The Estelle Grover Lecture 2015
Mitochondrial dynamics in pulmonary hypertension and cancer

Stephen L. Archer, MD Tier 1 Canada Research Chair in Mitochondrial Dynamics and Translational Medicine and Head of Medicine

COI: none
Funding NIH, CIHR and The American Heart Association

Queen’s University at Kingston Ontario, Canada
Mitochondrial Dynamics
Constriction, Fission, Fusion and Translocation

Leica gated STED, Resonant scan, normal human PASMC
TMRM, 63X, 1.7 X zoom
Estelle Grover
Enthusiastic mountaineer, avid scientist
and partner of Bob Grover in life and research
Reeves and Grover
Partners in Science from Mount Evans to Pikes Pike
Importance of Partners in Research

Bob and Estelle in Peru and Fiji
A New Partner: Elise
Why do we need partners and supportive colleagues in Science?
“In the light of knowledge attained, the happy achievement seems almost a matter of course, and any intelligent student can grasp it without too much trouble. But the years of anxious searching in the dark, with their intense longing, their alterations of confidence and exhaustion, and the final emergence into the light—only those who have themselves experienced it can understand that”… Einstein
University of Chicago
2007-2012
Lost Valley Ranch
Lofty goals at high altitude: The Grover Conferences, 1984–2011

E. Kenneth Weir¹, Wiltz W. Wagner Jr.², and Stephen L. Archer³

¹VA Medical Center 111C, 1 Veteran's Drive, Minneapolis, MN 55417. ²Department of Pharmacology and Center for Lung Biology, College of Medicine, University of South Alabama, Mobile, AL, 36688. ³Department of Medicine, The University of Chicago, 5841 South Maryland Avenue, Chicago, IL 60637, USA.
<table>
<thead>
<tr>
<th>Year</th>
<th>Conference Title</th>
<th>Publisher</th>
<th>Conference Director</th>
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<tbody>
<tr>
<td>1988</td>
<td>The Control of Cellular Proliferation</td>
<td>Futura, New York, NY</td>
<td>E.K. Weir, J.T. Reeves</td>
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<td>1990</td>
<td>The Diagnosis and Treatment of Pulmonary Hypertension</td>
<td>Futura, New York, NY</td>
<td>W.W. Wagner, E.K. Weir</td>
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<tr>
<td>2000</td>
<td>The Blood and the Pulmonary Circulation</td>
<td>Futura, New York, NY</td>
<td>J. Bhattacharya</td>
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<td>2002</td>
<td>Proinflammatory Signaling Mechanisms in the Pulmonary Circulation</td>
<td>Humana Press, Totowa New Jersey, NJ</td>
<td>T. Stevens</td>
</tr>
</tbody>
</table>

2013: Coupling of the Right Ventricle and Pulmonary Circulation

2015: The Pulmonary Circulation in the OMICS-Era

2015: The Pulmonary Circulation in the OMICS-Era

Pulmonary Circulation

Archer, SI, Ryan, J and Weir, EK

Austin, E, CHung, W, Best, H and Elliott, G
Grover meeting: HAPE
High Altitude Partnership Enabler
Grover meeting: HAPE
Grover meeting: HAPE
Are you an Equine Professional?

WARNING
Under Colorado Law, an equine professional is not liable for an injury to or the death of a participant in equine activities resulting from the inherent risk of equine activities, pursuant to section 13-21-120, Colorado Revised Statutes.
Grover meeting: 2013
Bob Grover on altitude-induced PH
When Mitochondria Attack
Rudolf Albert von Kölliker

- Swiss physiologist
- Studied under Henle
- 1857 identified *sarcosomes* (mitochondria) in skeletal muscle
Carl Benda 1857-1932

- German microbiologist used Crystal Violet to image mitochondria
- Heterogenous morphology, sometimes ball-shaped other times linear
- Created name *mitochondrion*: Greek, *mitos* (thread) + *chondrion* (granule)
Richard Altmann

"The Elementary Organism" 1852-1900

- German histopathologist & Prof Anatomy Leipzig
- Developed new staining techniques using osmium tetroxide and potassium dichromate to stain cells
- Observed filaments deriving from granules which he termed "bioblasts" and to which he attributed genetic and metabolic functions

Zeiss microscope 1879
I greatly admire Lynn Margulis’ sheer courage and stamina in sticking by the endosymbiosis theory, and carrying it through from being an unorthodoxy to an orthodoxy. I’m referring to the theory that the eukaryotic cell is a symbiotic union of primitive prokaryotic cells. This is one of the great achievements of twentieth-century evolutionary biology, and I greatly admire her for it…Richard Dawkins
Lynn could infuriate her colleagues, but at least one of her proposals changed the way that we think about life.

