

book
of
abstracts

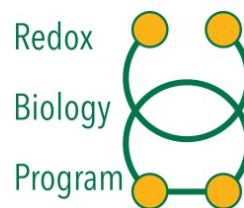
Proceedings of the First Redox Biology Program Meeting

Cardiovascular Redox Signaling Symposium

mcw.edu/redoxbiologyprogram



Medical College of Wisconsin
Milwaukee, Wis.
November 14, 2014



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First Redox Biology Program Cardiovascular Redox Signaling Symposium

Medical College of Wisconsin, Milwaukee, Wis.

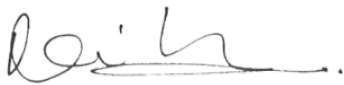
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Dear Symposium Registrants:

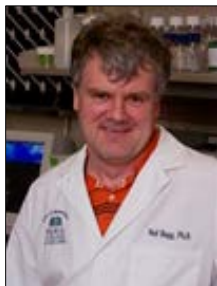
On behalf of the Organizing Committee, it is our pleasure to extend a warm welcome to the **Redox Biology Program Cardiovascular Redox Signaling Symposium**. We have assembled some prominent contributors addressing many topics: endothelial dysfunction, oxidant stress, redox signaling, nitric oxide applications, sirtuins, and antioxidant treatments. The accompanying poster presentations enhance, as well as expand, upon the many topics that will be discussed today. The program is an excellent opportunity for learning and is designed to encourage interaction and discussion.

The **Redox Biology Program Cardiovascular Redox Signaling Symposium** is jointly organized by the Department of Biophysics and the Redox Biology Program at the Medical College of Wisconsin. The Symposium would not have been possible without the generous support of our many sponsors. We are grateful for their investment in scientific research and education.

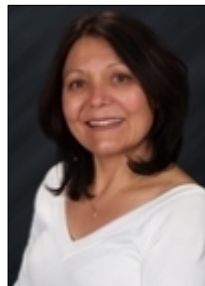
We thank you for your participation!



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Conference Program

November 14, 2014

- 8:00 **Welcome**
- 8:15 *Resetting the Redox Spectrum: Oxido-Reductive Stress in Health and Disease*
Ivor J. Benjamin, MD
- 8:45 *Role of NOXs in Hypertension Dissected Using Genetically Engineered Rat Models*
Allen W. Cowley Jr., PhD
- 9:15 *Modification of HDL by Reactive Aldehydes – The Prince Turns into a Frog*
Alexandra Chadwick
- 9:45 **Morning Break**
- 10:15 *Oxidative Pathways for the Generation of Dysfunctional HDL*
Jay Heinecke, MD
- 10:45 *Diabetic Vascular Endothelial Dysfunction – Role of Mitochondria*
Michael E. Widlansky, MD, MPH
- 11:15 *CD36 Functions as a Sensor of Oxidant Stress in the Vascular System*
Roy L. Silverstein, MD
- 11:45 **Lunch and Poster Session**
- 1:15 *Sirtuins: From Mitochondrial Redox and Metabolism to Chromatin Regulation*
John M. Denu, PhD
- 1:45 *Nitric Oxide Signaling through S-Nitrosoglutathione Transnitrosation*
Brian Smith, PhD
- 2:15 *Superoxide as a Regulator of Nitric Oxide Signaling in Beta Cells*
Kasia Broniowska, PhD & Control of Beta-Cell Fate by Nitric Oxide
Bryndon Oleson
- 2:45 **Afternoon Break**
- 3:15 *Redox Regulated Proteins and Proteome* **Richard A. Cohen, MD**
- 3:45 *Critical Role for Telomerase in Maintenance and Regulation of Flow Mediated
Dilation in the Human Microcirculation* **Andreas M. Beyer, PhD**
- 4:15 *Targeting NADPH Oxidases in Cardiovascular Diseases: Development of Assays for
High Throughput Screening of Nox2 Inhibitors* **Jacek Zielonka, PhD**
- 4:45 *Endothelial NOS Uncoupling: New Biochemical and Biophysical Developments*
Jeannette M. Vasquez-Vivar, PhD

Session Chairs

The following session chairs will be introducing speakers:

Morning Proceedings



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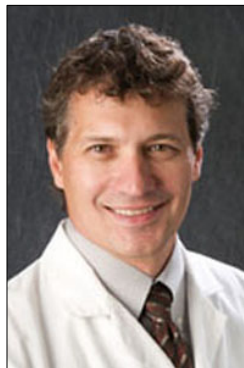


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Afternoon Proceedings



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**Part I:
Presentation Abstracts**

The Reductive Stress Hypothesis and the Antioxidant Treatment Paradox

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The reductive stress hypothesis in disease pathology was recently revisited by Benjamin and colleagues who have demonstrated that a protein-misfolding (R120G CryAB) cardiomyopathy was under **reductive stress**, as opposed to oxidative stress, from an over-active antioxidative system. Decreasing the function of glucose-6-phosphate dehydrogenase (G6PDH), which generates the reductant NADPH, “cures” the disease in a mouse model by ameliorating reductive stress, aggresome formation, hypertrophy, heart failure and death. Since this discovery, several laboratories have independently implicated the effects of reductive stress as causal mechanisms in hyperglycemic-induced metabolic syndrome, experimental ischemic injury (e.g., dominant negative Nox4 isoform), cardiomyopathy, and inheritable skeletal and cardiac myopathy. Of direct translational relevance, carriers of the Gd-Mediterranean allele of G6PD deficiency living on a coastal island in Sardinia, Italy are remarkably protected against ischemic heart disease, cerebrovascular strokes, retinal vein occlusion (RVO), nonarteritic anterior optic neuropathy (NAION), and perhaps, diabetic retinopathy, underscoring the far-reaching implications of this work in humans.

An alternative to the reductive stress hypothesis has been extensively pursued for almost five decades, beginning with the oxidative stress theory of aging, on the basis that free radicals and reactive oxygen species (ROS), the byproducts of oxidative phosphorylation, are deleterious in the setting of inadequate ROS scavenging by the antioxidative system. Oxidative stress has been proposed as a major mediator of vascular dysfunction and has been proposed as a pathological factor in almost every disease from glucotoxicity in pancreatic β -cells, inflammation in infection, cancer metastasis and survival, liver fibrosis, and neurodegenerative disease. Stroke, (not ‘classic’ neurodegenerative), affected 141 million people worldwide in 2012. Thousands of preclinical and clinical studies over decades have been inconclusive and failed to show efficacy of antioxidant therapeutics while other trials were prematurely terminated owing increased morbidity and mortality. What has not been clear are what factor(s) might account for such abysmal failure. Both proponents and opponents of oxidative stress have fueled the confusion and controversy — and both sides have overlooked the importance of “reductive stress,” as opposed to oxidative stress, as a causal mechanism in disease pathogenesis. What are the factor(s) that might increase the susceptibility for major adverse toxicity and fatal outcomes from antioxidant therapy? We postulate that either pro-reducing redox state among heterogeneous clinical cohorts and/or the pro-reducing effects of antioxidant agents will, either alone or combined, promote life-threatening reductive stress. Antioxidant therapeutics *per se* are not the culprits unless indiscriminately administered without regard for their biological context and/or consequence across the redox spectrum. Moreover, we think that the rationale for the similar use of current compounds in antioxidant clinical trials are seriously flawed and are unlikely to answer or overcome the existing deficiencies and major barriers in the field. Understanding the mechanism for basic redox reactivity and for biological redox effects is a *sine qua non* for the rational design of clinical trials using targeted oxido-reductive therapeutics in disease pathology.

