, 14, 15]. The main advantage of the first two strategies is that the physiological expression of the corrected gene is preserved. However, gene targeting into SH loci could lead to the treatment of different diseases using the same engineered nucleases and just swapping the donor transgene.

Genomic SHs are defined as intragenic or extragenic chromosomal locations where therapeutic transgenes can be integrated to facilitate their function in a predictable manner, without perturbing the activity of the endogenous or surrounding genes [16]. The three main SH used in GT are located in intragenic regions (AAVS1 and CCR5 in human and Rosa26 in murine) and follow three main criteria, consisting in facilitating sufficient transgene expression, not predisposing targeted cells to a malignant transformation or to alterations of their cellular functions and offering a predictable outcome of targeted cells [16]. However, extragenic locations of the cell genome constitute alternative SH

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✉ Guillermo Guenechea
g.guenetxea@ciemat.es

[1] Division of Hematopoietic Innovative Therapies, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT)/Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER)/Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD, UAM), 28040 Madrid, Spain

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