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*

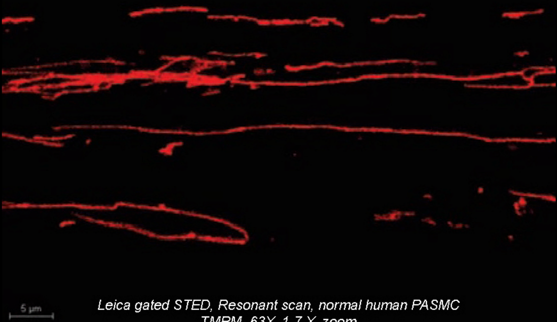
COI: none

Funding NIH, CIHR and The American Heart Association

Queen's University at Kingston Ontario, Canada

Mitochondrial Dynamics

Constriction, Fission, Fusion and Translocation



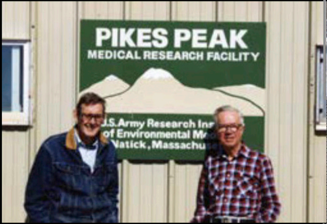
Leica gated STED, Resonant scan, normal human PASMC
TMRM, 63X, 1.7 X zoom

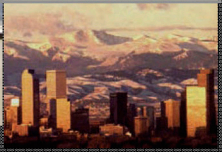
Estelle Grover

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



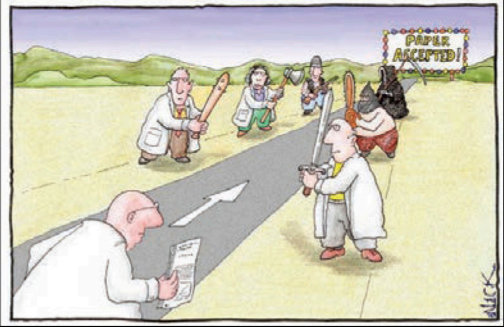


Importance of Partners in Research

Bob and Estelle in Peru and Fiji

A New Partner: Elise

Why do we need partners and supportive colleagues in Science?



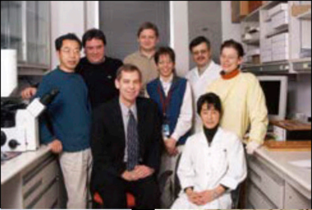
Partners in Science: E. Kenneth Weir

"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 Minnesota
Minneapolis VAMC
1988-1998

University of Alberta

1998-2007



University of Chicago
2007-2012

Queen's University
2012 -

Lost Valley Ranch

Lofty goals at high altitude: The Grover Conferences, 1984–2011

E. Kenneth Weir[1], Wiltz W. Wagner Jr.[2], and Stephen L. Archer[3]

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Website: www.pulmonarycirculation.org

DOI: [illegible]

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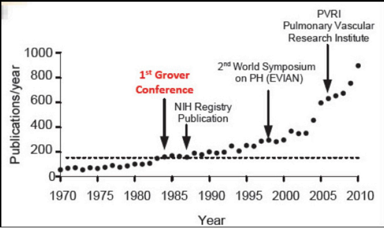
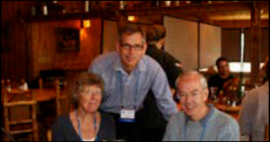


