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Transfection of human beta-globin gene into the AAVS1 locus of K562 cell line using CRISPR/Cas9 technology

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Beta thalassemia is a kind of hereditary illness, which is due to an impairment in production of globin chain .There is a need to find a suitable treatment for this disease in respect to the both high prevalence of the disease in Iran and the lack of proper remedies until now. CRISPR-Cas9 is a new promising genome editing technology having high efficiency in gene correction. In this study, we inserted the human beta-globin gene site specifically into the AAVS1 locus using AAVS1 Transgene knock-in vector kit (Origen). At first, we amplified the health beta globin gene from human blood sample with PCR. At the next step, the beta-globin amplicon was cloned in the pTG19-T vector. The fragment was then sub cloned into the pAAVS1-puro-DNR vector using NotI restriction enzyme and sequenced. Second part of our study was transfection of K562 cells with the kit vectors. For this purpose, the K562 cells were cultured in RPMI 1640, FBS 10% and 1% penicillin-streptomycin and incubated at 37 °C, 5% CO2 and 95% humidity. Transfection was optimized using pEGFP-C1 plasmid in electroporation by examining different electrical pulses and concentration of vectors. In addition, for minimal fatal concentration of puromycin, the K562 cells were exposed with different dose of the antibiotic. We confirmed the locus-specific integration of the transgene using PCR and specific primers. Now, we are evaluating the ability to express of beta-globin transgene.

Biography

Elahe Alikhani is currently pursuing his master's in medical biotechnology in Iranian Blood Transfusion Organization, Iran. She has completed his graduation from Tabriz University in Molecular Biology.

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