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EXPLORING THE PATHOGENETIC BASIS OF ICF SYNDROME WITH HUMAN INDUCED PLURIPOTENT STEM CELLS

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The Immunodeficiency, Centromeric Instability, Facial anomalies (ICF; OMIM 242860) syndrome is a genetically heterogeneous rare autosomal recessive immunological/neurological disorder characterized by loss of DNA methylation and chromosome instability, mainly involving the (peri)centromeric repeats (1,2). Hypomorphic biallelic mutations in DNMT3B gene impair the catalytic activity of the major de novo DNA methyltransferase active during early development, resulting in hypomethylation across the genome (3-6). These mutations account for approximately 60% of cases classified as ICF syndrome type1 (ICF1) patients (2). Explaining the molecular mechanisms underlying ICF1 pathogenesis has remained difficult due to the lack of well-suited cellular models. Recent generation of ICF1 patients'-derived induced pluripotent stem cells (ICF1-iPSCs) provided an appropriated model for studying the early-stage pathogenetic mechanisms of ICF1 syndrome and for deciphering the genomic targets that are affected during early development (7).
By performing gene-editing through the CRISPR/Cas9 technology we generated isogenic iPSC cell lines with corrected DNMT3B mutations from patients carrying missense and/or null mutations that disrupt the catalytic domain of DNMT3B.
Focusing on repetitive regions, we show that in contrast to pericentromeric repeats, which reacquire normal DNA methylation in corrected clones, the majority of subtelomeres acquire only partial DNA methylation and, accordingly, the telomeric ICF1 phenotype persists. Subtelomeres resistant to de novo methylation were characterized by abnormally high H3K4 trimethylation (H3K4me3), and short-term reduction of H3K4me3 by pharmacological intervention partially restored subtelomeric DNA methylation. These findings demonstrate that the abnormal epigenetic landscape established in ICF1 cells restricts the recruitment of DNMT3B (8).
To verify whether and at what extent the DNA methylation at early-stage DNMT3B targets is restored across the genome following the editing, the ICF1-iPSCs and their corrected counterparts are currently examined through an integrated epigenomic and transcriptomic approach.
ICF syndrome is considered primarily as a humoral immunodeficiency disease; however, this does not explain the high rate of opportunistic infections. It has been suggested that an additional intrinsic T-cell deficiency and a lymphocyte proliferation defect are present in individual ICF1 patients (9). Therefore, in vitro differentiation of ICF1-iPSCs towards hematopoietic progenitors and lymphoid lineage is anticipated to provide an efficient tool to model and understand disease-related mechanisms and in perspective to implement cellular transplantation therapeutic approaches (10). Accordingly, we generated CD45+ enriched progenitors from iPSCs and plan to develop suitable approaches to further differentiate the CD45+ cells toward the B-cell lineage.

La sindrome da Immunodeficienza, instabilità Centromerica e anomalie Facciali (ICF; OMIM 242860) è una malattia genetica a trasmissione autosomica recessiva estremamente rara descritta finora in

circa 70 pazienti. Ad oggi sono stati identificati quattro geni, le cui mutazioni spiegano quasi totalmente i casi di sindrome ICF finora noti (ICF 1-4). Oltre il 60% dei pazienti presenta mutazioni missenso e nonsenso in eterozigosi nel gene codificante la DNA metiltrasferasi de novo 3B (DNMT3B; ICF1), che interferiscono severamente con l'attività catalitica della proteina, determinando ipometilazione variabile nel genoma.
La malattia si manifesta prevalentemente nella prima infanzia con una grave immunodeficienza che comporta estrema suscettibilità alle infezioni respiratorie e/o del tratto gastrointestinale e morte in età pediatrica. Nei pazienti si riscontrano inoltre specifiche alterazioni cromosomiche, e frequentemente difetti di crescita, ritardo psicomotorio e lievi dismorfismi. La sindrome ICF è molto probabilmente sotto-diagnosticata, specialmente in casi con fenotipo incompleto e/o in casi sporadici.
Essendo una malattia estremamente rara il reclutamento di cellule primarie da pazienti è particolarmente difficile. A questa difficoltà si aggiunge che per questa sindrome i modelli murini umanizzati attualmente disponibili non riproducono i difetti immunologici caratteristici della patologia. Tenuto conto di queste forti limitazioni, abbiamo proposto l'applicazione di una strategia adatta a studiare i meccanismi patogenetici di sindromi complesse quali l'ICF, ovvero l'utilizzo di cellule staminali pluripotenti indotte (iPSCs). A tale scopo, sono state recentemente generate iPSCs da fibroblasti di individui affetti portatori di mutazioni nel gene DNMT3B. Queste cellule sono state utilizzate per studiare i meccanismi patogenetici alla base dei difetti della risposta immunitaria tipici della sindrome ICF. In particolare, è stato valutato il potenziale differenziativo delle ICF1-iPSC verso precursori ematopoietici, al fine di esaminare l'effetto delle mutazioni in DNMT3B durante il differenziamento. Inoltre, esse sono state impiegate in un protocollo di terapia cellulare per la correzione in vitro di tali mutazioni mediante la tecnologia CRISPR/Cas9. Tale modello cellulare fornirà strumenti di alto valore scientifico adatti a rispondere a questioni peculiari della malattia altrimenti non esplorabili nelle cellule del sangue derivanti dai pazienti.

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Sindrome da Immunodeficienza, instabilità Centromerica, anomalie Facciali (ICF)

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