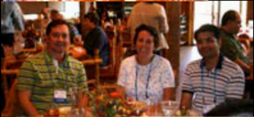
Table 1: The Grover Conferences on the Pulmonary Circulation, 1984-2011		Publisher	Conference Director
1984	Pulmonary Vascular Reactivity	Chest 88: 1995-2725, 1985	E.K. Weir, I.F. McMurtry, J.T. Reeves
1986	The Role of Lipid Mediators	Am. Rev. Respir. Dis. 136: 196-224; 782-788, 1987	E.K. Weir, J.T. Reeves
1988	The Control of Cellular Proliferation	Am. Rev. Respir. Dis. 140: 1093-1135; 1446-1493, 1989	E.K. Weir, J.T. Reeves
1990	The Diagnosis and Treatment of Pulmonary Hypertension	Futura, New York, NY	E.K. Weir, S.L. Archer, J.T. Reeves
1991	The Pulmonary Circulation and Gas Exchange	Futura, New York, NY	W.W. Wagner, E.K. Weir
1992	The Role of Ion Flux	Plenum Press, New York, NY	E.K. Weir, J. R. Hume, J.T. Reeves
1994	The Role of Radicals	Futura, New York, NY	E.K. Weir, S.L. Archer, J.T. Reeves
1996	The Pathogenesis and Treatment of Pulmonary Edema	Futura, New York, NY	E.K. Weir, S.L. Archer, J.T. Reeves
1998	The Fetal and Neonatal Pulmonary Circulations	Futura, New York, NY	E.K. Weir, S.L. Archer, J.T. Reeves
2000	The Blood and the Pulmonary Circulation	Futura, New York, NY	E.K. Weir, H.L. Reeve, J.T. Reeves
2002	Proinflammatory Signaling Mechanisms in the Pulmonary Circulation	Humana Press, Totowa New Jersey, NJ	J. Bhattacharya
2004	Genetic and Environmental Determinants of Pulmonary Endothelial Cell Function	http://www.groverconference.org/grover_past_conft.htm	T. Stevens
2006	Rho Family GTPases in Pulmonary Vascular Pathophysiology	http://www.groverconference.org/grover_past_conft.htm	K. Fagan, I.F. McMurtry
2008	Membrane Receptors, Channels and Transporters in Pulmonary Circulation	Humana Press-Springer, New York, NY Adv. Exp. Med. Biol. Vol. 661	J.P.T. Ward, J.X.-J. Yuan
2011	Risk Factors in Pulmonary Hypertension	Pulm. Circ. Vol. 1-2, 2011-2012	M.R. MacLean, N.W. Morrell, K. Fagan
2013:	Coupling of the Right Ventricle and Pulmonary Circulation	Pulmonary Circulation	Archer, Sl, Ryan, J and Weir, EK
2015:	The Pulmonary Circulation in the OMICS-Era	? Pulmonary Circulation	Austin, E, CHung, W, Best, H and Elliott, G

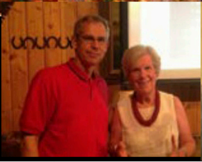
Grover meeting: HAPE

***H**igh **A**ltitude **P**artnership **E**nabler*



Grover meeting HAPE





Grover meeting: HAPE

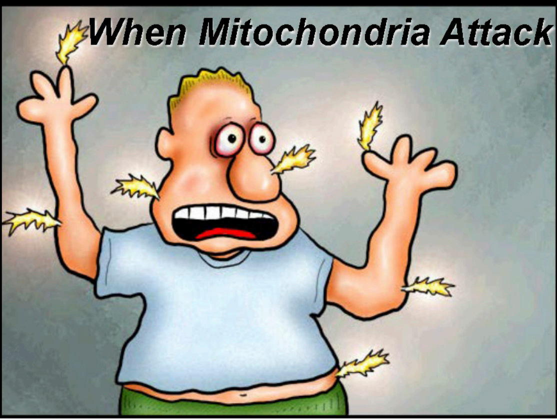
Grover meeting: HAPE

Are you an *Equine Professional*?

Grover meeting 2013

Grover meeting: 2013

Bob Grover on altitude-induced PH

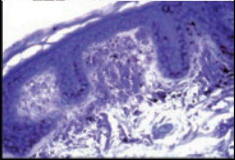

When Mitochondria Attack

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)



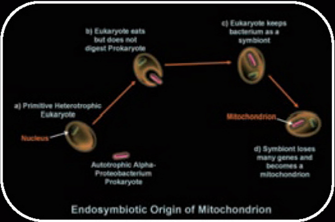
Richard Altmann
"The Elementary Organism" 1852-1900

- 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

Lynn Margulis (1938-2011)

The Origin of Mitosing Eukaryotic Cells; *The Journal of Theoretical Biology* :



*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

The Last Word

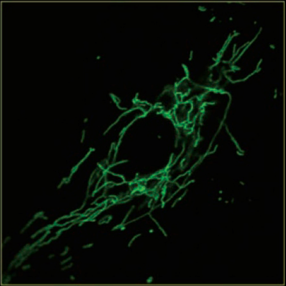
Lynn could infuriate her colleagues, but at least one of her proposals changed the way that we think about life.