When asked why she also proposed controversial ideas by an interviewer from Discover magazine, she replied:

“I don’t consider my ideas controversial. I consider them right.”

Knoll, A. H. 1022 | PNAS | January 24, 2012 | vol. 109 | no. 4
Mitochondria: *Powerhouse of the cell*

- Singular, stationary organelles that synthesize ATP

*Journal of Molecular Medicine 2010*
Traditional Role of Mitochondria

- Singular, stationary organelles that synthesize ATP

True Role of Mitochondria

- Networks of dynamically mobile organelles that synthesize ATP and act as oxygen sensors and regulators of cell proliferation and apoptosis
Noncanonical Mitochondrial Functions

Somatic Cell

Dividing Cell
A simple test

courtesy of BuzzFeed’s Dave Stoper (http://tinyurl.com/phccpac)
The Linkage of form and function in normal human PASMC
Imaging fission & fusion

Pre-activation

Photo-activation

Image Analysis
Back to the Future: Lewis and Lewis

Warren Harmon Lewis (1870-1964)

Margaret Reed Lewis (1881-1970)

MITOCHONDRIA (AND OTHER CYTOPLASMIC STRUCTURES) IN TISSUE CULTURES

MARGARET REED LEWIS AND WARREN HARMON LEWIS*

From the Anatomical Laboratory, Johns Hopkins Medical School, and the Marine Biological Laboratory, Woods Hole, Mass.
“Any one type of mitochondria such as a granule, rod or thread may at times change into any other type or may fuse with another mitochondrion, or it may divide into one or several mitochondria. Every type of mitochondria is continually changing shape and may assume as many as fifteen or twenty shapes in ten minutes.”

Mitochondria as Oxygen Sensors: Redox Theory

Fawn Hooded Rats: *Pseudohypoxia*  

Hypoxia-like:
1. Reduced HPV
2. PAH
3. Polycythemia

<table>
<thead>
<tr>
<th></th>
<th>SD</th>
<th>FHR</th>
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<tr>
<td><strong>Hemodynamics</strong></td>
<td></td>
<td></td>
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<tr>
<td>Mean PAP (mmHg)</td>
<td>11.0 ± 0.6</td>
<td>28.0 ± 0.4 *</td>
</tr>
<tr>
<td>TPR (mmHg.min.mL)</td>
<td>0.07 ± 0.01</td>
<td>0.30 ± 0.03 *</td>
</tr>
<tr>
<td>Mean SP (mmHg)</td>
<td>100 ± 6</td>
<td>95 ± 8</td>
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<tr>
<td>SVR (mmHg.min.Kg.L)</td>
<td>0.91 ± 0.07</td>
<td>0.81 ± 0.10</td>
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<tr>
<td>CO (mL/min)</td>
<td>108 ± 3</td>
<td>100 ± 7</td>
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<tr>
<td><strong>Blood Analysis</strong></td>
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<tr>
<td>HGB (g/L)</td>
<td>117 ± 8</td>
<td>133 ± 13 *</td>
</tr>
<tr>
<td>Creatinine (µM)</td>
<td>51 ± 20</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td>79 ± 1</td>
<td>78 ± 3</td>
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PAH (rat and human): *HIF-1α is on & mitochondria are SOD2 deficient and fragmented* Bonnet et al. Circ. 2006.
Mitochondria Are Fragmented in PAH

Control

- Red: Mitochondria
- Blue: Nuclei

PAH

- Green: Photoactivated GFP

Scale: 20 μm
HIF-activation triggers fission & proliferation

Normal PASMC

HIF-1α

% Proliferating PASMCs

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<th>DMSO 5</th>
<th>25</th>
<th>50</th>
<th>DMSO 5</th>
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PAH

** nM Chetomin

*** nM Chetomin
Fission/Fusion Imbalance in PAH?

Fragmented Mitochondria:
Mitosis Regulation (Cyclin B-CDK1)
Calcium Regulation (PDH)
Links to Ras/ERK (Mfn2)
Excess DRP-1 in Human PAH PASMC

**Fusogenic**

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<tr>
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<th>Mfn2</th>
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<tr>
<td>Ctr</td>
<td>1.0</td>
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<tr>
<td>PAH</td>
<td>0.5</td>
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**Fissogenic**

