Using a virus to fight a virus: gene therapy for HIV/AIDS using foamy virus

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Disclosure

• Dr. Trobridge has no actual or potential conflict of interest in relation to this program/presentation.

Objectives

• At the completion of this program, the participant will be able to:
  • Describe a stem cell gene therapy approach for blood diseases
  • List viral vector systems used for hematopoietic stem cell gene therapy
  • Explain what genotoxicity is in the context of viral vector gene therapy
  • Describe how a viral vector system can be modified to reduce genotoxicity
  • Discuss critically the promise and challenges of using stem cell gene therapy to treat HIV/AIDS
Overview

- Hematopoietic stem cell (HSC) gene therapy
- Foamy virus (FV) vectors (retrovirus) for HIV gene therapy
- Improving safety by reducing genotoxicity
- HSC gene therapy for SCID-X1

Stem cell definition (NIH)

Stem cells are distinguished from other cell types by two important characteristics:

- **Renewal**: they are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity
- **Differentiation**: under certain physiologic or experimental conditions, they can be induced to become tissue- or organ-specific cells with special functions

Types of stem cells

- **Embryonic stem cells (ES)**
- **Induced pluripotent stem cells (iPS)**
- **Tissue-specific (adult) stem cells**
  - hematopoietic (HSCs)
  - mesenchymal
  - muscle
  - neural
- **Germline-derived stem cells**
  - spermatogonial stem cells
  - oogonial stem cells
Hematopoietic stem cells: HSCs

- self-renew
- differentiate: produce the entire blood system

From: Stem Cell Amplification

Stem Cell Amplification

“Stemness” a molecular definition

- Transcriptional profiling has identified genes expressed in ES, HSC
- There are common molecular processes that underly self-renewal eg. Notch, Wnt (but no exact ‘fingerprint’)
- Distinct stem cell types have some common characteristics
“Stemness”
common characteristics

• Have the capacity to sense a broad range of growth factors and signaling molecules
• Can express downstream signaling components to respond to these signals
• Express many genes involved in regulating cell cycle and quiescence

HSCs: a model for other adult stem cell therapies

• HSCs have been used to treat many hematopoietic diseases since the first human bone marrow transplants reported in 1958
• The ease of collection and infusion has led to widespread use of HSC therapies
• Our understanding of HSC biology has influenced our knowledge of other tissue-specific stem cells

HSCs: autologous transplantation
eg. Non-Hodgkins Lymphoma (NHL)

1. harvest CD34+ and cryopreserve
2. chemotherapy to kill leukemic cells
3. thaw and infuse autograft
4. infused HSCs repopulate recipient and enter remission
E. Donnall Thomas and HSC transplantation

1955: Six patients were treated with radiation and chemotherapy followed by infusion of normal donor marrow. These were only transient grafts and none lived beyond 100 days.

1960s: By the mid-1960s, they could reliably transplant canine littermates. Meanwhile, methods for human leukocyte antigen typing became available and, with these advances, he returned to the challenge of human marrow grafting.

1969: began clinical trials of allogeneic transplantation from matched siblings for patients with advanced leukemia. Most of the initial patients died of progressive leukemia or complications of transplantation, but a few survived.

Challenges faced by E. Donnall Thomas

“Because normal marrow was easily destroyed by irradiation, shouldn’t it be possible to destroy an abnormal marrow and replace it with marrow from a normal donor?”

“Early attempts were unsuccessful, either because the transplanted marrow was rejected, or because the marrow rejected its new host. These failures led many to criticize Don’s efforts as dangerous and futile. But his persistence paid off in the development of a therapy that has saved hundreds of thousands of lives.”

Thomas wins 1990 Nobel

1990 Nobel Prize in Physiology or Medicine:

awarded jointly to: Joseph E. Murray and E. Donnall Thomas for their discoveries concerning "organ and cell transplantation in the treatment of human disease".

