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P1434 CORRECTION OF CONGENITAL NEUTROPENIA-RELATED GENES IN NAÏVE IPS CELLS DERIVED FROM PATIENTS USING VIRAL-FREE CRISPR/CAS9 GENE-EDITING SYSTEM

Topic: 24. Gene therapy, cellular immunotherapy and vaccination - Biology & Translational Research

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Background:

Severe congenital neutropenia (SCN) is a rare haematological condition with a heterogeneous genetic background that often leads to severe impairment of the immune system. For patients who do not respond to standard treatment, the only possible therapy is allogeneic haematopoietic stem cell transplantation. SCN patients have an increased risk of the development of myelodysplastic syndrome or acute myelogenous leukaemia. Rare genetic disorders, such as SCN, require prioritising bone marrow failure diagnosis and therapy tailored to individual patients. Human induced pluripotent stem (iPS) cells are a promising prospect for future cell therapy in a wide range of diseases like congenital neutropenia for which there are currently no cures or effective therapies.

Aims:

The aim of this study was to develop potential treatment procedure based on the use of iPS cells generated from epithelial cells of patients with congenital neutropenia. Using mutation-specific gRNA and CRISPR-Cas9 system, we made an attempt to repair patients-derived iPS cells which may have potential for renewal of the corrected granulocyte population in the bone marrow.

Methods:

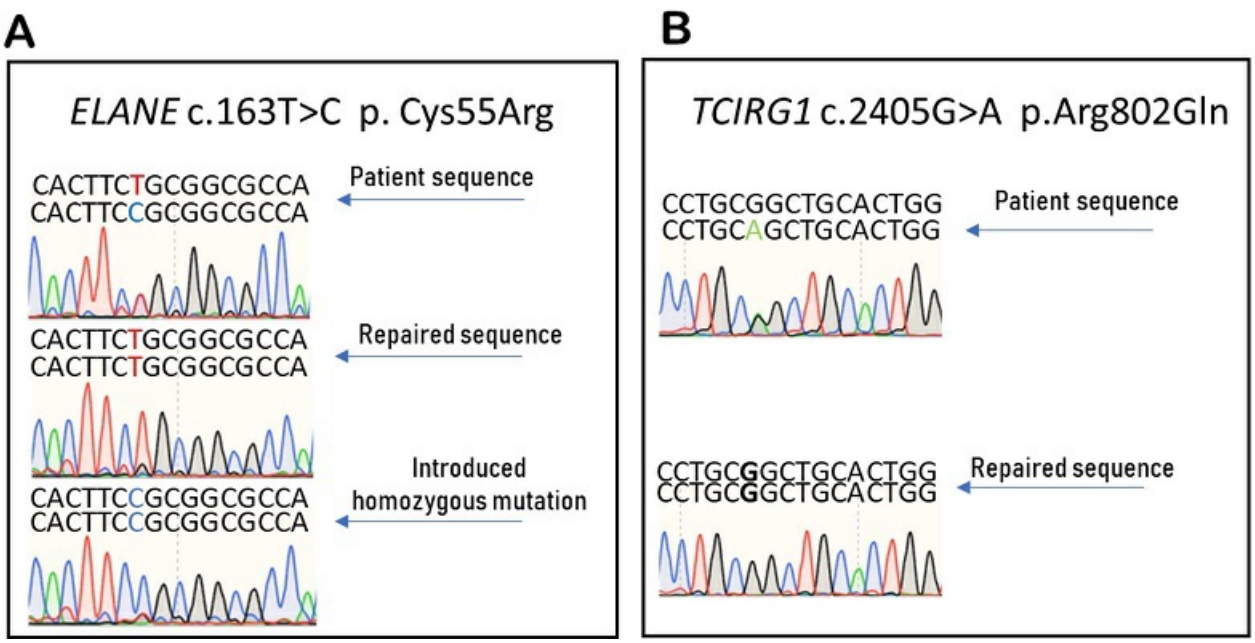
Patient-derived epithelial cells were reprogrammed with the EBV-derived oriP/EBNA-1 system carrying transcription factors. The transition of these cells into the naïve state was accomplished with the use of cell culture media supplemented with Leukaemia inhibitory factor, an inhibitor of Protein kinase C, small molecule activators of WNT, and inhibitors of ERK1/2 and JNK signaling pathways. CRISPR-Cas9 was used to repair mutations in patients-derived naïve iPS cells. Into the target cells, a specific sgRNA combined with eSpCas9 protein and HDR template was introduced via nucleofection with Lonza 4D Nucleofector, as a CRISPR enhancer, brefedrin A was used. To verify the efficiency of transfection and gene edition, the targeted region were analysed with Sanger sequencing (Fig. 1).

Results:

We have generated primed and naïve iPS cells from 7 patients with different severity of neutropenic phenotype and genetic background, 4 another samples are pending. The morphology of the iPSCs line (primed cells) displayed a typical ESC-like colony appearance comprised of tightly packed cells. Alkaline phosphatase and immunocytochemical staining confirmed that the reprogrammed cells express pluripotent stem cell markers and can differentiate into the three germ layers.

We successfully repaired a mutation in *ELANE* gene in 4 out of 30 screened colonies as well as *TCIRG1* gene in 3 out of 14 screened colonies. Additionally, for further functional studies, we generated a homozygotic *ELANE* p.Cys55Arg mutant, that might serve as a unique model of the genotype-phenotype gradient of elastase activity.

Image:



Summary/Conclusion:

We have successfully generated and repaired iPS cells derived from patients with congenital neutropenia which might serve as a procedure with a great potential in the field of cell therapy tailored to individual patients.