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<tr>
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<th>Drp1</th>
<th>Fis1</th>
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<tbody>
<tr>
<td>Ctr</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>PAH</td>
<td>2.0</td>
<td>1.5</td>
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**DRP1 Ser616-P**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PAH</th>
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<tbody>
<tr>
<td>No Antibody</td>
<td></td>
<td></td>
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<tr>
<td>Plexiform lesion</td>
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**DRP1 P-Ser616**

<table>
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<tr>
<th></th>
<th>% DRP1 P-Ser616 positive blood vessels</th>
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<tbody>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>PAH</td>
<td>30</td>
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</table>

**B**
Mitochondrial division is coordinated with mitosis by a shared regulatory kinase: Cylin B-CDK1

- Hyperproliferative states require rapid mitochondrial division
- Mitochondrial division is a mitotic checkpoint
- Inhibition of mitochondrial division causes G2-M arrest
- Forced fusion is an antiproliferative strategy useful in PAH and cancer
Consequences in PAH?

PASMC
Chronic CoCl₂ Treatment Causes PAH Which Can Be Treated by DRP1 Inhibition

- Control
- CoCl₂
- CoCl₂ + Mdivi-1

Walking Distance

- Control
- CoCl₂
- CoCl₂ + Mdivi-1

PVR

Endothelial cells
Smooth muscle cells
Nuclei
Excessive mitochondrial fission and cell proliferation
Lessons from NSCL Cancer

A549  MFC 3.87

Human lung cancer cell

PAEC MFC = 0.96

Primary healthy human lung cells

A549  H358  H1993  HCC827

hSAEC  hBEC  hPAEC  hPASMC

Inhibition of mitochondrial fission prevents cell cycle progression in lung cancer

Javier Rebitera,⁎ Harry J. Zhang,⁎ Peter T. Topk,① Yantao Zhang,① Glenn Markbreirth,① Zhengyong Song,① Ravi Selips①,② Alys N. Bosin,① Christian Wiedenb,① and Stephen L. Archer①
Mitochondrial Fission in Cancer
Stage IV Non Small Cell Lung Cancer
A newly recognized mitochondrial disorder regulates proliferation and apoptosis
Inhibiting Fission (or enhancing fusion) regresses lung cancer in a murine xenotransplantation model.
The Sweet Tooth of Pulmonary Hypertension

Inhibiting Pyruvate Dehydrogenase Kinase: treating PAH and Cancer
Aerobic glycolysis **uncoupled** from glucose oxidation

*(Warburg O et al, J Gen Physiol 1927;8:519-530)*

*(Warburg O Science 1956;123:309-312)*
The Warburg Players: SOD2-HIF-PDK-PDH
Warburg’s Organelle: The mitochondria
Dichloroacetate Reverses Hypoxic PHT

Chronic Hypoxic PHT

Control

Chronic Hypoxic + DCA

Michelakis et al Circulation 105, 244-250, 2002

PVR

DCA 0.75g/L in Drinking water

(n=5 each)
DCA reduce proliferation and increases apoptosis in MCT PAH

McMurtry, MS et al. Circ Res 2004
DCA benefits the hibernating right ventricle in PAH
Piao et al J Mol Med 2010
Michelakis and Wilkins
UNPUBLISHED DATA FROM DCA Trial in PAH

Representative images of mean transit time (average time for tracer to travel from the arterial input to the lung parenchyma) show a significantly shorter transit time 6 months following DCA therapy, which corresponds to an increase in perfusion.

<table>
<thead>
<tr>
<th>patient</th>
<th>Δ PVR</th>
<th>Δ 6mw</th>
<th>Δ RVmass</th>
<th>Δ RVEDV</th>
<th>DCA mg/Kg</th>
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<tr>
<td>38 F</td>
<td>-7%</td>
<td>+29m</td>
<td>-0%</td>
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<tr>
<td>58 F</td>
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<td>-16%</td>
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<tr>
<td>42 F</td>
<td>-5%</td>
<td>+10m</td>
<td>-2%</td>
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<tr>
<td>41 F</td>
<td>-45%</td>
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<tr>
<td>39 F</td>
<td>-28%</td>
<td>+79m</td>
<td>-17%</td>
<td>-19%</td>
<td>3 bid</td>
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DCA inhibits growth of human cancer
A Mitochondrial-Redox Mechanism of Regulating Cell Proliferation/Apoptosis & RV Contractility

HPV

PAH

Cancer

SOD2
HIF-1α
PDK
Kv1.5
Kathie Doliszny, Ken Weir, Liz Weir