Regenerative Therapy for Pulmonary Hypertension

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PHPN Symposium
September, 2015
Disclosures

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- Grants/research support:
  - Northern Therapeutics Inc.
  - United Therapeutics, Inc.

- Equity/interest
  - Northern Therapeutics

- Speaker’s Bureau: N/A
- Honoraria from Industry: N/A

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At the conclusion of this activity, the participant will be able to:

1. Provide a understanding of the rationale for “regenerative” therapy for PAH
2. Explore the ways to produce lung vascular repair and regeneration in experimental models of PAH
3. Review the results of the early clinical experience with cell and gene therapies for PAH
4. Discuss future opportunities to develop more effective and robust regenerative therapies for PAH
Question 1

• What is the mechanism(s) that results in pulmonary arterial hypertension
  1. Narrowing of lung arteries
  2. Blockage of arteries in the lung by uncontrolled vascular cell growth
  3. Degeneration of small vessels in the lung
  4. All of the above
  5. Don’t know

Correct answer is ‘4’
(but I would accept ‘5’ since we really don’t know which of these is the most important!)
PAH is a rare disease resulting from loss of lung micro-vasculature

Advances in Pulmonary Hypertension. 2013;11:4: 171-182
Hypoxia/SU5416 produces severe PAH in rats: role of EC apoptosis

Effect of VEGF receptor antagonist (SU5416)

Effects of SU5416 reversed by caspase inhibition (z-ASP)

Activated caspase 3

PCNA

Apoptosis

Proliferation

Complex lesions up to 14 wks in the rat chronic hypoxia-SU5416 model

“… sustained exposure to very high blood pressure may be the major factor required for their development and that the lesion may be the consequence rather than the cause of the hypertension”

Abe at al. Circulation. 2010;121:2747-2754
Target for Therapy: Proliferative versus Degenerative mechanism?

Hemodynamic Stress is Essential to the Development and Maintenance of Occlusive Vascular Lesions in a Rat Model of Severe PAH; Kohtaro Abe et al. Abstract A1974
Question 2

- Why does understanding the mechanism of vascular disease in PAH matter?
  1. It is important for publishing more papers and getting more grants
  2. It is of interest scientifically but probably not of practical value for the treatment of this disease
  3. It is essential for designing new and more effective treatments
  4. We already have good therapies so we don’t need to waste time on this

Correct answer is ‘3’
Therapeutic implications

• Vascular narrowing
  – Vasodilators
    • Prostaglandins, ERAs, PDE and soluble guanylate cyclase stimulators (Riociguat)

• Arterial obliteration from dysregulated cell growth (the “cancer hypothesis”)
  – Growth inhibitors/cytotoxic agents
    • Imatanib, DCA (dichloroacetate)

• Loss of lung microcirculation from arteriolar degeneration and dropout
  – Blood vessel regeneration → angiogenesis and vasculogenesis
    • Cell (EPC) and gene therapies
Question 3

• Can the pulmonary blood vessels undergo angiogenesis?

1. No – all neovascularization in the lung (tumours, abscesses) originate for the bronchial (systemic) circulation

2. Yes – the pulmonary circulation has a well developed capacity to repair and regenerate the lung microcirculation

Correct answer is ‘2’
(but the respiratory text books still maintain the dogma that new blood vessels can arise only from the bronchial circulation)
Can the pulmonary circulation regenerate its microvasculature?

Fluorescent microangiography of Matrigel implants containing fluorescently-labelled MSCs

Bronchial circulation

Pulmonary c.

Normal lung

Matrigel plug

Dutly et al. 2005
Angiogenic cell (FB)-based gene therapy for PAH

Zhao et al. AJRCMB VOL 35 2006
Repair and Regeneration of Lung Microvasculature with EPCs?

Role of EPCs in pulmonary vascular regeneration?