Role of NOXs in Hypertension Dissected in Rat Knockouts

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Evidence will be summarized showing that redox imbalance of NO, O₂⁻ and H₂O₂ occurs in the renal outer medulla (OM) of the Dahl salt-sensitive (SS) rat when fed a high salt diet, which leads to hypertension and renal injury. This evidence will include:

1. Data showing that genetic variances in the promoter region of the NAD(P)H oxidase p67^{phox} subunit of SS rats (compared to the salt-resistant congenic control strain) results in enhanced expression of the p67^{phox} mRNA protein and enzyme in the OM of SS rats fed a high salt diet (noting that no differences were seen in protein expression of gp91^{phox}, p22^{phox}, p47^{phox} or Rac1).

2. Development and application of an inbred line of SS p67^{phox}^{-/-} mutant rats demonstrating the functional relevance in the progression of hypertension in SS rats. These rats exhibited a nearly 50% reduction of OM tissue H₂O₂, O₂⁻ (microdialysis), NAD(P)H oxidase activity and a 40% reduction of salt-induced hypertension compared to wild-type (SS-WT) littermates.

3. Data from two series of studies showing that excess ROS production in the renal outer medulla, resulting from excess p67^{phox}, was responsible for salt-sensitivity in the SS rats. First, chronic renal medullary interstitial infusion of apocynin and lower interstitial ROS levels reduced salt-induced hypertension in SS nearly 50%. Second, transgenic overexpression of p67^{phox}, specifically in the outer medullary thick ascending limb of Henle (mTAL) of a salt-resistant congenic strain, resulted in an enhancement of salt-sensitivity and increased renal injury compared to SS-WT rats.

4. Results from studies indicating that Nox4 also contributes importantly to increased oxidative stress in the outer medulla and to the development of hypertension and renal injury in the SS rat.

5. Results from recent studies based on *in vivo* fluorescence imaging of isolated perfused mTAL indicating that Nox4 contributes specifically to intracellular H₂O₂ production but not O₂⁻ production in the mTAL.

Modification of HDL by Reactive Aldehydes — the Prince Turns Into a Frog

A. C. Chadwick

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Cardiovascular disease (CVD) is primarily caused by atherosclerosis, a condition characterized by buildup of cholesterol and fatty lesions in the arteries. High density lipoprotein (HDL) combats atherosclerosis by transporting excess cholesterol from the periphery to the liver for excretion via reverse cholesterol transport (RCT). Mounting evidence suggests that HDL “function”, rather than HDL-cholesterol levels, is a better indicator of CVD risk. In fact, oxidative modifications to HDL may render the particles “dysfunctional” and reduce HDL’s athero-protective functions. Acrolein, a reactive aldehyde found in tobacco smoke, was recently shown to increase CVD risk in humans, however its effects on HDL function have not been fully characterized. We hypothesized that acrolein modification of HDL generates a dysfunctional particle that would be unable to facilitate cholesterol clearance, and rather, promote pathways that lead to atherogenesis. To test this, we first modified HDL with acrolein (acro-HDL) and confirmed the presence of acrolein adducts on apoA-I and apoA-II, the two major HDL proteins, by immunoblot and mass spectrometry analyses. We determined that in the early steps of RCT, acro-HDL was unable to accept free cholesterol from cultured cells or reduce foam cell formation in murine peritoneal macrophages as compared to native HDL. We next determined that acro-HDL’s functionality at the end of RCT was impaired, resulting in 10-25% less efficient delivery of HDL-cholesteryl esters to cells expressing SR-BI, the HDL receptor. Quantitative RT-PCR was used to demonstrate that acro-HDL, but not native HDL, increased mRNA expression of scavenger receptors (CD36 and LOX-1) and inflammatory markers (ICAM-1 and TNF- α) in human THP-1 differentiated macrophages, paralleling the effects of oxidized LDL in stimulating atherogenic pathways. HDL dysfunction was also observed for HDL modified by other aldehydes such as 4-hydroxynonenol or malondialdehyde. Together, our findings reveal that modification of HDL produces a dysfunctional particle that is pro-atherogenic.

Myeloperoxidase and the Generation of Dysfunctional HDL

B. Shao, J.W. Heinecke

School of Medicine, Division of Metabolism, Endocrinology, and Nutrition, University of Washington, Seattle, WA

Inflammation and metabolic disorders have been proposed to convert high-density lipoprotein (HDL) to a dysfunctional form lacking anti-atherogenic properties. Consistent with this proposal, the ability of serum HDL to promote sterol efflux from cultured macrophages varies markedly, despite similar levels of HDL-C and apoA-I, HDL's major protein. Importantly, the sterol efflux capacity of serum HDL with cultured macrophages associates strongly and negatively with CAD status, and that association is independent of HDL-C and apoA-I levels. However, the factors that control the efflux capacity of serum HDL remain poorly understood.

In the current study, we isolated HDL from control subjects and subjects with stable coronary artery disease (CAD) or acute coronary syndrome (ACS) and used tandem mass spectrometry to explore the relationship between site-specific oxidation of apoA-I and the protein's ability to promote cholesterol efflux by the ABCA1 pathway. We found that levels of 3-chloroTyr192 and Met(O)148 were significantly higher in apoA-I of HDL isolated from CAD or ACS subjects than in HDL from apparently healthy control subjects. We also found that serum HDL in CAD and ACS subjects was significantly less able to promote cellular sterol efflux by the ABCA1 pathway. There was a strong inverse association between ABCA1 efflux capacity with CVD status, and this association persisted after adjustment for HDL-C. Levels of 3-chloroTyr192 and Met(O)148 positively associated with CVD status. Moreover, serum HDL's ability to promote sterol efflux by the ABCA1 pathway correlated inversely with levels of 3-chloroTyr192 and Met(O)148.

Because chlorination of Tyr192 in concert with oxidation of Met residues *in vitro* impairs the ABCA1 activity of apoA-I, and 3-chlorotyrosine is a characteristic chemical fingerprint of myeloperoxidase, our observations suggest that the heme protein contributes to the generation of dysfunctional HDL with impaired ABCA1 efflux capacity in humans with atherosclerotic vascular disease.

