The Official Journal of the Italian Society for the Study of Hemostasis and Thrombosis
SISET
Società Italiana per lo Studio dell'Emostasi e della Trombosi
Corso Nazionale SISET
Bergamo | 23-25 ottobre 2025

29TH NATIONAL CONFERENCE OF THE ITALIAN SOCIETY FOR THE STUDY OF HEMOSTASIS AND THROMBOSIS, 2025

ABSTRACT N. CS06

LE MALATTIE EMORRAGICHE CONGENITE

PATIENT SPECIFIC IPSCS-DERIVED SINUSOIDAL ENDOTHELIAL CELLS FOR PHENOTYPIC CORRECTION OF HAEMOPHILIA A.

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Background and Aims: Hemophilia A (HA) is a rare X-linked recessive bleeding disorder caused by variants in the *F8* gene, which encodes coagulation factor VIII (FVIII). It affects approximately one in 6,000 males. The severity of HA is determined by the level of plasma FVIII activity and is classified as severe (<1%), moderate (1%–5%), or mild (5%–40%). In patients with HA, increasing FVIII activity to above 2–5% can significantly improve quality of life.
The aim of this study was to restore FVIII levels to prevent spontaneous bleeding by correcting FVIII variants using CRISPR-Cas9 base editing in liver sinusoidal endothelial cells (LSECs), which are recognized as a key FVIII producers' cells.

Methods: Peripheral blood mononuclear cells (PBMC) from 3 HA patient and 2 healthy subjects were isolated and reprogrammed into induced pluripotent stem cells (iPSC) after transduction in a feeder-free culture with a non-integrative system with Sendai virus. The clones obtained were stabilized using an enzymatic method with EDTA and characterised for their pluripotency markers expression. Gene correction of the HA-iPSCs will be carried out using CRISPR-Cas9 DNA base editing technologies with specially target of FVIII variations. The following gene variants NM_000132.4:c.6593G>T, NM_000132.4:c.4285C>T and NM_000132.4:c.5373+1G>A were considered. Subsequently, HA-iPSCs will be differentiated into liver sinusoidal endothelial cells (LSECs) and hepatocyte-like cells (HLCs) using an optimized differentiation protocol involving BMP4 and VEGF and others growth factors. Specific cells markers were analysed and the expression of FVIII, LSEC and HLCS markers, were evaluated using immunofluorescence (IF) and real-time PCR (RT-qPCR).

Results: Obtained iPSCs was positive for alkaline phosphatase staining and immunofluorescence showed the expression of stem cells markers (Oct4, SeV, Sox2, Klf4, KOS and cMyc). The LSECs and HLCs differentiated obtained cells were stained positive for the expression of CD31, STAB2 and LYVE1 and cytokeratin 18 respectively. Following RT-qPCR and flow cytometry analysis expression of the above markers confirmed the obtained cells phenotype. Furthermore, the expression of FVIII were assessed by IF and flow cytometry in the HA-patients obtained cells before and after gene editing and the correction was confirmed.

Conclusions: In this study, we generated specific iPSCs from HA-patients carrying different *F8* gene variants, derived from PBMCs. These iPSCs were then differentiated in vitro into LSECs and HLCs using optimized protocols. CRISPR-Cas9 mediated gene correction enabled us to demonstrate that specific disease-causing variants can be effectively repaired, resulting in the restoration of FVIII expression.
The LSECs and HLCs obtained through these protocols represent a useful in vitro model for evaluating FVIII restoration in HA-patients with different *F8* gene variants. Given the limitations of current treatments, new strategies for a definitive cure are urgently needed. Gene and cell therapies offer promising avenues and could provide a powerful, personalized therapeutic approach to optimize treatment for each individual HA-patient.

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