“E. Donnall Thomas managed to diminish the severe reaction that the graft can cause in the recipient, i.e. the so-called "graft-versus-host" reaction (GVH). In addition, Thomas could show that intravenously infused bone marrow cells were able to repopulate the bone marrow and produce new blood cells."
Leukemias and lymphomas, including:
- Acute myelogenous leukemia (AML)
- Acute lymphoblastic leukemia (ALL)
- Chronic myelogenous leukemia (CML)
- Chronic lymphocytic leukemia
- Juvenile myelomonocytic leukemia
- Waldenström’s macroglobulinemia
- Non-Hodgkin lymphoma
- Multiple myeloma and other plasma cell disorders

Marrow failure states, including:
- Severe aplastic anemia
- Fanconi anemia
- Paroxysmal nocturnal hemoglobinuria (PNH)
- Pure red cell aplasia
- Aplastic anemia / congenital thrombocytopenia

Myelodysplastic and myeloproliferative disorders, (MDS/MPS) including:
- Refractory anemia (all types)
- Chronic myelomonocytic leukemia
- Agnogenic myeloid metaplasia (myelofibrosis)

Inherited immune system disorders, including:
- Severe combined immunodeficiency (SCID)
- Wiskott-Aldrich syndrome

Hemoglobinopathies, including:
- Beta thalassemia major
- Sickle cell disease

Inherited metabolic disorders, including:
- Hurler’s syndrome (MPS-IH)
- Adrenoleukodystrophy
- Metachromatic leukodystrophy

Indications for HSCs transplantation

HSC transplantation is a routine lifesaving procedure

- ~ 50,000 worldwide

- main indications:
  - lymphoproliferative disorders / leukemias

The nature of our blood system has facilitated HSC transplantation

- Easily harvest and infuse HSCs
- Hematopoietic system is sensitive to irradiation and chemotherapy: can ablate recipients
- Repopulation does not require complex 3D tissue structures
- HSC transplantation is unique in that HSCs form the immune system:
  - transplanted T cells enhance transplantation
  - Can 'reset' the immune system to facilitate allogeneic stem cell transplantation
Allogeneic HSC transplantation has limitations

- Allogeneic transplantation requires a matched donor
- Severe side effects of allogeneic transplantation:
  - Transplant rejection
  - GVHD (graft versus host disease)

How can we avoid GVHD?

Hematopoietic stem cell (HSC) gene therapy

goal: deliver a therapeutic gene (e.g., γc) to an autologous HSC
(autologous = no GVHD)
How do we identify/isolate HSCs?

HSCs must be identified using a functional assay: the ability to reconstitute the hematopoietic system of a lethally irradiated host.

- There are imperfect markers for human HSCs
  - CD34
  - CD133
  - Rho^a

- Currently we can enrich but not purify human HSCs using CD34.

Part I: HSC gene therapy summary

- HSCs can be easily harvested, manipulated ex vivo
- Used to treat many hematopoietic/immune diseases
- Delivery of a therapeutic gene allows for autologous cells
- Autologous cells avoids use of donor allogeneic cells which can lead to GVHD

Part II: Foamy virus vectors for HIV gene therapy

(using a virus to fight a virus)
Global AIDS pandemic

- estimated 18 million infected with HIV in 2016
- 1.1 million died of AIDS, 2.1 million become infected
- antiretroviral therapy has reduced morbidity, mortality
- no effective vaccine available
- alternative approaches are needed

AIDS stem cell gene therapy approach

anti-HIV vector

HSC → HSC

T lymphocytes
macrophages
microglial cells
dendritic cells
HSC gene therapy for HIV/AIDS
- goal: deliver a therapeutic anti-HIV gene to HSCs

Could HSC transplantation cure HIV/AIDS?

Yes: the Berlin patient
- Diagnosed HIV positive 1996
- Acquired acute myeloid leukemia
- Received HSC transplant in 2007
- HSCs from HIV-resistant donor (CCR5<sup>-/-</sup>)
- Functionally cured of HIV for >9 years
  - discontinued antiretroviral therapy
  - no infectious virus

Long-Term Control of HIV by CCR5 Delta32/Delta32 Stem-Cell Transplantation
Why HSC gene therapy instead of allogeneic CCR5⁻/⁻ transplantation?