Diabetic Vascular Endothelial Dysfunction — Role of Mitochondria

M.E. Widlansky

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Diabetic vascular complications cause significant morbidity and mortality and contribute significantly to growing healthcare costs. While the causes of diabetic vascular disease remain incompletely elucidated, phenotypic data strongly suggest a unique pathophysiology for diabetic vascular disease relative to that seen in non-diabetic individuals. This presentation will review our data and understanding of the role mitochondrial dysfunction plays in the dysregulation of vascular endothelial function in humans with diabetes, focusing on potential targets for therapies specifically aimed to reduce the disease burden of diabetic vascular disease.

CD36 Functions as a Sensor of Oxidant Stress in the Vascular System

R.L. Silverstein, MD

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CD36 is a type B scavenger receptor expressed on platelets, macrophages, and other vascular cells. Our lab and others have shown that CD36 promotes athero-thrombosis in settings of hyperlipidemia, diabetes, chronic inflammation, and oxidant stress by functioning as a signaling receptor for danger associated molecular patterns (DAMPs) including oxidized LDL, cell-derived microparticles, glycated proteins, and pro-inflammatory peptides. CD36-mediated signals promote platelet activation, inhibit macrophage migration, and are required for macrophage uptake of oxidized LDL (oxLDL) and foam cell formation and for atherosclerotic lesion formation in mouse model systems. Activation of CD36 pathways leads to disruption of normal cytoskeletal dynamics and inhibition of migration and may contribute to macrophage trapping in atheromatous plaque. Combining cell imaging with biochemical studies of cells from wild-type and CD36 null mice, we have dissected the CD36 signaling pathway in vascular cells and identified key roles for specific src-family tyrosine kinases (Fyn and Lyn), MAP kinases (JNK1 and 2), Vav family guanine nucleotide exchange factors, and intracellular ROS generation in CD36 signaling downstream of DAMPs. Recent studies have revealed that CD36 signaling often involves participation of other cell surface receptors, including tetraspanins, toll-like receptors, and integrins. Targeting CD36 or its downstream signaling partners holds promise for developing novel therapies for athero-thrombotic disorders.

Sirtuins: From Mitochondrial Redox and Metabolism to Chromatin Regulation

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Over the last several years, accumulating evidence suggests protein-lysine acetylation is a post-translational modification that rivals phosphorylation in its broad coverage of the proteome. Acetyl-proteomic studies have catalogued thousands of acetylation sites on hundreds of proteins that represent nearly all aspects of cellular function. Highest among cellular compartments, more than 60% of all mitochondrial proteins have at least one known acetylation site, and most show multiple sites. Surprisingly, more is known about the role of mitochondrial deacetylation than the mechanisms that drive acetylation. The NAD⁺-dependent protein deacetylase sirtuin 3 (SIRT3) is the major mitochondrial deacetylase. SIRT3 is one of 7 mammalian deacetylases (SIRT1-7) that collectively regulate genome maintenance, metabolism, cell survival, and lifespan. We and others have demonstrated that SIRT3 regulates several metabolic pathways. We previously reported that caloric restriction (CR) reduces oxidative DNA damage in multiple tissues and prevents age-related hearing loss in wild-type mice but fails to modify these phenotypes in mice lacking SIRT3. In response to CR, SIRT3 directly deacetylates and activates mitochondrial isocitrate dehydrogenase 2 (Idh2), leading to increased NADPH levels and an increased ratio of reduced-to-oxidized glutathione in mitochondria, protecting cells from oxidative stress-induced cell death. To date, there is no direct evidence of dedicated protein acetyltransferases in mitochondria. This curious observation has fueled the idea that mitochondrial protein acetylation is non-enzymatic and is driven by chemical reaction of acetyl-CoA with the lysine side chain. Such a mechanism could lead to both spurious and functionally important modification, suggesting that some lysine residues evolved with enhanced reactivity to 'sense' acetyl-CoA levels. We have recently developed methods to interrogate acetylation stoichiometry and lysine reactivity, which have provided new insight into the mechanisms that drive protein acetylation. A general mechanism that controls sirtuin activity is unknown. Recently, we discovered that nuclear SIRT6 is allosterically activated by certain long-chain, free fatty-acids and suggests that this regulation induces transcriptional repression of genes involved in carbohydrate and fat metabolism.

Nitric Oxide Signaling Through S-nitrosoglutathione Transnitrosation

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Nitric oxide (NO) synthesized by nitric oxide synthase (NOS) plays essential roles in cellular signaling. The best-described role for NO is activation of soluble guanylate cyclase (sGC). Mounting evidence has revealed the importance of a separate, sGC-independent NO signaling pathway involving cysteine S-nitrosation. However, the cellular pathways that lead to selective S-nitrosation of only a subset of cellular cysteines remain largely unknown. Solution chemistry of S-nitrosation includes NO oxidation to N₂O₃ followed by reaction with thiolates, radical recombination of NO and thiyl radicals, and transition metal catalyzed pathways. Once formed, nitrosothiols can be transferred between small molecule or protein thiols through transnitrosation reactions. Selectivity may be conferred through colocalization with NOS isoforms, protein-protein interaction driven transnitrosation reactions, regulation of S-nitrosoglutathione (GSNO) levels, or directed denitrosation of protein nitrosothiols. Here, we provide evidence through detailed kinetic analyses that transnitrosation signaling pathways can be initiated through auto-S-nitrosation of NOS and subsequent transnitrosation of S-nitrosated NOS with glutathione to form GSNO. In cells, GSNO is thought to be the primary small-molecule nitrosating agent. We show that thioredoxin exhibits site-specific and redox-controlled transnitrosation by GSNO. However, the remaining targets of GSNO transnitrosation have yet to be fully identified and characterized. Therefore, we used a competitive activity-based profiling method to quantify the reactivity of GSNO against >1,200 cysteines in parallel in a human proteome. Using this approach, we identified a subset of cysteine residues that are sensitive to GSNO transnitrosation. S-nitrosation of one of the GSNO transnitrosation targets, 3-hydroxyacyl-CoA dehydrogenase type-2 (HADH2), inhibits the enzymatic activity of HADH2 and, potentially, fatty acid breakdown in the mitochondria. S-nitrosation of another target, cathepsin D (CTSD), affects the ability of CTSD to be proteolytically processed to its active form and, potentially, the involvement of CTSD in the pathogenesis of several diseases, including breast cancer and Alzheimer's disease.

