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TARGETED GENOME EDITING IN RECOMBINATION ACTIVATING GENE 1 (RAG1): A PRECISE CORRECTION OF THE GENETIC DEFECT IN HUMAN SCID

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Defects in Recombination activating gene 1 and 2 (RAG) result in a broad spectrum of clinical manifestations including a complete block in T and B cells differentiation, leaky severe combined immunodeficiency (SCID) or atypical SCID with granuloma1. Hematopoietic stem cell transplantation (HSCT) is the treatment of choice, however its success is limited by the availability of compatible donors and the severe side-effects caused by the myeloablative conditioning required to achieve long-term immune reconstitution 2,3. Conventional gene therapy is an attractive therapeutic option; but its applicability is constrained by the need of a physiological expression of RAG1 gene. Preclinical data indicate that a low RAG1 expression results in severe immune dysregulation, while high vector copy number may lead to the risk of insertional mutagenesis caused by integrating viral vector 4,5,6To overcome these problems, we set up a gene editing platform based on engineered nucleases to restore the expression of the corrective human RAG1 cDNA under the physiological control of its endogenous promoter. To this purpose, we developed a gene editing strategy by targeting the intronic region located at the 5' of the second exon of the gene, which contains the entire coding sequence thus allowing the correction of all pathological mutations. We identified the best performing CRISPR-Cas9 ribonucloprotein complex that allows a high level of cutting activity (by NHEJ-mutagenesis assay) and efficient editing (homology directed repair assay) in K562 and NALM6 cell lines. In parallel, we have developed an adeno associated virus type 6 (AAV6) donor DNA carrying the human codon optimized RAG1 cDNA followed by BGH polyA sequence. Currently, we are validating this platform in human CD34+ cells obtained from cord blood and mobilized peripheral blood of normal donors and RAG1 patients by in vitro and in vivo analyses. In parallel, we identified the minimal number of gene-targeted cells necessary to achieve therapeutic levels of immune reconstitution by competitive transplantation in Rag1-/- mice and in parallel we tested novel conditioning regimen. We demonstrated that 10-20% of wild type Lineage negative cells is required to obtain immune reconstitution in Rag1-/- mice. Finally, mice treated with non genotoxic conditioning mediated by antiCD45-Saporin7 showed robust immunological reconstitution comparable to fully myeloablative conditioning, while preserving central lymphoid organs.

Difetti nei geni Rag1 e Rag2 causano un ampio spettro di manifestazioni cliniche che vanno dalla assenza di linfociti T e B, alle forme di SCID atipica alle immunodeficienze con granuloma. Il trapianto di midollo osseo è la terapia di elezione, tuttavia il suo successo è limitato dalla disponibilità di donatori compatibile e da possibili effetti secondari del condizionamento. La terapia genica potrebbe costituire una possibile strategia, tuttavia il suo successo è limitato in quanto il gene RAG1 è regolato finemente durante il ciclo cellulare. Esperimenti di terapia genica hanno dimostrato come bassa espressione del gene Rag1 porti a immune disregolazione, mentre elevate espressione causata da alto vector copy number potrebbe portare a instabilità genomica. Per superare tali problemi abbiamo

quindi allestito una piattaforma di editing, targettando la regione al 5' dell'esone 2 che contiene tutta la regione coding di RAG1 e dove mappano la maggior parte di tutte le mutazioni finora riportate in letteratura. Abbiamo pertanto identificato la migliore complesso ribonucleoproteico CRISPR-Cas9 con alto livello di cutting valutato come test di mutagenesi NHEJ ed efficiente editing (homology directed repair assay) in linee cellulari K562 e NALM6. In parallelo abbiamo sviluppato un vettore Adeno associato (AAV6) che porta un donor DNA contenente il cDNA umano di RAG1 codon optimized seguito dalla sequenza di polyA. Stiamo ora validando tale piattaforma in cellule CD34 ottenute da cordone e da mobilizzato facendo sia saggi in vitro che in vivo. In parallelo abbiamo identificato il minimo numero di cellule necessarie per raggiungere livelli terapeutici di immunoricostituzione mediante esperimenti di trapianto competitivo in topi Rag1-/-. Inoltre abbiamo testato nuovi regimi di condizionamento usando un composto non genotossico mediante anticorpo diretto contro CD45 coniugato con immuno tossina (saporin). I topi trattati con tali composti hanno dimostrato un buon livello di immunoricostituzione simile a quello raggiunto da regimi di condizionamento mieloablativo, ma al contempo preservando la morfologia degli organi linfoidi7.)

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Immunodeficienza combinata da difetto RAG1

Coordinator: Anna Villa
Duration (N. Years): 5
Starting year: 2016

Telethon Project (nr):

TGT16E02

Disease Name:

RAG1 Severe Combined Immunodeficiency

Keywords:

RAG1 defect, Gene Editing, Severe Combined Immunodeficiency