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

Endothelial Cell

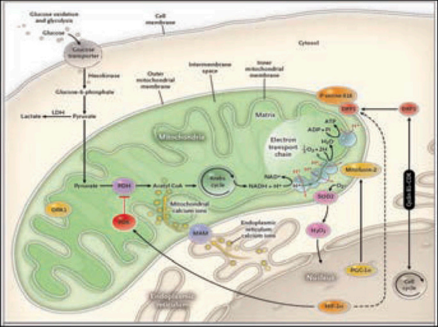


Mitochondria: *Powerhouse of the cell*

Journal of Molecular Medicine 2010

- Singular, stationary organelles that synthesize ATP

Mitochondrial Metabolism



Traditional Role of Mitochondria

- Singular, stationary organelles that synthesize ATP

Journal of Molecular Medicine
2010

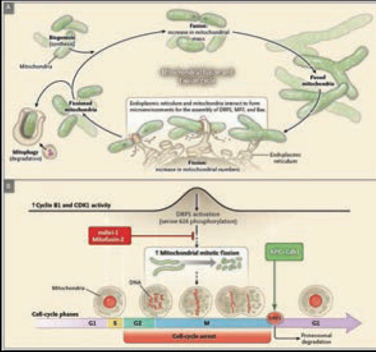
True Role of Mitochondria

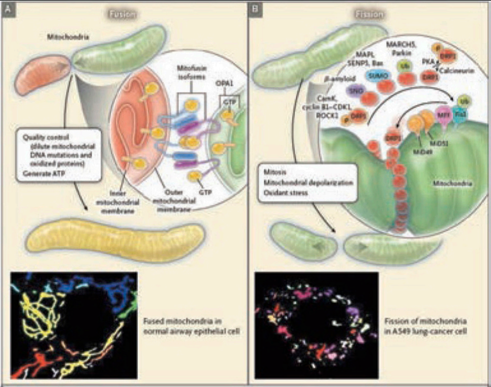
- Networks of dynamically mobile organelles that synthesize ATP and act as oxygen sensors and regulators of cell proliferation and apoptosis

Noncanonical Mitochondrial Functions

Somatic Cell

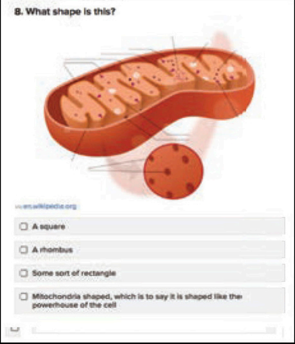
Dividing Cell



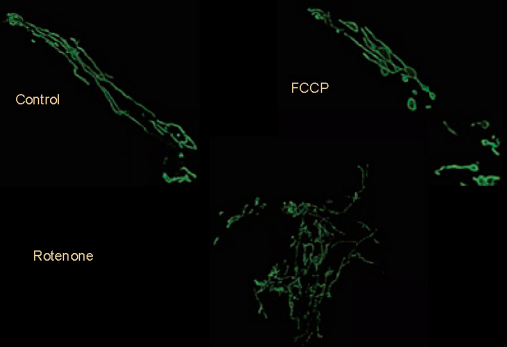

A
Fusion
Mitochondria
Mitofusin isoforms
OPA1
GTP
Quality control (dilute mitochondrial DNA mutations and oxidized proteins)
Generate ATP
Inner mitochondrial membrane
Outer mitochondrial membrane
GTP
Fused mitochondria in normal airway epithelial cell
B
Fission
MARCH5, Parkin
MAPL SENP5, Bax
SUMO
Ub
P
DRP1
PKA
Calcineurin
DRP1
β-amyloid
SNO
CamK, cyclin B1–CDK1, ROCK2
P
DRP1
Ub
MFF
Fis1
DRP1
MiD51
MiD49
Mitosis
Mitochondrial depolarization
Oxidant stress
Mitochondria
Fission of mitochondria in A549 lung-cancer cell

