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Efficiency of ubiquitous chromatin opening elements in driving the expression of human CD18 within self-inactivating lentiviral vectors for gene therapy applications

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Abstract

Patients with leukocyte adhesion deficiency type 1 (LAD1) suffer from recurrent bacterial infections due to mutations in the common β_2 integrin subunit (CD18/ITGB2 gene). Treatment options include long-term administration of antibiotics, repeated granulocyte infusions, allogeneic bone marrow or stem cell transplantations, all of which have considerable limitations. Gene therapy could bring about a potential permanent cure for LAD1. We tested different fragment of the ubiquitous chromatin opening element (UCOE) from the human HNRPA2B1-CBX3 locus for their efficiency in driving the expression of human CD18 gene. Twelve new self-inactivating (SIN) lentiviral vectors were constructed, 10 of which incorporating various fragments of the UCOE, two others containing the long and short fragments of the elongation factor 1 alpha promoter (EF1αL, 1169 bp; EF1αS 248 bp) and a murine stem cell virus (MSCV) promoter within the context of the same lentiviral vector. These vectors were tested *in vitro* for the expression of human CD18 on the surface of $CD34^+$ hematopoietic stem cells (HSCs) isolated from both moderate and severe LAD1 patients. Among the promoters tested in moderate patient's $CD34^+$ HSCs, 3'631 bp, 3'652 bp, 3'1262 bp, A2UCOE and EF1αS resulted in higher percentage of $CD18^+CD34^+$ cells (11.4% to 15.1% at MOI 10; 12.7% to 16.5% at MOI 100), comparable to the expression driven by the MSCV promoter (15.2% at MOI 10; 16.1% MOI 100). The 5'655 bp, 5'723 bp, 5'1296 bp, 2598 bp and EF1αL promoters resulted in comparatively lower levels of CD18 expression (10.4% to 11.1% at MOI 10; 5.7% to 10.9% at MOI 100). All the 3' promoter fragments of the UCOE were further tested in a severe LAD1 patient's $CD34^+$ HSCs (1.6% to 5% at MOI 10 and 1% to 3.4% at MOI 100). Results obtained from this study would be useful in examining the human CD18 expression on murine LAD1 $CD34^+$ HSCs *in vitro* followed by *ex vivo* studies to demonstrate the phenotypic correction of LAD1 in a murine model. Efforts are underway to compare the efficiency of gene correction using this conventional gene therapy approach with CRISPR-mediated gene correction of CD18 in human LAD1 $CD34^+$ HSCs *in vitro*.

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