Superoxide as a Regulator of Nitric Oxide Signaling in β -Cells

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Inflammatory cytokines impair pancreatic β -cell function by stimulating the expression of inducible nitric oxide synthase (iNOS) and production of nitric oxide (NO). In addition to NO, excessive formation of reactive oxygen species, such as superoxide and H_2O_2 , has been shown to cause β -cell damage. Here, we determined the forms and functions of reactive species produced in β -cells in response to cytokines. Cytokine treatment of isolated islets or an insulinoma cell line results in the production of nitric oxide but does not stimulate the formation of peroxynitrite (assessed using the coumarin-7-boronate). Exposure of β -cells to the NADPH oxidase activator phorbol 12-myristate 13-acetate fails to stimulate superoxide generation. Addition of either NO donor, menadione (to produce superoxide), or H_2O_2 alone results in impaired mitochondrial oxidation and causes β -cell death. In contrast, peroxynitrite does not impair oxidative metabolism or decrease β -cell viability. Surprisingly, when NO-treated β -cells are forced to produce superoxide (via treatment with menadione), peroxynitrite is generated, but oxidative metabolism and β -cell viability are preserved. In the cells exposed to NO, when superoxide is generated in the extracellular medium (via xanthine oxidase system), peroxynitrite is formed, but ATP levels and β -cell viability remain compromised. These findings demonstrate that NO is the toxic species produced in response to cytokines and that the location of radical generation and the site of radical reactions are key determinants that regulate the effect of reactive species on β -cell function and viability. Extrapolation of these results to islet inflammation during the development of diabetes could predict that radical generation in β -cells might be a key determinant in impairment of function.

Control of β -Cell Fate by Nitric Oxide

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Type-1 diabetes mellitus (T1D) is an autoimmune disease characterized by selective destruction of the pancreatic β -cells. The pro-inflammatory cytokine interleukin-1 (IL-1) is believed to participate in the development of T1D by inhibiting β -cell function and inducing β -cell death. The damaging effects of IL-1 on β -cells are dependent on expression of inducible nitric oxide synthase (iNOS) and subsequent β -cell generation of nitric oxide (NO). While β -cells can recover from short cytokine exposures (0-24 h), longer cytokine exposures (36 h and longer) result in β -cell death by apoptosis. The cellular mechanisms that control β -cell fate following cytokine exposure remain unclear.

We have recently identified the DNA damage response kinase ataxia telangiectasia mutated (ATM) as a key regulator controlling the commitment of β -cells to death during prolonged cytokine exposure. ATM, when activated in response to DNA double-strand breaks (DSBs), phosphorylates and activates many targets to promote DNA repair and coordinate cell fate decisions. One such target is histone H2AX, which is termed γ H2AX when phosphorylated by ATM. In both the insulinoma cell line INS 832/13 and isolated rat islets, we found that IL-1-induced ATM activation, measured by γ H2AX formation, temporally correlates with and promotes β -cell apoptosis after a 36 h treatment with IL-1. Interestingly, though it is the DNA damage induced by nitric oxide that is required for γ H2AX formation, we do not observe γ H2AX under conditions where nitric oxide levels are high. Instead, γ H2AX formation in response to the nitric oxide donor DEA/NO is a delayed event that occurs 2 hours after treatment, well after the nitric oxide is gone (after 45 minutes of DEA/NO treatment), and a nearly 4-fold increase in DNA damage has occurred (after 15 minutes of DEA/NO treatment). In this study, we show that the presence of nitric oxide inhibits γ H2AX formation and that it is not until the levels of nitric oxide have decreased that γ H2AX formation can occur. We find that nitric oxide inhibits γ H2AX formation induced not only in response to the DNA damage caused by nitric oxide, but also in response to other DSB-inducing agents such as hydrogen peroxide and camptothecin. The inhibition of γ H2AX is an effect unique to nitric oxide, as γ H2AX formation induced by camptothecin is restored when nitric oxide is scavenged or converted into peroxynitrite. In addition to nitric oxide liberated from donor compounds, we show that the nitric oxide produced endogenously by the β -cell via iNOS in response to a cytokine treatment is capable of inhibiting γ H2AX formation in response to the DNA damaging agent camptothecin. In addition to the inhibition of γ H2AX, nitric oxide also prevents the activation of DNA damage-dependent signaling pathways involved in apoptosis, as phosphorylation of ATM, phosphorylation of p53, and cleavage of caspase-3 induced by camptothecin are completely eliminated by nitric oxide cotreatment.

Based on our findings, we hypothesize that the inhibition of ATM by nitric oxide is a protective mechanism that prevents the induction of ATM-dependent β -cell apoptosis under conditions where cytokine-induced damage is reversible (0-24 h), and it is not until the levels of nitric oxide decrease after extended treatments with cytokines (36 h and longer) that ATM-dependent apoptosis can occur. We are currently investigating signaling mechanisms activated by nitric oxide that inhibit ATM.

Redox Signaling in Ischemic Angiogenesis

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Reactive oxygen species (ROS) arising during ischemia may affect the recovery of blood flow by inducing cysteine (Cys) thiol oxidizations that initiate angiogenic signaling. Our recent studies have focused on identifying the functionally important thiol adducts and their major protein targets using a standard hindlimb ischemia (HLI) model induced by surgical femoral vascular excision. In previous studies by others, abrogation of the normal, gradual return of blood flow over 3-4 weeks has demonstrated the essential roles of vascular endothelium growth factor, PI3 kinase, Akt, and nitric oxide signaling. Because in vitro studies in human endothelial studies suggested the importance of glutathione (GSH) adducts, we have studied HLI in mice that genetically overexpress or lack the enzyme glutaredoxin-1 (Glx), the major cytosolic regulator of GSH protein adducts. During HLI, GSH protein adducts increased during the first 24d and remained increased for at least 3 days. Mice with 3-fold global overexpression of Glrx had fewer GSH adducts and significantly more severe ischemia. Mice with endothelial cell-specific Glrx overexpression experienced severe ischemia leading to a high incidence of limb loss. Glrx deficient mice had increased GSH protein adducts and a significant improvement in HLI compared with wild-type. Studies in cultured endothelial cells point to potential GSH- and Glrx-regulated proteins, including NFkB p65, p21Ras, and the sarco(endo)plasmic Ca²⁺ ATPase-2. In the case of SERCA2, GSH adducts on Cys-674 increase during ischemia or hypoxia and increase its Ca²⁺ uptake activity. A SERCA2 C674S knockin mouse had impaired HLI recovery, and its EC showed impaired Ca²⁺ rises and angiogenic responses to VEGF. Thus, GSH adducts increase during ischemia on Cys thiols, affecting protein function and HLI. Some, like those on SERCA Cys-674 appear to be essential for normal angiogenesis. Angiogenic signaling in ischemia depends upon the levels of ROS and GSH protein adducts, the reactivity of the specific Cys thiol, as well as the subcellular localization and the change in function of the specific protein.

Critical Role for Telomerase in Maintenance and Regulation of Flow Mediated Dilatation in the Human Microcirculation

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Rationale: We have previously shown that flow-induced dilation of human coronary arterioles is mediated in part by nitric oxide in subjects without coronary artery disease (CAD) and by hydrogen peroxide (H₂O₂) derived from mitochondria in subjects with CAD. However, the mechanism of this transition in mediators of FMD from health to disease is not known. Telomerase, traditionally described as a nuclear enzyme crucial for telomere maintenance, translocates to the cytosol and to mitochondria during stress where it can reduce mitochondrial oxidative stress. Since telomerase activity is decreased in disease, we hypothesized that a primary reduction in telomerase activity contributes to the decrease in NO, driving a compensatory dilation to H₂O₂ as the mediator of FMD.