A simple test

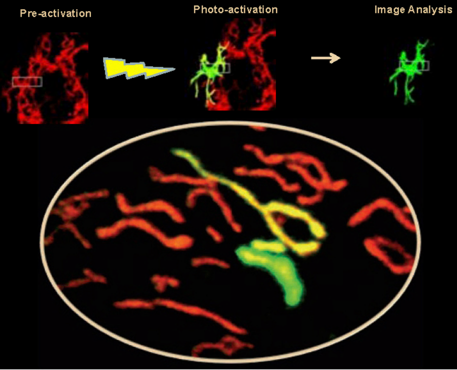
..courtesy of BuzzFeed's Dave Stoper (**http://tinyurl.com/phccpac**)



The Linkage of form and function in normal human PASMC

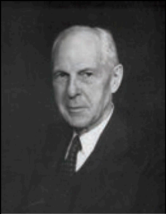


Imaging fission & fusion



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[1]

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."

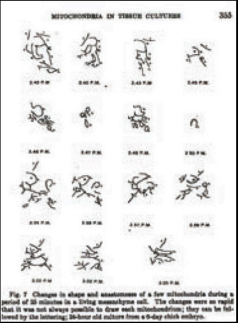

MITOCHONDRIA IN TISSUE CULTURES 355

Fig. 7 Changes in shape and anastomoses of a few mitochondria during a period of 25 minutes in a living mesenchyme cell. The changes were so rapid that it was not always possible to draw each mitochondrion; they can be followed by the lettering. 24-hour old culture from a 5-day chick embryo.

Lewis MR, Lewis WH. Mitochondria in tissue culture. *Science*. 1914;39:330-333

Oxygen Homeostatic System

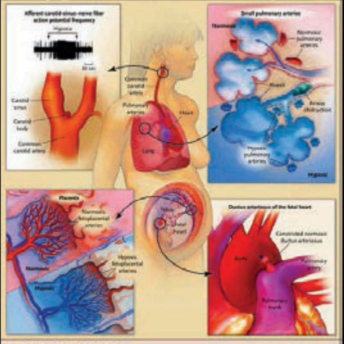
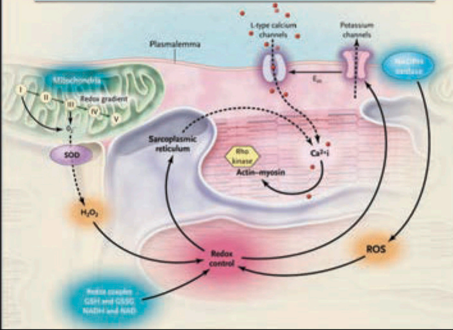


Figure 2. Homeostatic Oxygen-Sensing System.

Weir et al. NEJM, 2005.

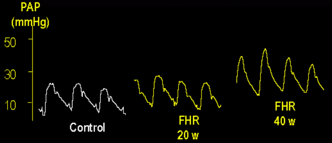
Mitochondria as Oxygen Sensors
Redox Theory

N Engl J Med. 2005;353(19):2042-2055



Fawn Hooded Rats: *Pseudohypoxia*

Bonnet et al. Circ. 2006.

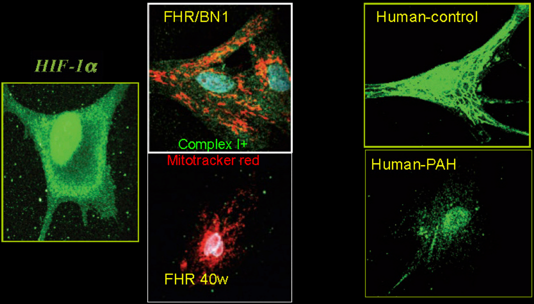


N=5 to 12/group	SD	FHR
Hemodynamics		
Mean PAP (mmHg)	11.0 ± 0.6	28.0 ± 0.4 *
TPR (mmHg.min.mL)	0.07 ± 0.01	0.30 ± 0.03 *
Mean SP (mmHg)	100 ± 6	95 ± 8
SVR (mmHg.min.Kg.L)	0.91 ± 0.07	0.81 ± 0.10
CO (mL/min)	108 ± 3	100 ±7
Blood Analysis		
HGB (g/L)	117 ± 8	133 ± 13 *
Creatinine (μM)	51 ± 20	33 ± 4
PO_2 (mmHg)	**79 ± 1**	**78 ± 3**