- Not enough CCR5⁻ donors
- Risk of lethal graft versus host disease
- HSC gene therapy with gene-modified autologous cells can expand clinical use

Challenges for HSC gene therapy

- Quiescent HSCs reside in a specialized niche and are quiescent (G0)
  - When HSCs do divide:
    - some daughter cells remain HSCs (self-renew) and re-enter G0
    - others enter the cell cycle and start down the differentiation pathway to produce mature blood cells
Steady-state hematopoiesis requires differentiation and also massive expansion

- The adult marrow releases $\sim 3 \times 10^9$ new red blood cells, per kg body weight per day.
- The marrow produces granulocytes, especially neutrophils, at a prodigious rate to supply the baseline needs of circulating cells that survive in the peripheral blood only 3 to 6 hours.

How can we deliver a therapeutic transgene to all blood cells?

- nanoparticles?
- liposomes?
- other?

We must deliver the transgene to the HSC in a way that ensures all daughter cells are transduced.
Retroviral vectors for HSC gene therapy

- to date retroviral vectors are the only effective method
- retroviral vectors integrate, ensuring transmission to daughter cells via mitosis

Integration ensures transmission to daughter cells

Retroviruses developed as vectors

- PFV: spuma- (Foamy)
- MLV: gamma-
- HIV-1: lenti-

Morozov et al., 1997
Coffin et al., Retroviruses
Otago University (NZ) Microbiology Department
**Foamy name:** cytopathic effect in cell cultures

Tobaly-Tapiero et al. 2000 Journal of Virology

**Foamy retroviruses**
- zoonotic transfer from nonhuman primates to humans
- non-pathogenic
- retrovirus life cycle, but functional virion is dsDNA

**Foamy virus (FV) vector design:**
Production of FV vectors

Transduction by FV vectors

Transgenes that inhibit HIV replication
Inefficient delivery has limited HIV gene therapy

- previous clinical trials with MLV-based vectors:
  - ribozyme marking of 0.01% to 0.001% (Amado, et al. Hum Gen Ther, 2004)
  - ribozyme marking less than 0.38% (Mitsuyasu et al. Nat Med, 2009)
  “OZ1 DNA did not reach the quantifiable range of the assay in any blood cell sample at any time point.”

Membrane-bound C46 HIV fusion inhibitor

- peptide derived from C-terminal of HIV gp41
- based on enfuvirtide (T20, fuzeon)
- inhibits R5 and X4 strains

C46 fusion inhibitor blocks HIV-1 infection
We would like to combine transgenes to overcome HIV escape

- transmembrane fusion inhibitor C46
- shRNAs targeted to HIV, CCR5
- HIV integration cofactor LEDGF:IBD
- humanized TRIM5α cyclophilinA fusion (TCypA)

Combinatorial anti-HIV vector

shRNAs targeted to HIV and CCR5

anti-HIV C46 fusion inhibitor

EGFP for efficient tracking of gene marking in vivo

Anti-HIV transgenes reduce HIV vector titers but do not reduce FV vector titers
Foamy vectors are better than HIV vectors for delivering anti-HIV transgenes

Potent inhibition of viral replication by foamy combinatorial anti-HIV vectors

Efficient foamy transduction of CD34+ progenitors and mesenchymal stem cells (MSCs)
FV vectors are effective for gene delivery to HSCs

- mouse model
  - Vassilopolous, Trobridge et al., Blood, 2001
  - Josephson et al., PNAS, 2002
  - Josephson, Trobridge and Russell, Human Gene Therapy, 2004

- dog model
  - Kiem et al., Blood, 2007
  - Trobridge et al., Human Gene Therapy, 2008
  - Trobridge et al., PLOS One, 2012

- FV vectors efficiently transduce G0 cells that later divide
  - Trobridge and Russell, Journal of Virology, 2004

How can we test our Foamy anti-HIV vectors before treating patients?

Will they negatively impact HSCs?

Can we deliver them efficiently to HSCs?

Can we protect HSC-derived CD4 cells from HIV infection?

