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RESEARCH ARTICLE

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Creation of an in vitro model of GM1 gangliosidosis by CRISPR/Cas9 knocking-out the *GLB1* gene in SH-SY5Y human neuronal cell line

Kamran Hosseini[1] | Jafar Fallahi[1] | Hadi Aligholi[2] | Zahra Heidari[1] | Elham Nadimi[1] | Fatemeh Safari[3] | Mohsen Sisakht[1] | Amir Atapour[4] | Sahar Khajeh[5] | Seyed Mohammad Bagher Tabei[6] | Vahid Razban[1,7]

[1]Department of Molecular Medicine, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran

[2]Department of Neuroscience, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran

[3]Diagnostic Laboratory Sciences and Technology Research Center, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran

[4]Department of Medical Biotechnology, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran

[5]Bone and Joint Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

[6]Department of Medical Genetics, School of Medical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran

[7]Stem Cells Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Correspondence

Vahid Razban, Department of Molecular Medicine, Faculty of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran.
Email: razban_vahid@yahoo.com

Seyed Mohammad Bagher Tabei, Department of Medical Genetics, School of Medical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran.
Email: tabeismb@sums.ac.ir

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Abstract

GM1 gangliosidosis is one type of hereditary error of metabolism that occurs due to the absence or reduction of β-galactosidase enzyme content in the lysosome of cells, including neurons. In vitro, the use of neural cell lines could facilitate the study of this disease. By creating a cell model of GM1 gangliosidosis on the SH-SY5Y human nerve cell line, it is possible to understand the main role of this enzyme in breaking down lipid substrate and other pathophysiologic phenomena this disease. To knock-out the human *GLB1* gene, guides targeting exons 14 and 16 of the *GLB1* gene were designed using the CRISPOR and CHOP-CHOP websites, and high-efficiency guides were selected for cloning in the PX458 vector. After confirming the cloning, the vectors were transformed into DH5α bacteria and then the target vector was extracted and transfected into human nerve cells (SH-SY5Y cell line) by electroporation. After 48 h, GFP^+ cells were sorted using the FACS technique and homozygous (compound heterozygous) single cells were isolated using the serial dilution method and sequencing was done to confirm them. Finally, gap PCR tests, X-gal and Periodic acid-Schiff (PAS) staining, and qPCR were used to confirm the knock-out of the human *GLB1* gene. Additionally, RNA sequencing data analysis from existing data of the Gene Expression Omnibus (GEO) was used to find the correlation of *GLB1* with other genes, and then the top correlated genes were tested for further evaluation of knock-out effects. The nonviral introduction of two guides targeting exons 14 and 16 of the *GLB1* gene into SH-SY5Y cells led to the deletion of a large fragment with a size of 4.62 kb. In contrast to the non-transfected cell, X-gal staining resulted in no blue color in *GLB1* gene knock-out cells indicating the absence of β-galactosidase enzyme activity in these cells. Real-time PCR (qPCR) results confirmed the RNA-Seq analysis outcomes on the GEO data set and following the *GLB1* gene knock-out, the expression of its downstream genes, *NEU1* and *CTSA*, has been decreased. It has been also shown that the downregulation of *GLB1-NEU1-CTSA* complex gene was involved in suppressed proliferation and invasion ability of knock-out cells. This study proved that using dual guide RNA can be used as a simple and efficient tool for targeting the

NEU1, a finding validated by qPCR. These observations are consistent with the RNA-Seq data and support previously published reports.[19,34-36] Therefore, until now, no study has been reported using bioinformatics analysis to accurately examine the relationship between*GLB1* gene expression and downstream genes, and for the first time, this study investigated the relationship between the expression of these genes using RNA-Seq data.

As explained in the previous sections, the ternary complex of elastin binding protein, neuraminidase-1, and cathepsin-A acts as an elastin receptor complex (ERC) on the cell surface (in addition to their lysosomal activity) and plays an important role in the maintenance and stability of the ECM. ERC acts in the formation of elastin fibers through the EBP subunit of the ternary complex and following the reduction of EBP expression in the cell, the function of the ternary complex is disrupted and its connection with the ECM is interrupted.[37] A decrease in EBP gene expression leads to a reduction in cell movement and cell migration. The results of the present investigation confirmed the previous studies on the role of each component of the ternary complex in the invasion, proliferation, and migration of different cancer cells.[38-41] Hu et al. also showed that decreased expression of cathepsin A protein (*CTSA* gene), one of the proteins involved in the ERC complex, reduced the proliferation and invasion of lung adenocarcinoma cells.[38] similarly, Zhou et al. found that following the knockdown of NEU1 protein in melanoma cells, the proliferation and invasive activity of cells in vitro decreased, as well as the growth and progression of cancer tumors in vivo following the change in the expression of proteins such as CDK2, CD44, CASP3, and CASP8 decreased. On the other hand, the reason and novelty behind choosing these two genes (*NEU1* and *CTSA*) is bioinformatic analysis of *GLB1* downstream genes which have been proved experimentally by real time PCR in the present study.

5 | CONCLUSION

In summary, we developed and phenotyped a knock-out cell model (*GLB1*$^{-/-}$) of GM1 gangliosidosis that reflects the events in the cells of GM1 gangliosidosis patients, such as the absence or extensive reduction of the β-gal enzyme activity. The present study is the first reported study (in vitro) on human nerve cells (SH-SY5Y cell line) to create a cell model of GM1 gangliosidosis and investigate the possible consequences of *GLB1* gene knock-out functionally and at the transcription level in cells and confirm these consequences by X-gal staining, scratch assay, transcriptome analysis, and qPCR assay. Previous studies have mostly focused on in vivo disease models (including *Glb*$^{-/-}$ mouse and organoid models),[24] and iPSCs[25] and have aimed to increase lifespan and reduce pathological symptoms of the disease. Therefore, our cell model can be used as a tool for further studies by researchers in the field of diagnosis and treatment of GM1 gangliosidosis.

AUTHOR CONTRIBUTIONS

The core idea of this study came from KH and VR. They also directed the other authors and analyzed the collected papers. KH is primarily responsible for writing the manuscript of the article. Researchers such as J.F, Z.H, E.N, F.S, and M.S have cooperated during this project. Final editing was done by JF, VR, H.A, S.K and SMBT. "All authors reviewed the manuscript."

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. All data generated or analyzed during this study are included in the final published article.

ORCID

Kamran Hosseini http://orcid.org/0000-0003-1189-7143
Fatemeh Safari http://orcid.org/0000-0001-6792-8987
Sahar Khajeh http://orcid.org/0000-0003-1614-2962
Seyed Mohammad Bagher Tabei http://orcid.org/0000-0001-9923-6969
Vahid Razban http://orcid.org/0000-0002-8966-6081

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