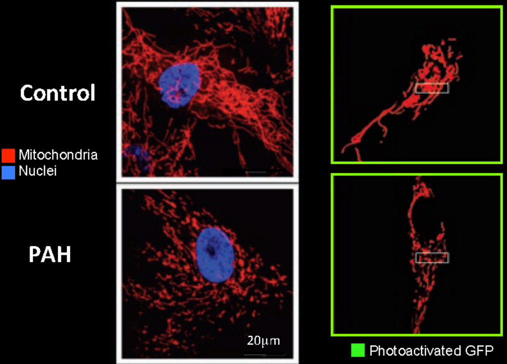
Hypoxia-like:

1. Reduced HPV
2. PAH
3. Polycythemia

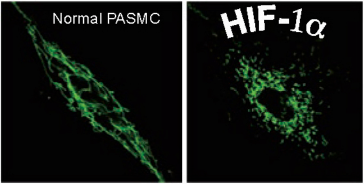
PAH (rat and human): *HIF-1α is on &mitochondria are SOD2 deficient and fragmented* Bonnet et al. *Circ.* 2006.

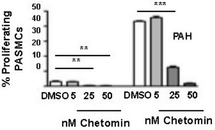


Mitochondria Are Fragmented in PAH

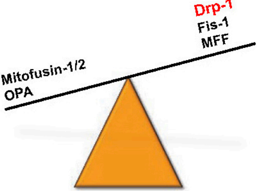


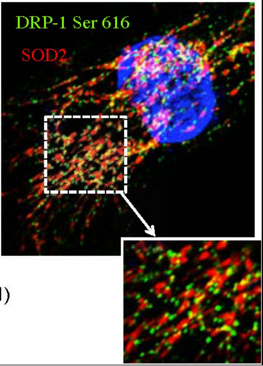
HIF-activation triggers fission & proliferation





Fission/Fusion Imbalance in PAH?





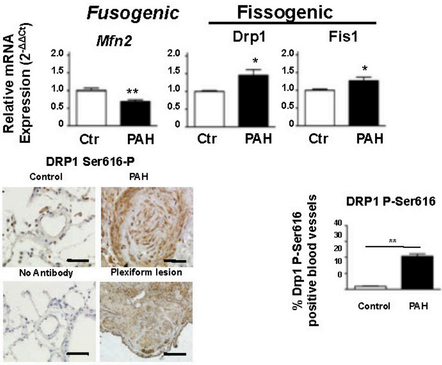
Fragmented Mitochondria:

Mitosis Regulation (Cyclin B-CDK1)

Calcium Regulation (PDH)

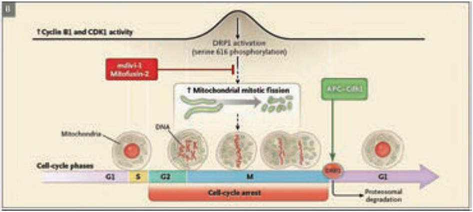
Links to Ras/ERK (Mfn2)

Excess DRP-1 in Human PAH PASMC



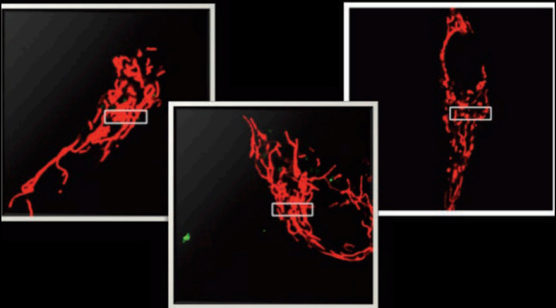
Mitochondrial division is coordinated with mitosis by a shared regulatory kinase: *Cylin B-CDK1*