Methods: Small resistance vessels (human coronary and adipose arterioles; ~200 μm) were isolated from discarded intraperitoneal and subcutaneous adipose or discarded human atrial sections. Arterioles were cannulated and prepared for video microscopy. Dilation to graded degrees of shear was measured in vessels constricted with endothelin-1, before or after treatment with the telomerase inhibitor BIBR 1532. The mediator of FMD was determined using pharmacological scavengers of NO (L-NAME) and H₂O₂ (PEG-Catalase).

Results: Exposure of arterioles from healthy subjects to the telomerase inhibitor BIBR 1532 had no effect on the magnitude of FMD but changed the mechanism from an L-NAME-inhibitable to a catalase-inhibitable one (% max dilation at 100 cm H₂O Atrial Vehicle 64.4±5, L-NAME 83±4, Catalase 33±5; Adipose Vehicle 70±4, L-NAME 75±2 Catalase 37±7 P<0.05 N =5). Mitochondria are the main source of H₂O₂ mediating the relaxation, as rotenone and MitoTEMPOL inhibited most of the FMD after telomerase inhibition (% max dilation at 100 cm H₂O rotenone 29±8; MitoTEMPOL 4±17). In order determine the level of mtH₂O₂, we used a novel fluorescent dye (mitoPY1, Torcirs) targeted to mitochondria and specific for H₂O₂. Similar to the functional data, flow increased mtH₂O₂ with pharmacological decreased telomerase. Exposure of adipose or atrial vessels to the specific telomerase activator (AGS 499, 15-20 h) reversed the phenotype associated with CAD as it turned a catalase inhibitable dilation to one mediated by NO (% max dilation at 100 cm H₂O Adipose Vehicle 80±4; L-NAME 4±18; Catalase P<0.05 N =5).

Conclusions: Our data show a previously undiscovered role of telomerase in preventing the switch from NO to H₂O₂ as the mediator of FMD. Activation of telomerase reverses the pathological mechanism of FMD in arterioles from subjects with chronic CAD.

Targeting NADPH Oxidases in Cardiovascular Diseases: Development of Assays for High Throughput Screening of Nox2 Inhibitors

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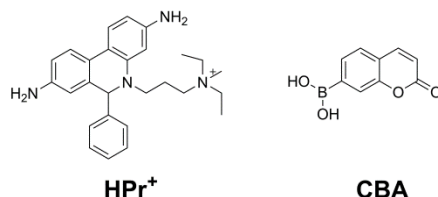
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NADPH oxidases (NOX) have been implicated as a major source of superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) in several inflammatory diseases (e.g., cardiovascular and neurodegenerative diseases and cancer). Intense research is currently underway in many laboratories to discover potent and specific inhibitors of NOX enzymes. With the development of new and site-specific probes for detecting $O_2^{\cdot-}$ and H_2O_2 , it is now feasible to search and discover potent inhibitors of NOX using a high-throughput screening (HTS) approach. Newly synthesized probes with well-defined redox chemistry include a cell-impermeable analog of hydroethidine, namely hydropropidine (HPr⁺), for extracellular $O_2^{\cdot-}$ and boronate-based fluorogenic probe, coumarin boronic acid (CBA) for H_2O_2 . Additionally, HPLC-based detection of conversion of hydroethidine into 2-hydroxyethidium and HRP-catalyzed oxidation of Amplex Red to resorufin serve as orthogonal assays for $O_2^{\cdot-}$ and H_2O_2 , respectively.



Here, we present the development and application of those assays for screening of a small library of over 2,000 compounds of known biological activity to find small molecule inhibitors of Nox2 isoform. The selected positive hits are validated further via confirmatory assays, including oxygen consumption measurements, EPR spin trapping of $O_2^{\cdot-}$, dose response and determination of cytotoxicity. Confirmed hits will be a subject for further mechanistic studies of enzyme inhibition and may provide new tools in the research on NADPH oxidases and potential candidates for drug development.

Endothelial NOS Uncoupling: New Biochemical and Biophysical Developments

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Defective endothelial nitric oxide synthase (eNOS) activity is one significant cause of endothelial dysfunction and vascular disease. eNOS is a flavo- and heme containing enzyme that generates nitric oxide and citrulline from L-arginine oxidation. Post-translational modifications (PTM) of serine, threonine, tyrosine in eNOS polypeptide sequence influence NO production rates. The NO production is however strictly dependent on tetrahydrobiopterin (BH4) cofactor, which binds in close proximity to the heme-iron group in the oxygenase domain. We showed that activation of eNOS under limiting BH4 availability promotes the uncoupled oxidation of NADPH with superoxide release from heme-group. Recently, S-glutathionylation of eNOS (eNOS-SG) has been proposed also to stimulate superoxide production from eNOS by facilitating the reduction of oxygen by reduced flavins. We have revisited these studies to establish the differences in superoxide yield from heme- (BH4-free eNOS) and flavin (eNOS-SG) by EPR spin trapping methodologies. Glutathionylation of recombinant bovine eNOS BH4 free was verified by immunotechniques and LC MS/MS as previously described (Chen et al. Nature. 2010; 468: 1115-8). We identified several residues as target of glutathionylation including Cys910 and Cys691 corresponding to Cys908 and Cys689 in human eNOS. We showed that superoxide production from eNOS-SG is lower than eNOS and inhibited by reconstitution of the enzyme with BH4 and cyanide, a heme-iron-ligand. These results indicated that eNOS-SG generates uncoupled superoxide from heme-group. Additional studies examining the effects of glutathionylation on the rates of reduction of chemical flavin-acceptors such as ferricyanide (FAD) and cytochrome c (FMN) and NADPH reduction were performed. These studies lead us to identification of a glutathione reductase-like activity of eNOS, which indicate another significant yet unrealized role for eNOS in redox control in the vasculature.

**Part II:
Poster Abstracts**

How the Tyrosyl Radical EPR Signal from RR and Iron-Sulfur Signals in Mitochondria Can Contribute to Redox Signaling

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Either the reduced or oxidized species (complex) gives an Electron Paramagnetic Resonance (EPR) signal depending on which species is paramagnetic.

Here one example for the detection of the stable tyrosyl radical signal from RR (ribonucleotide reductase) is illustrated. The tyrosyl radical signal is proportional to the RR activity. This signal, which is weak in normal cells, is detected only in cancer cells. Loss of the RR tyrosyl radical signal implies that RR is inhibited, RR concentration is lowered, or that fewer cells are in S-phase.

Another example of an EPR marker is the detection of Fe-S (iron-sulfur clusters) in mitochondria. Are these Fe-S signals arising from Complex I and II and aconitase bio-markers for insults from ROS (reactive oxygen species)? Spectra are shown for the EPR signals that are routinely detected from mitochondria.