Culture for ~7 days
- Fibronectin matrix
- EGM-2 media
- Endothelial GFs

Early outgrowth EPCs or Circulating Angiogenic Cells

Transfection with heNOS 24 hrs prior to cell Tx

Apheresis

Electroporation
Occasional “Engraftment” of EPCs into distal pre-capillary arterioles

Efficacy of EPCs in the reversal of MCT-induced PAH (RVSP)

Survival analysis following cell therapy in the treatment MCT-PAH model

Question 4

Which of the following is **NOT** required to begin clinical testing of a novel therapy?

1. A whole lot of money
2. A full understanding of the mechanism of action of the therapy
3. Preclinical evidence that the therapy is safe
4. Preclinical evidence that the therapy is effective
5. More enthusiasm than brains

**Correct answer is ‘2’**

(In general, regulatory authorities are much more concerned about safety and efficacy, than mechanism)
THE PHACeT TRIAL

Clinical Track

Endothelial NO-Synthase Gene-Enhanced Progenitor Cell Therapy for Pulmonary Arterial Hypertension: the PHACeT Trial

John Granton,* David Langleben,* Michael B. Kutryk, Nancy Camack, Jacques Galipeau, David W. Courtman,* Duncan J. Stewart*

Editorial

Cell-Based Gene Therapy in Pulmonary Arterial Hypertension
Translational Journey

Vikram Gurtu, Evangelos Michelakis
The Pulmonary Hypertension And Cell Therapy (PHACeT) Trial

Principle Investigators:
- John Granton (Toronto); David Langleben (Montreal)

Safety study
- I^o EP: tolerability of cell transplantation in patients with PAH refractory to all standard therapies

Cell delivery
- eNOS transfected autologous early growth EPCs
- Delivery via SG catheter
  - Pacing port (i.e. RV delivery)
  - allows continuous monitoring of PA pressure
- Dose ranging for eNOS transfected cells given over 3 days in divided doses
Overlapping, dose escalation protocol

Panel 1
- Day 1: 1x10^6
- Day 2: 3x10^6
- Day 3: 3x10^6

Panel 2
- Day 1: 3x10^6
- Day 2: 10x10^6
- Day 3: 10x10^6

Panel 3
- Day 1: 10x10^6
- Day 2: 20x10^6
- Day 3: 20x10^6

3 patients/panel

Trial stopped after 7 patients because of slow enrolment
Primary safety EP: Hemodynamic changes during cell delivery

Circulation Research 2015; provisional acceptance
Long-term functional endpoints

6MWD

Circulation Research 2015; provisional acceptance

*p<0.05; **p<0.01
Long-term functional endpoints

SF-36 QOL

Hemodynamics

Circulation Research 2015; provisional acceptance
PHACeT – Conclusions

- Delivery of eNOS-transfected EPCs was well tolerated hemodynamically in patients with refractory PAH
  - Strong trend towards short-term improvement
    - Role of NO produced by eNOS-transfected cells?
  
- Significant improvement in 6MWD and QOL assessment (in this non controlled study)

- No persistent hemodynamic improvement at 3 months after a single course of cell therapy
Early experiments in transportation
Question 5

What is a “disruptive” technology/innovation?

1. Something that causes problems and should be abandoned
2. Malware that is designed to disrupt computer software systems like a “worm” and a “virus”
3. A technology that lacks refinement, often has performance problems and may not yet have a proven practical application
4. Has the potential to replace existing technologies
5. Answers 3 and 4

Best answer is ‘5’
(though I would accept ‘4’ as well which is the essential element of the definition)
Disruptive vs. Sustaining Technologies

Adapted from "The Innovator’s Dilemma" by Clayton M. Christensen

Andreas Grunzig
1977
Moving the needle on gene and cell therapy for PAH

- Multiple cell dosing to achieve better long-term outcomes
- Selection of a more “regenerative” cell type
  - Late-outgrowth EPCs (ECFCs)?
  - Combination of early and late EPCs?

Technologies to achieve greater cell persistence and engraftment
  - i.e. cell “cocooning”

Use of “minicircle” DNA vector to avoid innate immune response to bacterial sequences

- Use of viral vectors for efficient and stable transfection
- Incorporation of conditional vectors to turn on and off transgene expression
Question 6

What happens when attached cells are lifted off the culture dish and put into suspension for delivery to the patient?

