

Construction and evaluation of a specific nonviral vector for ex-vivo gene correction of human β -globin gene defects

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Background and Aims: We have designed and constructed a nonviral tissue-specific vector, named pHBB, containing a copy of wild type β -globin gene for ex-vivo gene transfer and expression of β -globin chain to compensate hemoglobin production defects in hematopoietic precursors of patients with β -thalassemia or sickle cell anemia.

Methods: This vector was designed and constructed containing two main parts: a bacterial backbone for vector amplification in *E. coli* and a puromycin resistance ORF as a eukaryotic selectable marker. Second part contains an expression cassette including β -globin gene enhancers, promoter and complete β -globin gene sequence along with both UTRs. Moreover this vector includes a specific phiC31 integrase site. pHBB co-transfection with another vector encoding phiC31 integrase enables pHBB to integrate into specific sites in the human genome. Also two loxP sites flanking of bacterial backbone and puromycin resistant ORF were designed to facilitate deletion of these sequences by Cre recombinase after integration of pHBB into the genome of target cells that reduces possible host immune responses and potential genotoxicity.

Results: The structure of the vector was confirmed through several steps of digestion experiments and sequence analysis. Functional analysis of the recombinant plasmid was successfully achieved by stable transfection of the vector into genome of a hematopoietic cell line, K562, which is β -globin promoter specific cell line and potentially able to express β -globin gene. The expression of target gene in this cell line was evaluated by immunocytochemistry, real-time PCR and western blot methods.

Conclusions: Regarding to high rate and persistent expression of β -globin by this vector in the K562 cell line, it seems pHBB can be assumed as an appropriate vector for ex vivo gene transfer into hematopoietic precursors of patients with β -thalassemia or sickle cell anemia to improve β -globin expression that results hemoglobin concentration increment in red blood cells in this patients.

Keywords: β -thalassemia; Nonviral vector; β -globin; phiC31; K562