A gene therapy approach for the threatment of Inborn Errors of Immunity caused by mutations in large genes

Elisabetta Toriello[1], Antonio De Rosa[1], Carmen Rosano[1], Cosimo Abagnale[1], Rosa Maritato[1], Alessia Medugno[1], Roberta Romano[1], Emilia Cirillo[1], Enrico Maria Surace[1], Claudio Pignata[1], Giuliana Giardino[1]

[1]University of Naples "Federico II", Department of Translational Medical Sciences, Naples, Italy

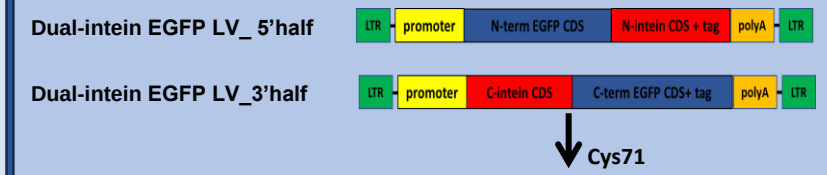
Mail to elisabetta.toriello@unina.it, giuliana.giardino@unina.it

BACKGROUND AND AIM

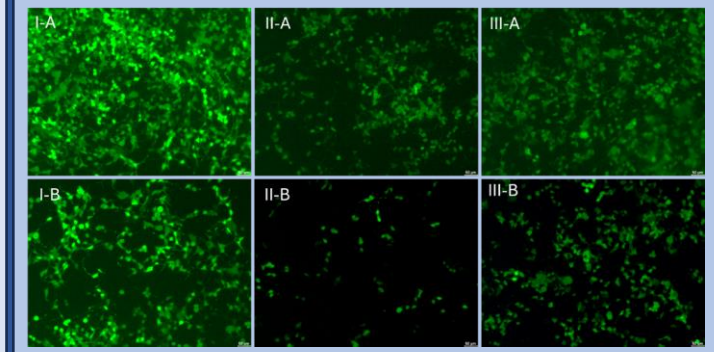
Inborn errors of immunity (IEIs) are rare genetic disorders caused by mutations in genes critical for immune cell function, development or signaling pathways. Gene therapy represents a potential curative treatment for these conditions, with lentiviral vectors (LVs) emerging as a promising tool in this field. However, one of the significant limitations related to the use of LVs relies in their cargo capacity limited to around 8 kilobases. This prevents their application for the treatment of conditions requiring the delivery of larger DNA sequences and the stable expression of the transgene in actively proliferating cells. Recent studies have shown that protein trans-splicing is a powerful tool for expanding the cargo capacity of AAV vectors upon co-infection of a host cell with different vectors, each expressing a split-intein flanked portion of the full lenght- protein. Aim of the present study is to demonstrate the feasibility of the split-inteins-mediated protein trans-splicing technology to the platform of LVs.

RESULTS

1) As proof-of-principle we developed dual- intein LVs, each expressing either the N- or the C-terminal half of the EGFP reporter protein fused to the N- and C- terminal halves of the DnaE split-inteins from the cyanobacterium Nostoc punctiforme, under the control of EF1A promoter. EGFP CDS was splitted at Cys 71, that has been previously demonstrated by Tornabene et al., 2019 to be very efficient in the trans-splicing process.

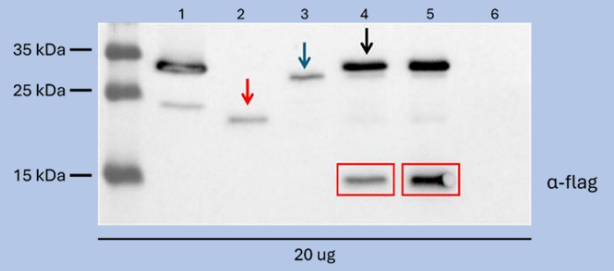


2) Dual intein EGFP LVs were used to infect HEK293 cells at different Multiplicity of infection (MOI) and evaluate the production of the full-length EGFP protein. As expected, 72 hours post-infection EGFP fluorescence was detected in all cells infected either with a single LVs that encodes full-length EGFP or with the combination of the two EGFP intein LVs at all the tested MOIs.



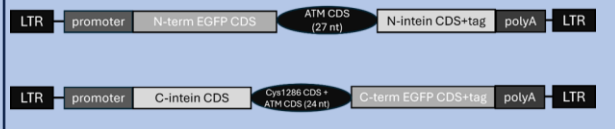
I: HEK293 cells infected with EGFP LV at **A)** MOI 5 and **B)** MOI 3; **II:** HEK293 cells co-infected with the dual-intein EGFP LVs at **A)** MOI 2,5 each vector **B)** MOI 1,5 each vector; **III:** HEK293 cells co-infected with the dual-intein EGFP LVs at **A)** MOI 5 each vector **B)** MOI 3 each vector. The image is representative of N=3 independent experiments.

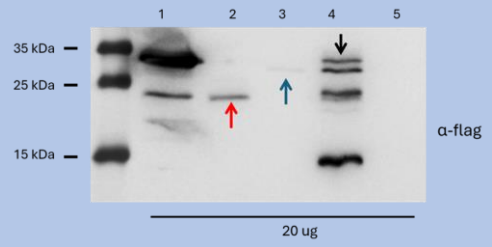
3) Trans-spliced EGFP protein of the expected size (~30 kDa) was detected by Western blot analysis of HEK293 cell lysates following co-infection only with both pLenti-EGFP intein LVs.



1.EGFP full-lenght LVs at MOI=5;
2. dual-intein N-term EGFP LV at MOI=5;
3. dual-intein C-term EGFP LV at MOI=5;
4. dual-intein N-term and C-term EGFP LVs at MOI= 2,5 aech vector;
5. dual-intein N-term and C-term EGFP LVs at MOI= 5 aech vector;
6. untreated cells.

4) Four potential splitting points for the ATM transgene were selected and tested using EGFP reconstitution as a reporter system. For Cys1286, although no fluorescence was detected in co-transfected HEK293 cells, Western blot analysis confirmed successful splicing and production of full-length EGFP fused to 18 ATM-derived amino acids.





CONCLUSIONS

Intein-mediated protein trans-splicing enables efficient reconstitution of full-length EGFP, validating this approach as a proof-of-concept for targeting large genes. This platform holds promise for the development of lentiviral gene therapies for Inborn Errors of Immunity i.e. LRBA deficiency or Ataxia Telangiectasia, caused by mutations in genes exceeding LVs packaging limit.