Lentiviral vectors: approaches and possible use in bone marrow failure syndrome

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INTRODUCTION

Gene therapy

Consists in the transfer of genetic material into cells or tissues of interest to prevent or cure a disease

Approaches:

- In vivo gene therapy → Vector (with the therapeutic gene) administration directly into the patient. Minimal number of cells are required.
- Ex vivo gene therapy → Transduction in cell culture and reintroduction of cells into the patient.

IN VIVO / EX VIVO → Hematopoietic stem cell (HSC) as a target for gene therapy in hematological diseases

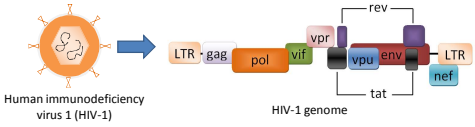
Lentiviral vectors pseudotyped to transduce CD34 cells → Bone marrow failure syndrome (reduced number of cells in bone marrow)

LENTIVIRAL VECTORS PRODUCTION

LVs are based on human immunodeficiency virus (HIV-1). This virus presents envelope, capsid and two molecules of single strand RNA.

HIV-1 genome presents:

-LTR (long terminal repeat): it contains the promoter
-gag: encodes for the capsid
-pol: encodes for viral enzymes involved in reverse transcription and virus integration.
-env: encodes for the envelope glycoprotein (GP)
-rev: encodes for a protein necessary for RNA transport to the cytosol from the nucleus.
-Accessory regulatory proteins (Nef, Vpr, Vif and Vpu).

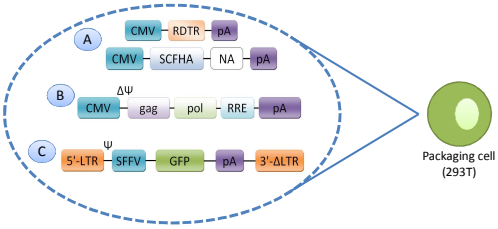


Human immunodeficiency virus 1 (HIV-1) — HIV-1 genome

Is critically important to ensure that the corresponding LV is replication-defective.

The self-inactivating (SIN) LV configuration reduces the risk of expression of coding sequences located adjacent to the vector integration site by abolishing the promoter activity of the LTR.

For LV production, packaging cells are transfected with 3 different constructs. 293T (human embryo kidney cell line) is mainly used as packaging cell.



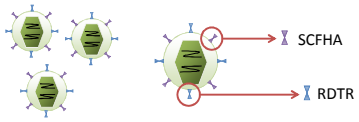
A- Glycoprotein RDTR (mutant RD114 glycoprotein of an endogenous feline virus) under control of cytomegalovirus (CMV) promoter .
• SCFHA: SCF (stem cell factor) + HA (influenza hemagglutinin).
• NA (neuraminidase) allows efficient release of virus from the producer cell.

SCFHA and NA: under control of CMV promoter

B- *gag-pol* genes necessaries for virus assembly under control of cytomegalovirus (CMV) promoter. ΔΨ: packaging signal mutated.

C- SIN vector with the green fluorescent protein (GFP) under control of spleen focus-forming virus (SFFV) promoter. LTR mutated.

Viral particles



RDTR

▪ RDTR glycoprotein allows vector-cell fusion and is resistant to degradation by human complement.

▪ RDTR pseudotyped LVs efficiently transduce CD34⁺ HSCs.

▪ CD34 is a membrane protein that regulates cellular adhesion and define a group of undifferentiated quiescent cells (G_0 phase from cell cycle).

SCFHA

▪ SCF cytokine allows specific binding to c-kit⁺ hCD34⁺ cells.

▪ HA allows functional presentation of the LV particles.

▪ SCFHA glycoprotein in RDTR/SCFHA-LVs serves mainly to target the vector specifically to hCD34c-Kit primary cells, allowing specific binding/ stimulation.

EX VIVO GENE THERAPY

HSC is a good target for gene therapy in some human diseases

• They have differentiation and expansion capabilities.

• Transduction of small number of HSC results in gene correction of much greater numbers of cells.

HSCs are often quiescent, which makes it difficult to transfer genes into these cells with high efficiency. LVs target nondividing cells including quiescent HSC.

Two different sources of HSCs:
- Umbilical cord blood (UCB)
- Bone marrow(BM)

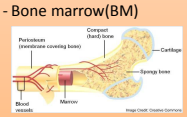


Image from: http://www.omnimedicalsearch.com/conditions-diseases/images/bonemarrow.jpg

Image from: http://www.compartiendomio pinion.com/2011/11/celulas-madre-el-futuro-de-la-medicina.html

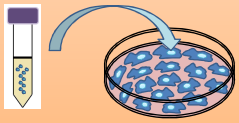
Cell culture of HSC

CD34⁺ cells are isolated from BM or UCB blood samples and cultured in plaque.