1. The cells begin to proliferate uncontrollably
2. They are fine as long as the syringe is shaken gently all the time
3. They undergo rapid form of programmed cell death because of the loss of contact with extracellular matrix
4. They fuse together into large multicellular aggregates

Correct answer is ‘3’
ANOIKIS
“Cocooning” to enhancing cell survival and engraftment

Single-cell encapsulation with incorporation of integrin binding partners to prevent “anoikis” and promote migration through capsule and into surrounding tissue.
“Cocooning” Bone Marrow Stromal Cells

Cocooned cells (15 minutes)

fibrinogen

MSCs

Cocooned cells

24 h

20 x

7 days

60 x

Golnaz et al. Biomaterials. 2009 Oct;30(29)
Microfluidics “on chip” platform for high throughput cell cocooning

Custom microfluidic chamber designed for:
A. hydrogel polymerization
B. enrichment of cell containing droplets
C. serial amplification for high throughput

Michel Godin, Dept of Physics
Minicircle DNA transfection of late outgrowth EPCs (ECFCs)
Potential for durable high efficiency gene transfer with viral vectors

Transgene expression level vs. Duration of expression for Lenti viral vector, Minicircle DNA, and Plasmid DNA.
Co-Activator Peptide (CAP)  Ligand-controllable transcription factor (LTF)

“OFF”

RXR  VP16  Gal4  EcR  Inducible Gene Program

Activator Ligand (Veledimex)

“ON”

Basal Transcription Proteins

Inducible Gene Program

Chan et al. (2013) AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics.
Science – Breakthrough of the year for 2013

*T-Cell Chimeric Antigen Receptors*

*Cancer Immunotherapy*
Rebuilding the lung?


Decellularization using detergents: i.e. 8mM CHAPS, 1M NaCl and 25mM EDTA in PBS
Implantation of recellularized lung scaffolds

Transplantation of Engineered Rat Lung

Orthotopic left lung transplantation was performed into sygnetic Fischer 344 rats
Question 7

- What is the major limitation to long-term *in vivo* survival of recellularized lung scaffolds post implantation?
  1. Lack of oxygen
  2. Immune rejection
  3. Incomplete vascular recellularization
  4. Incomplete re-epithelialization of airways

Correct answer is ‘3’
Vascular Failure of “recell” Lung Scaffold

T H Petersen et al. Science
2010;329:538-541

Published by AAAS
Induced pluripotent stem cells (iPSC)

Generation of iPSC
Differentiation of iPSC-derived Smooth Muscle Cells (iPS-SMCs)

Embryoid body Formation
- Growth on low-adherence plate with EB media
- D1-10

Embryoid body Differentiation
- Growth on gelatin-coated plates with EB media
- D11-16

VSMC Culture
- Growth on matrigel-coated plates with VSMC media
- Continual passage
Morphology of iPSC-derived Endothelial and Smooth Muscle Cells

iPS-SMC

iPS-EC

Scale bar represent 100μm

SMA

CD31
iPSC EC-SMC Co-culture 24Hrs Post-Seeding on Matrigel
“Decel/Recel” lung scaffold

Native

Decel.

Revascularized
Summary/Conclusions

- Stem/progenitor cell therapy shows promise for patients with severe PAH refractory to current treatments
- Innovative strategies are needed to refine this “disruptive technology” and increase efficacy
  - Multiple (monthly) dosing
  - Stable, robust transgene expression (next gen viral vectors)
  - Enhance cell engraftment and transdifferention
- The nascent field of Regenerative Medicine is rapidly evolving and novel technologies are on the horizon that could be transformative
  - Decel/recel scaffolds for lung transplantation
Thank you!

- PHACeT Trial
  - Study Coordinators:
    - Nancy Camack
    - Rosemary Dunne
  - Investigators
    - Mike Kutryk
      - St Michael’s Hospital, Toronto
    - David Langleben
      - Jewish General Hospital, Montreal
    - John Granton
      - University Health Network, Toronto
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