Our recent publications where Fe-S signals are detected:

Gadicherla, A.K., Stowe, D.F., Antholine, W.E., Yang, M., Camara, A. Damage to mitochondrial complex I during cardiac ischemia reperfusion injury is reduced indirectly by anti-anginal drug ranolazine. *Biochim. Biophys. Acta* 1817:419-29, 2012.

Myers, C.R., Antholine, W.E., Myers, J.M. The Pro-Oxidant Chromium(VI) Inhibits Mitochondrial Complex I, Complex II, and Aconitase in the Bronchial Epithelium: EPR Markers for Fe-S Proteins. *Free Radicals Biol. Med.* 49(12):1903-15, 2010.

Ghosh, A., Chandran, K., Kalivendi, S., Joseph, J., Antholine, W.E., Hillard, C.J., Kanthasamy, A., Kanthasamy, A., Kalyanaraman, B. Neuroprotection by mitochondria-targeted drug in Parkinson's disease model. *Free Radic. Biol. Med.* 49(11):1674-84, 2010.

A recent review by us that discusses EPR detection of the tyrosyl radical in RR is:

Chitambar, C.R., Antholine, W.E. Iron-targeting antitumor activity of gallium compounds and novel insights into triapine®-metal complexes. Forum Review. *Antioxid. Redox Signal.* 18:956-72, 2013.

Using iPCells with Dystrophin Gene Mutations to Compare Differences in Oxidative Stress Responses between Cardiac and Skeletal Myocytes

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Mechanisms of oxidative stress contributing to muscular dystrophy are not well understood. We studied two patient-specific iPCell lines with Dys gene mutations (D-iPCs) to study the mechanism of stress induced injury in derived cardiomyocytes (iCM) and skeletal myocytes (iSkM). D1 (exon 3-6 deletion), D2 (exon 45-53 deletion), and wild-type (N)-iPC lines were differentiated into iCM or iSkM. mRNA expression for SOD1 and SOD2 was reduced in D1/D2-iCM as compared to N-iCM. Stress conditioning significantly elevated reactive oxygen species (ROS) in D1/D2 vs. N-iCM, induced loss of mitochondrial membrane potentials ($\Delta \psi_m$), and cell injury. To check ROS source, iCM were pretreated with inhibitors against Nitric Oxide Synthase (L-NAME), NADPH Oxidase (Apocynin), Xanthine Oxidase (Allopurinol), and mitochondria (Rotenone). The allopurinol (XO-blocker) pretreatment on iCM normalized ROS levels, $\Delta \psi_m$, and reduced cell injury after stress but had no effect on elevated ROS in D1/D2 iSkM. Our results suggest elevated ROS in iCM after stress is due in part to decreased expression of anti-oxidants and increased ROS derived from XO. XO does not contribute to increased ROS levels in iSkM. Allopurinol may be a treatment of choice for dystrophic cardiomyopathy but is unlikely to have therapeutic benefits for skeletal myopathy.

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Spatial and Temporal Restriction of Human Cardiomyocyte Cell Surface N-Glycoproteins During *in vitro* Differentiation of Human Pluripotent Stem Cells

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Human pluripotent stem cell (PSC) differentiation into the cardiac lineage offers a valuable system for modeling early stages of cardiac development and for the *in vitro* generation of cell types valuable for drug testing, disease modeling, and in the long-term, cell therapy. The success of these applications will rely on the ability to identify and isolate stem cell derivatives of the appropriate stage and type without relying on genetic modifications. Current differentiation protocols generate mixed populations of cardiomyocytes, including atrial and ventricular-like cells. Currently, few surface accessible markers are available for identifying cells in the cardiac lineage, and none of them are cell type or commitment-stage specific. The aims of this study are: 1) to identify surface accessible proteins that are temporally restricted during various stages of cardiac differentiation, *in vitro*, and thus may be useful for identifying stage-specific cells, and 2) to investigate whether the *in vitro* data corroborate with *in vivo* data at the genetic and protein level. Human embryonic and induced pluripotent stem cells were differentiated towards the cardiac lineage *in vitro*, and cell surface proteins were identified using the Cell Surface Capturing (CSC) Technology, an antibody-independent strategy that uses affinity enrichment of cell surface N-glycoproteins and high mass accuracy mass spectrometry to achieve >85% specificity for authentic cell surface proteins while simultaneously determining N-glycosite occupancy and membrane topology. Peptides were analyzed by LC-MS/MS using an LTQ-Orbitrap Velos and data analyzed using the Socerer™2 Sequest platform. Comparison of these data to that contained within the Cell Surface Protein Atlas, a dataset of CSC-Technology data from >70 cell types which currently contains >3000 proteins, reveals >100 proteins potentially restricted to the cardiac lineage. The temporal restriction patterns of six novel targets during cardiomyocyte differentiation were characterized in comparison with standard and previously reported markers. The onset of these markers during *in vitro* differentiation was refined, and their presence on human tissue was investigated using qRT-PCR, immunofluorescence, and flow cytometry. We also have evidence of the presence of these targets in human heart tissue at the transcript and protein levels.

Protein Tyrosine Phosphatase 1B Regulation of Argonaute 2 in Cardiac Hypertrophy

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The disruption of Protein Tyrosine Phosphatase (PTP) function has been shown to be an underlying cause of several cancers. Despite this, the majority of the members of the PTP superfamily have yet to be characterized. Given the importance of reactive oxygen species (ROS) in the transition from cardiac hypertrophy to heart failure and the documented inhibition of PTPs by cellular oxidants, we hypothesized that specific PTPs could act as checkpoints in this process. Using the cysteinyl-labeling assay to detect reversible oxidation of PTPs, we have identified PTPIB as a target of ROS in cardiomyocytes and hearts undergoing hypertrophy. Since we have recently identified a novel signaling pathway linking PTPIB inactivation to Argonaute 2 (AGO 2) phosphorylation and to the inhibition of gene silencing downstream of RAS (Yang et al. (2014) Mol Cell 55(5):782-90), we explored whether the RAS-ROS-PTPIB-AGO2 pathway was involved in cardiac hypertrophy. We confirmed that phosphorylation of AGO2 at tyrosine-393 was increased in TAC hearts and that AGO2 was a substrate of PTPIB in H-RASV12-expressing myocytes as well as in TAC hearts of transgenic mice expressing a PTPIB trapping-mutant. We generated cardiomyocyte-specific PTPIB knockout (PTPIB cKO) mice to study the role of PTPIB in the heart while avoiding the systemic effects of a global PTPIB KO or of global PTPIB inhibition. Preliminary results of PTPIB cKO mice subjected to TAC for 30 days showed a dramatic left ventricular dilation, systolic and diastolic dysfunction, and overall impairment of left ventricular performance compared to control mice subjected to TAC for the same period. In addition, the inhibitory phosphorylation of AGO2 on tyrosine-393 was increased in the absence of PTPIB, confirming the involvement of the PTPIB-AGO2 pathway in hypertrophy. We propose that ROS-mediated inhibition of PTPIB contributes to AGO2 inhibition and regulation of gene silencing in cardiac hypertrophy.

