Dissertation Project – 2nd Cycle

Student´s Name:
Student email address: No.
Supervisor(s): Ana Sofia Coroadinha
Supervisor(s) email address: avalente@itqb.unl.pt
Lab/Institution: Cell Line Development and Molecular Biotechnology, UTCA, iBET and ITQB-UNL
Av. da República (EAN), 2781-901 Oeiras. Web: http://tca.itqb.unl.pt

TITLE: Chimeric lentiviral vectors for gene therapy

BACKGROUND

Gene therapy, the treatment or prevention of diseases by the transfer of genetic material, is considered a revolutionary methodology in medicine. After two decades of research Gene Therapy started to become a real form of treatment. The use of gene therapy started to gain credibility in 1999 when the first clinical trial was initiated to treat SCID, a severe immunodeficiency of genetic origin, using retroviral vectors derived from murine leukemia virus (MLV). In November of 2012 the first Gene Therapy product was approved by the European regulatory entity EMA, the Alipogene Tiparvec (Glybera®). Since then several gene therapy products were approved for the treatment of both hereditary monogenic diseases as well as cancer diseases.

The transfer of genetic material into patient cells can be performed using different methods, being viral vectors transduction the one presenting the highest efficiency. From the later, lentiviral vectors are very efficient, presenting high transfer rate and long term expression. The number of clinical trials using this vector is currently increasing.

In order to use lentiviral vectors for gene therapy they need to be efficiently produced complying with quality and safety required to be used in clinical trials. Despite of their high potential, lentiviral vectors are difficult to produce. This is mainly due to the toxicity of some of the viral components that induce cell apoptosis. By mutating the lentiviral protease our group generated a novel generation of lentiviral vectors and lentiviral vector packaging cell lines (LentiPro) [1].

OBJECTIVES

This work aims to study and characterize LentiPro cell lines to elucidate the key factors enabling viral vector production, as well as, generate novel improved lentiviral vector packaging cell line for constitutive production. The novel packaging cell lines to be designed will improve stable lentiviral vector manufacture by (i) minimizing or eliminating viral cytotoxicity using molecular biology tools to introduce mutated or chimeric viral proteases and/or envelopes and (ii) enhancing yields by means of cell engineering in order to re-design the virus and producer cells.

PROJECT DESCRIPTION

Work Plan:

Task 1 – Learning animal cell culture techniques: working in laminar flow, propagation of cells, determining cell concentration, long term storage of cells. Reading work related bibliography.

Task 2 – Learning techniques required for lentiviral vector manipulation: culture of producer cells and production of lentiviral vectors in t-flask, quantification of infectious and total particles. LentiPro cell lines: characterization of cell growth, viability and viral production. Viral vectors analysis by flow cytometry, RT-PCR, Nanosight, Western-Blot and ELISA.

Task 3 – Learning the methodologies related to molecular biology: construction and cloning plasmids to express the genes required for the work. Transfection of mammalian cells, cell engineering and modification of lentiviral vectors. Designing different protease and/or envelope proteins. Designing expression cassettes for CRISPR/Cas9 knockout or for the over-expression of cellular genes.

Task 4 – Modifying and establishing novel packaging LentiPro cell lines. Studying how the modification introduced in the lentiviral vector constructs or in the producer cell line affected cell behavior (cytotoxicity, growth, etc) and lentiviral vector quality/yield. Further development and characterization of the packaging cell lines and lentiviral vectors will be pursued according to the obtained results.

Thesis – Master thesis writing.

Recommended references:

[1] Tomás, H.A., Rodrigues, A.F., Carrondo, M.J.T., Coroadinha, A.S. (2018) LentiPro26: novel stable cell lines for constitutive lentiviral vector production. Scientific Reports, 2018. 8(1): p. 5271. DOI:10.1038/s41598-018-23593-y

[2] Hélio A. Tomás, Ana F. Rodrigues, Paula M. Alves and Ana S. Coroadinha (2013). Lentiviral Gene Therapy Vectors: Challenges and Future Directions, Gene Therapy - Tools and Potential Applications, Dr. Francisco Martin (Ed.), ISBN: 978-953-51-1014-9, InTech, DOI: 10.5772/52534 (Available from: http://www.intechopen.com/books/gene-therapy-tools-and-potential-applications/lentiviral-gene-therapy-vectors-challenges-and-future-directions)

TIMELINE

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1										
Task 2										
Task 3										
Task 4										
Thesis										