- 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

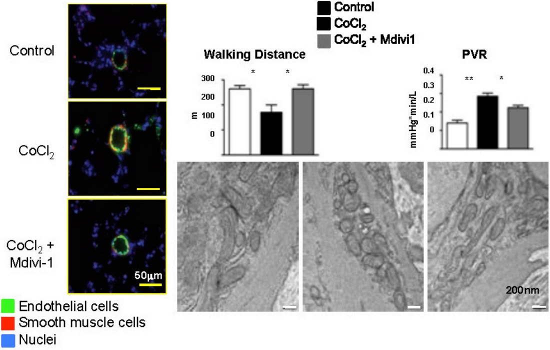


Consequences in PAH?

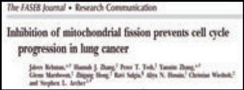
PASMC



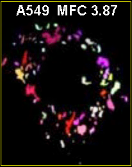
Chronic $CoCl_2$ Treatment Causes PAH Which Can Be Treated by DRP1 Inhibition

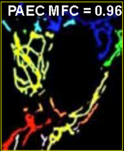


Excessive mitochondrial fission and cell proliferation
Lessons from NSCL Cancer


The FASEB Journal • Research Communication

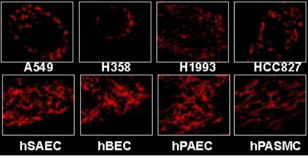
Inhibition of mitochondrial fission prevents cell cycle progression in lung cancer





Human lung cancer cell

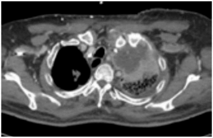
Primary healthy human lung cells

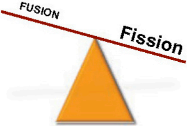


Mitochondrial Fission in Cancer

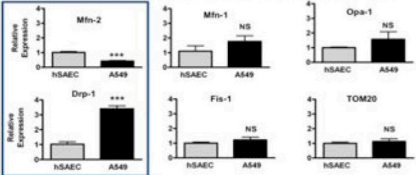
Stage IV Non Small Cell Lung Cancer

A newly recognized mitochondrial disorder regulates proliferation and apoptosis

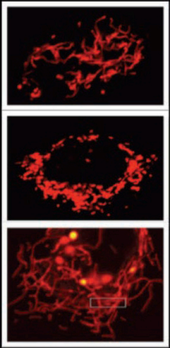


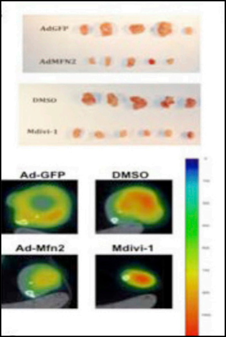


Expression of Mitochondrial Networking Mediators in Cultured Cells



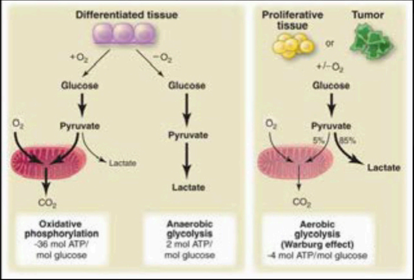
Inhibiting Fission (or enhancing fusion) regresses lung cancer in a murine xenotransplantation model





The Sweet Tooth of Pulmonary Hypertension

Inhibiting Pyruvate Dehydrogenase Kinase: treating PAH and Cancer



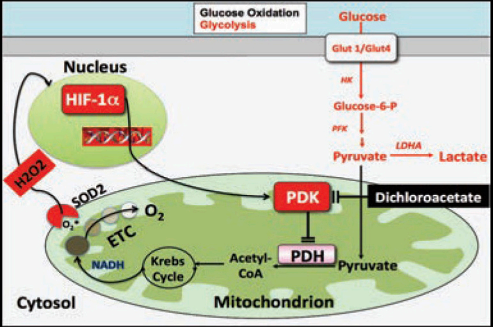
Otto Warburg

Aerobic glycolysis uncoupled from glucose oxidation

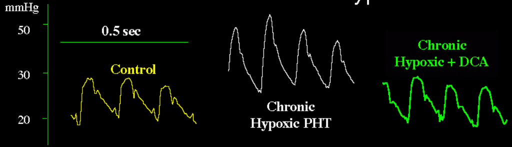
(Warburg O et al, J Gen Physiol 1927;8:519-530)