Nitric Oxide Abolishes the Effects of HDAC Inhibitors: Implications for Chemoresistance

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The identification of Nitric Oxide (NO) as an endogenous epigenetic regulatory molecule, and its role in the progression of numerous types of cancer, exemplifies the importance of NO in disease and disease-free states. Dysregulation of post-translational histone modifications contribute to cancer etiology by altering chromatin structure and regulating gene expression. Acetylation state of histone lysine residues is determined by two enzyme classes: Histone Acetyltransferases (HAT) and Histone Deacetylases (HDAC). Our lab has demonstrated that NO both decreases acetylation and increases methylation of histone lysine residues, yet the reasons behind this relationship are not currently understood. Due to the significant effect NO had on decreasing global acetylation levels, we hypothesized that clinically used HDAC inhibitors might reverse these effects of NO. We treated MDA-MB-321 Breast Cancer carcinoma cells with various NO concentrations in the presence of the HDAC inhibitor, Suberanilohydroxamic acid (SAHA). Western blot analysis measured changes in histone acetylation, specifically histone 3 lysine 9 (H3K9). NO abolished the effects of HDAC inhibitors in a dose dependent manner. Using a real-time cell growth/viability assay (xCELLigence), we were able to look at phenotypic responses to these treatment conditions. NO stimulated cell growth (dose dependently) yet at later time points ultimately lead to cell death. SAHA had low toxicity with no effect on cell growth. However, the stimulatory effects of NO are not seen during co-treatment with SAHA. These results highlight the complex relationship between NO and HDAC inhibitors in regulating the histone acetylation status and reveal an important opportunity for therapeutic intervention.

Macrophages Cause Reduced Biosynthesis of H₂S in Mesenteric Resistance Arteries during Obesity

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Impaired signaling of the potent vasodilator, hydrogen sulfide (H₂S), has recently emerged as a contributor to numerous cardiovascular diseases, but its potential role in obesity-induced microvasculature dysfunction has yet to be assessed. Obesity is also associated with increased chronic inflammation and recruitment of macrophages to the vascular adventitia. The current study was designed to test the hypothesis that microvascular H₂S is depleted in a mouse model of diet-induced obesity by a mechanism that involves an interaction with perivascular macrophages. Mesenteric arterioles were isolated from 30 week-old lean and obese mice and mounted in a pressure myography chamber. Vessels were loaded with the H₂S indicator SF7-AM, and smooth muscle and endothelial layers were imaged using confocal microscopy. Lower concentrations of H₂S were recorded in arterioles from obese mice compared to those from lean mice. Generation of H₂S is dependent on the substrate L-cysteine and the enzyme cystathionine gamma-lyase (CSE). The protein expression of CSE was comparable in mesenteric arterioles from lean and obese mice. When compared to vessels from lean mice, however, the addition of L-cysteine to arterioles from obese animals produced a smaller elevation in smooth muscle H₂S, which was correlated with reduced relaxation in vessels pre-contracted with phenylephrine. Co-culturing arterioles from lean mice with peritoneal macrophages isolated from obese mice overnight resulted in vessel H₂S depletion. In contrast, co-culture of vessels from lean mice with macrophages from lean controls had no effect on vessel H₂S levels. Taken together, these data support a model in which H₂S biosynthesis is compromised in the obese microvasculature by a mechanism dependent on crosstalk with perivascular macrophages.

Mechanical Shear Stress Restores Mitochondrial Cytoarchitecture in Human Endothelial Cells Exposed to Angiotensin II by Modulating Activity of the Fission-Inducing Protein Dynamin Related Protein

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The mitochondria exist in a state of balance between fission (fragmentation) and fusion (joining of mitochondrial segments). Disease is characterized by excessive fission, leading to an overproduction of mitochondrial reactive oxygen species (ROS) which mediate pathological changes in vascular endothelium. Dynamin-related protein-1 (DRP-1) is the primary mediator of mitochondrial fission. The therapeutic benefit of exercise is largely due to increased endothelial shear; however, the effects of shear stress on DRP-1 expression and mitochondrial structure are unknown.

Hypothesis: Shear stress reduces mitochondrial fission in cultured human umbilical vein endothelial cells (HUVECs) exposed to the fission-inducing stimulus angiotensin II (ANG II) by reducing DRP-1 expression and activity.

Methods: HUVECs were treated with MitoRFP for 24 hours to fluorescently label the mitochondria. They were then exposed to ANG II (10 pM; 4 hours), fixed, and 25 random cells/group were imaged with a confocal microscope. Separate groups of cells were sheared (15 dyn/cm²; 24 hours) immediately following ANG II exposure using the Ibidi Pump System. To quantify mitochondrial fission, images were converted to binary color, and ImageJ was used to count non-contiguous mitochondrial fragments. This value was divided by the number of pixels in the mitochondrial network and multiplied by 1000 to yield a Mitochondrial Fragmentation Index (MFI). Total DRP-1 expression and phosphorylation of the S616 activator and S637 suppressor sites were analyzed by Western blot.

Results: ANG II increased MFI (1.13 ± 0.25 vs. 0.75 ± 0.06), DRP-1 expression (+48%), and phosphorylation of the S616 activator site (+141%; $p < 0.05$ for all) compared to static controls. Conversely, ANG II-treated cells sheared for 24 hours showed reduced mitochondrial fission (MFI = 0.50 vs. 0.75 ± 0.06), DRP-1 expression (-24%) and phosphorylation at S616 (-11%) compared to control. ANG II treated cells exposed to shear also showed a robust increase (+528%) in phosphorylation of the S637 suppressor site of DRP-1.

Conclusions: High shear stress restores mitochondrial network integrity in HUVECs exposed to ANG II, suggesting exercise may promote vascular health through modulating mitochondrial fission.