Competitive repopulation approach in a mouse model

| Human CD34⁺ | Vector 1 control green | Vector 2 anti-HIV red |

Analyze peripheral blood for ratio of red to green cells

Challenge with HIV to determine if there is protection
Summary part II: Foamy anti-HIV vectors

- anti-HIV transgenes do not adversely affect foamy titers
- developed high titer combinatorial FV anti-HIV vector
- established competitive repopulation assay with HIV challenge

Next Steps: Foamy anti-HIV vectors

- identify a potent anti-HIV cassette that efficiently engrafts
- demonstrate protection in vivo:
  - protected (red) cells survive after HIV challenge in vivo
- remove fluorescent marker and translate into clinic
  - AIDS lymphoma patients who receive a HSC transplant to treat lymphoma (FHCRC)

Gene therapy adverse event

LMO2-Associated Clonal T Cell Proliferation in Two Patients after Gene Therapy for SCID-X1

S. Hasein-Seyyed Abina,1,2 C. Yun Kalle,3,4 M. Schmidt,6,7 M. P. McCormack,8 N. Wolffraat,9 P. Lebood,10 A. Lin,11 C. S. Oshanna,12 R. Faulk,13 E. Morillon,13 B. Sorensen,14 A. Forster,15 P. Fraser,16 J. I. Cohen,18 G. de Saint Basile,1
I. Alexander,19 U. Wintergerste,16 T. Freiburg,20 A. Aries,21 D. Stopa-Lannet,22 S. Ramana,2 I. Radford-Weiss,2 F. Gross,2 F. Valenz,2 E. Delahesse,2 E. Macleiffy,4 F. Sigaux,21 J. Soulier,21 L. E. Lau,22 M. Wissler,22 C. Prive,1,2 T. H. Rablieta,2 F. Le Deist,1 A. Fischer,1,2,3,4 M. Cavazzana-Calvo,1,5,6,7
SCID-X1
(X linked severe combined immunodeficiency)

- presents in male infants as life-threatening infection and failure to thrive
- deficiency in γc shared cytokine receptor subunit
- absence of T and NK lymphocytes
- dysfunctional B cells


SCID-X1 HSC gene therapy
- goal: deliver therapeutic γc gene to HSCs

HSC gene therapy
- Harvest CD34+
- Add vector
- γc
- infuse γc

Genotoxicity: vector proviruses can cause leukemia

- 18 out of 20 patients had clinical benefit but 5 developed leukemia
- 4/5 responded to chemotherapy and continue to have clinical benefit
Vector genotoxicity mechanisms

What is the integration profile of foamy vectors?
- are they safer than the MLV vectors used in the SCID-X1 trial?
- how frequently do they integrate in or near oncogenes?

The chromosomal DNA sequence next to a vector allows identification of the nearby gene
Distribution of FV integration sites

Foamy vectors have a distinct integration profile

Can we make FV vectors safer?
Retargeting foamy vector integration

Retargeted FV vectors integrate less often near genes

Retargeted FV vectors integrate within or near peaks of H3K9me3 regions in human CD34 cells. * p<0.001
Retargeted FV vectors are safer

Table 3. Retargeted FVs integrate farther away from genes and proto-oncogenes in human cord blood CD34+ cells.

<table>
<thead>
<tr>
<th>Vector</th>
<th>Total sites</th>
<th>&lt; 10 kb from Refseq TSS</th>
<th>&lt; 50 kb from proto-oncogene TSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4217</td>
<td>24.5</td>
<td>6.5</td>
</tr>
<tr>
<td>Retargeted</td>
<td>1534</td>
<td>12.5***</td>
<td>2.5***</td>
</tr>
</tbody>
</table>

*** statistically significant at p < 0.001 compared to control vector.

Within or less than 1 kb from a CpG island. RIS, retroviral vector integration sites; TSS, transcription start site.

VISA: a webserver for Viral Integration Site Analysis

Hocum et al., 2013
BMAC bioinformatics

Beard et al., 2010 Journal of Clinical Investigation
Adair et al., 2012 Science Translational Medicine
Olszko et al., 2015 Gene Therapy
Hocum et al., 2016 Scientific Reports
Browning et al., 2017 Gene Therapy

Summary part III: genotoxicity

- Genotoxicity is a major challenge for HSC gene therapy
- FV vectors have a favorable integration profile
- Retargeted foamy vectors appear to be safer
- Next step: use CRISPR/Cas to direct integration to a specific locus
Can foamy HSC gene therapy be used in underdeveloped countries?

- HSC gene therapy currently requires harvest, ex vivo culture of HSCs
- Direct injection of vector is a simpler approach

Canine SCID-X1 cured by foamy gene therapy with a single intravenous injection

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