(Warburg O Science 1956;123:309-312)

The Warburg Players: SOD2-HIF-PDK-PDH
Warburg's Organelle: The mitochondria

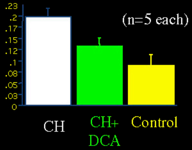


Dichloroacetate Reverses Hypoxic PHT

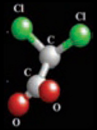


Michelakis et al Circulation 105, 244-250, 2002

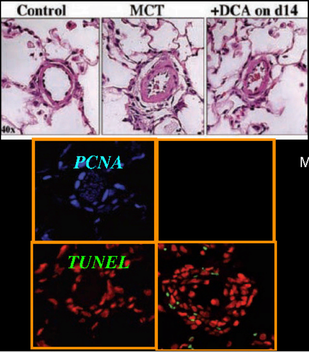
PVR



DCA 0.75g/L in
Drinking water



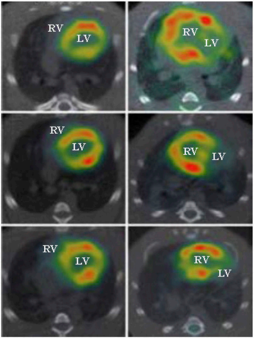
DCA reduce proliferation and increases apoptosis in MCT PAH



McMurtry, MS et a
Circ Res 2004

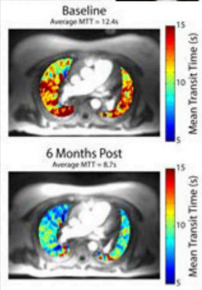
DCA benefits the hibernating right ventricle in PAH

Piao et al J Mol Med 2010



Michelakis and Wilkins UNPUBLISHED DATA FROM DCA Trial in PAH

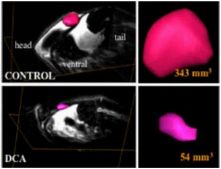
Representative images of mean transit time (average time for tracer to travel from the arterial input to the lung parenchyma) show a significantly shorter transit time 6 months following DCA therapy, which corresponds to an increase in perfusion.

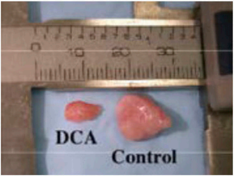


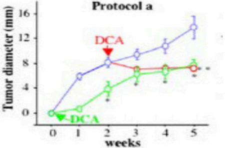
patient	Δ PVR	Δ 6mw	Δ RVmass	Δ RVEDV	DCA mg/ Kg
38 F	-7%	+29m	-0%	-30%	3 bid
58 F	-34%	+92m	-19%	-16%	3 bid
42 F	-5%	+10m	-2%	+1%	3 bid
41 F	-45%	+90m	-20%	-15%	6.25 bid
39 F	-28%	+79m	-17%	-19%	3 bid

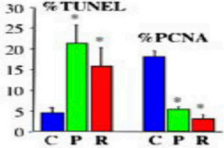
DCA inhibits growth of human cancer

Bonnet et al *Cancer Cell* 11: 37-51, 2007

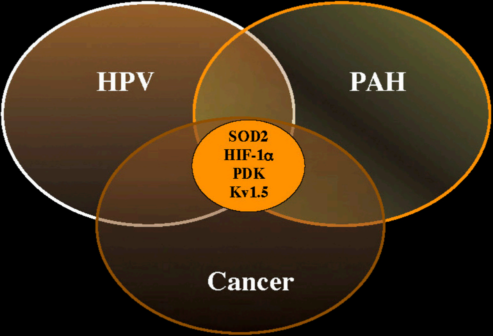








A Mitochondrial-Redox Mechanism of Regulating Cell Proliferation/Apoptosis & RV Contractility



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