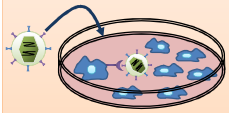
Disadvantages

• A minimal number of cells are required (not possible in some diseases).

• Culture of HSC results in impaired homing capabilities and functional deficits. Furthermore, ex vivo culture potentially exposes the cells to infectious agents, serum, and other risks related to manipulation.



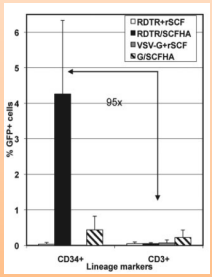
Introduction of LV particles in cell culture



RDTR/SCFHA -LV binds to c-Kit and efficiently transduces c-Kit⁺CD34⁺ cells:

• RDTR/SCFHA-LVs transduce hCD34 fraction in CB and BM at low vector doses and with a significantly selectivity to this cell population.

• Transduction with RDTR/SCFHA vectors is independent of retronectin.



Obtained from: Frecha C., et al 2012

Transduced cells expressing the therapeutic gene are selected from cell culture and reintroduced into the patient.

Advantages

• No rejection: patient receives his own cells expressing the therapeutic gene.

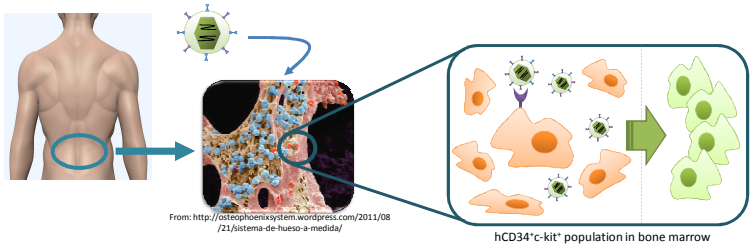
• Alternative to allogeneic hematopoietic stem cell transplantation

Obtained from: http://anatomiadelcrimen.blogspot.com.es/2011/11/toxicologia-forense-ii-parte.html

IN VIVO GENE THERAPY

In in vivo gene therapy LV particles are introduced directly into the patient.
Cells are in their microenvironment which regulates both self-renoval and differentiation capacities.

Key aspects in in vivo gene therapy:
- Evaluation of the safety and efficacy in in vivo models of human hematopoiesis
- Biodistribution
- Engraftment and ability to differentiate
- Administration → intra-femoral injection / systemic administration



From: http://osteophoenixsystem.wordpress.com/2011/08/21/sistema-de-hueso-a-medida/

hCD34⁺c-kit⁺ population in bone marrow

BONE MARROW FAILURE SYNDROME

Bone marrow failure syndrome (BMFS) is a group of disorders with abnormal hematopoiesis which causes defective production of different cell types

Manifestation

Cytopenia (failure in one cell line)
- •Diamond-Blackfan anemia
- •Shwachman-Diamond syndrome
- •Thrombocytopenia absent radius (TAR) syndrome

Erythroid
Myeloid
Megakaryocytic

Pancytopenia (failure of 3 cell lines)
- • Fanconi Anemia (FA)
- •Dyskeratosis congenita

The only long-term curative treatment at present is allogenic HSC transplantation but there are many adverse effects.

In vivo gene therapy could replace ex vivo handling and allow the correction of some hematopoietic diseases directly into the niche of HSC in BM. It could be a good strategy in BMFS because of the low number of cells in BM of these patients.

CONCLUSIONS

• Hematopoietic stem cell is a useful target in gene therapy for hematopoietic disorders.
• Lentiviral vectors transduce HSC both in vivo and ex vivo.
• RDTR-SCFHA-LVs transduce efficiently hCD34⁺ cells and resist human complement.
This new generation of HSC-targeted lentiviral vectors should simplify gene therapy protocols through transduction of hCD34 cells directly in the bone marrow.

• RDTR-SCFHA-LVs could be use as in vivo gene therapy for bone marrow failure syndrome because minimal number of cells are not necessary.
• In the future in vivo gene therapy could replace ex vivo handling allowing the correction of some human diseases.
• Clinical trials are needed (biosafety and production/protocols improvement)

References

Frecha Cecilia et al. A novel lentiviral vector targets gene transfer into human hematopoietic stem cells in marrow from patients with bone marrow failure syndrome and in vivo in humanized mice (2012). Blood, 119(5):1139-1150
Mátrai Janka et al. Recent advances in lentiviral vector development and applications (2010). Molecular Therapy, 18 (3): 477–490