PROGETTO

Supervisore:: Vania Broccoli

Titolo/*Title*: Isolating and validating new engineered AAV vectors for efficient brain transduction and efficacious gene therapy in a Rett disease mouse model

Curriculum: Terapia Genica e Cellulare/*Gene and Cell Therapy*

Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento: https://research.hsr.it/en/divisions/neuroscience/stem-cells-and-neurogenesis/vania-broccoli.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

Adeno-associated virus (AAV) vectors are commonly employed delivery systems for *in vivo* gene therapy in preclinical and clinical studies. However, they have a poor tropism for the brain while accumulating in the liver when administrated intravenously raising important concerns of toxicity (Hudry and Vandenberghe, 2019). We generated a new peptide display library and performed an Iterative *in vivo* selection for superior neurotropic viruses after intravenous delivery. With this method we identified two novel variants, named AAV-Se1 and AAV-Se2, that outperformed the parental natural capsid and maintained high transduction efficiency in mice (C57BL/6 and BALBc strains) and in adult marmosets (Giannelli, Luoni et al., iScience 2024).

Rationale and hypothesis

Novel AAV vectors will be exploited for establishing a gene therapy protocol for treating a mouse model of Rett syndrome. This is a serious neurodevelopmental disease leading to cognitive disabilities, loss of speech and autistic behavior lacking of a cure. Thus, this project will evaluate the therapeutic potential of these novel viruses to rescue morphological, behavioral and functional deficits exhibited by these mice over time. Moreover, novel AAV capsid library screenings will be carried out to identify more AAV variants with desiderable properties to enhance their transduction efficiency in the brain and selective neural cell populations.

Objectives and specific aims

We will establish a new gene therapy approach based on AAV-Se2 intravascular delivery expressing a functional copy of Mecp2 in Rett mutant mouse brains (Luoni et al., 2020). Symptomatic rescue will be scored in adult Rett animals treated with either standard AAV9 or AAV-Se2 to determine the advantages of using an engineered capsid for reverting the neurological deficits. Since MeCP2 gene duplication leads to severe brain dysfunctions in patients (Lioy et al., 2011), the AAV-Se2 capsid will be used to overexpress Mecp2 in vivo either in

neurons or astrocytes to determine the transcriptional changes and the phenotypic alterations in the treated mice. More AAV-Se2 viral doses will be inoculated in the mice to reach different levels of Mecp2 overexpression to determine the exact range of transgene expression where no side effects are observed and can be considered safe. Finally, more AAV viral screenings will be carried out with different strategies to further select competitive AAV vectors with new features to improve brain transduction and enhance transduction of selective neural cell types inclusind neural and glial cells.

Expected outcomes

This project will establish a new gene therapy approach with relevant translational potential to rescue neurological deficits in Rett mutant mice. This investigation will determine the superiority of the new viral vectors in achieving significant benefits in the Rett mice at the symptomatic stage. Moreover, subsequent AAV library screenings will isolate novel viral variants with enhanced properties in crossing biological barriers or infect specific neural cell types.

Competenze che deve acquisire lo studente (Max 600 caratteri spazi inclusi):

The student will develop strong expertise in:
- Molecular cloning and gene engineering
- AAV vector cloning, production and quality assessment
- Manipulation and analysis of mouse models of Rett syndrome
- Brain tissue isolation and histological analysis
- Genome-wide bulk and single-cell transcriptomics studies
- Basic knowledge of bioinformatics tools
- Immunofluorescence and image analysis
- Statistics and data interpretation

Bibliografia (max. 15)

- Giannelli G, Luoni M, Bellinazzi B, Iannielli A, Philippens I, Korbelin J., Broccoli V. New AAV9 engineered variants with enhanced neurotropism and reduced liver off-targeting in mice and marmosets. iScience 2024.

- Hudry E and Vandenberghe LH. Therapeutic AAV gene transfer to the nervous system: a clinical reality. Nueron 101, 839-862, 2019.

- Lioy DT, Garg SK, Monaghan CE, Raber J, Foust KD, Kaspar BK, Hirrlinger PG, Kirchhoff F, Bissoette JM, Ballas N, Mandel G. A role for glia in the progression of Rett's syndrome. Nature 475: 497-500, 2011.

- Luoni M, Giannelli S, Indrigo MT, Niro A, Massimino L, Iannielli A, passeri L, Russo F, Morabito G, Calamita P, Gregori S, Deverman B, Broccoli V. Whle brain delivery of an instability-prone Mecp2 transgene improves behavioral and molecular pathological defects in mouse models of Rett syndrome. Elife 9e52629, 2020.