Beta-hydroxybutyrate Improves Cardiac Excitation-Contraction Coupling (ECC) and Mitochondrial Function in Type-2 Diabetic Hearts

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The risk of cardiovascular disease (CVD) and mortality in type-2 diabetic patients is twice as high as in age-matched healthy subjects. However, the mechanisms linking type-2 diabetes with CVD remain poorly understood. We studied changes in ECC and mitochondrial function in control and diabetic (db/db) mice. We found no major changes in ECC (Ca transients, cell shortening, sarcoplasmic reticulum Ca²⁺ load) under basal conditions despite decreased heart-to-body weight. However, under stress conditions (beta-adrenergic stimulation) ECC was significantly impaired, and increased ROS generation and a shift of the redox environment towards oxidation were observed. Furthermore mitochondrial Ca²⁺ uptake was increased leading to enhanced mPTP opening. Mitochondrial proton leak was increased while ATP-coupled respiration, monitored using the Seahorse extracellular flux analyzer, was decreased in diabetic hearts. We determined that despite elevated blood glucose levels (395 vs 157 mg/dl in control) and obesity (BMI was 5.50 vs 3.10 in control) blood levels of beta-hydroxybutyrate (a ketone body formed in the liver) remained unchanged (821 vs 784 μ M in control). Ketone body oxidation becomes a significant contributor to overall energy metabolism in extrahepatic tissues under numerous physiological conditions (neonatal period, starvation, post-exercise, low carbohydrate diet) when circulating ketone body concentrations increase from micromolar levels in normal fed state to 7 mM and can rise to ~20 mM under pathological conditions like diabetic ketoacidosis. We evaluated the effect of elevated beta-hydroxybutyrate levels on ECC and mitochondrial function in diabetic hearts. Exposure to the elevated levels of beta-hydroxybutyrate (2-5 mM) alone or in the presence of low concentrations of pyruvate (0.1-1 mM) improved cardiac ECC, preserved the redox environment and decreased proton leak, ROS generation, and mPTP opening with no effect on mitochondrial Ca²⁺ uptake. Therefore, these data suggest that type-2 diabetes patients potentially could benefit from a ketogenic (low carbohydrate) diet that increases beta-hydroxybutyrate generation.

830 nm Photobiomodulation Preserves Retinal Mitochondrial Redox Potential and Protects Against Retinal Degeneration in a Rodent Model of Retinitis Pigmentosa

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Retinitis pigmentosa (RP) is the most common cause of inherited blindness in the developed world. RP is caused by the progressive photoreceptor cell degeneration. Mitochondrial dysfunction and oxidative stress play a significant role in the pathogenesis of RP. Low energy photon irradiation in the far-red to near-infrared (NIR) range of the spectrum, (photobiomodulation or PBM) has been shown to act on mitochondria-mediated signaling pathways to improve mitochondrial function, attenuate oxidative stress, and prevent cell death. We tested the hypothesis that PBM acts in the retina to promote mitochondrial integrity and function, prevent photoreceptor cell death, and preserve retinal function in an established rodent model of retinitis pigmentosa, the P23H rhodopsin transgenic rat. Retinal function, structural integrity, surviving photoreceptors, and the mitochondrial redox state were assessed. PBM did not alter the structural and functional characteristics of retina in a non-dystrophic animal strongly supporting the safety of PBM. 830 nm PBM profoundly attenuated retinal degeneration in P23H rats resulting in the preservation of retinal mitochondrial redox potential, retinal function, and retinal morphology in comparison to the sham-treated group. These findings provide evidence supporting the therapeutic utility of PBM in the treatment of retinal degenerative disease. They also further our understanding of the mechanism of action of PBM by showing that it improves mitochondrial function in the retinae of RP animals. By exploiting the cells own mechanism of self-repair, PBM has the potential for translating into clinical practice as an innovative, non-invasive stand-alone or adjunct therapy for the prevention and treatment of retinal diseases.

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Global Gene Expression Profiling in PAI-1 Knockout Murine Heart and Kidney: Molecular Basis of Cardiac-Selective Fibrosis

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Cardiac fibrosis, an abnormal matrix remodeling in stressed or injured hearts, is a major contributor to cardiovascular disease-related morbidity and mortality. At present, there is no effective therapy for fibrosis. In order to develop novel therapeutic approaches, it is important to identify the molecule(s) which ignite(s) the onset of fibrogenesis. We and others have demonstrated that aged plasminogen activator inhibitor-1 (PAI-1) knockout mice develop spontaneous cardiac-selective fibrosis without affecting any other organs including kidney, lung, and liver. Therefore, the PAI-1 knockout model of cardiac-selective fibrosis provides an excellent opportunity to find novel contributors to cardiac fibrogenesis. Here, we hypothesized that differential expressions of profibrotic and antifibrotic genes in PAI-1 knockout hearts and unaffected organs lead to cardiac-selective fibrosis. To test our hypothesis, we performed genome-wide gene expression profiling of transcripts derived from aged wild-type and PAI-1 knockout hearts and kidneys. Results revealed that while myocardial tissues derived from aged PAI-1 knockout mice showed significantly elevated levels of collagen accumulation compared to age- and sex-matched wild-type controls, collagen accumulation in kidneys derived from aged PAI-1 knockout mice were insignificant and comparable with age- and sex-matched wild-type controls suggesting PAI-1 deficiency is associated with age-dependent cardiac-selective fibrosis. Analysis of Illumina-based microarray data and qPCR data revealed that while the expression of several genes, involved in profibrogenic pathways, including *Ankrd1*, *Egr1*, and *Timp1* are upregulated in PAI-1 knockout hearts, the expression of those genes are downregulated in PAI-1 knockout kidneys compared to controls. The protein level of *Ankrd1*, a transcriptional cofactor, is significantly elevated in myofibroblasts and AngII-mediated hypertension-induced fibrotic hearts. Importantly, TGF-beta failed to induce collagen synthesis in *Ankrd1* deficient cardiac fibroblasts indicating *Ankrd1* may be an igniter of cardiac-selective fibrosis. Therefore, the results of the present study have identified new molecular target(s) for the prevention and treatment of cardiac fibrosis associated with a wide-variety of cardiovascular diseases.

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SGLT3a Activity in Mouse Blood Vessels: A Novel Link to Diabetic Nephropathy

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Uncontrolled hyperglycemia in type 2 diabetics increases their risk of developing hypertension and kidney damage. Patients with comorbidity of diabetes and hypertension are a high-risk group for the progression to diabetic nephropathy. However, the exact mechanism is not well understood. SGLT3 transports sodium across the cell membrane in a glucose-dependent mechanism. Mouse SGLT3a is highly homologous to human SGLT3, and our preliminary immunohistochemical studies have shown SGLT3a expression in mouse kidney arterial vasculature. We hypothesized that hyperglycemia up-regulates SGLT3 activity in kidney vasculature, leading to the development of hypertensive arterial vessels and ultimately kidney damage. We investigated the role of SGLT3a in vascular reactivity using mouse thoracic aorta as our model. Aortas were cut into ~ 3mm rings, and each ring was mounted on tungsten triangles in a physiological salt solution at 37 °C and constantly aired with 95% O₂/5% CO₂. Rings were equilibrated at 0.5 g passive force, and responses to deoxynojirimycin (DNJ, potent SGLT3 agonist) were analyzed with Digi-Med tissue force analyzers (Micro Med). As control, experiments were also conducted with D-glucose (20.5 mM). Mean values ± standard errors were determined using SigmaPlot 11.0 software. In response to 50 and 100 μM DNJ treatment, aortic force tension increased by 0.05 ± 0.01 g at 0.12 mg/sec (N=4) and 0.05 g ± 0.01 (N=4) at 0.13 mg/sec, respectively. An increase of 0.06 g ± 0.02 (N=12) at 0.08 mg/sec was observed in response to 150 μM DNJ treatment. Similarly, D-glucose exposure increased force tension by 0.14 g ± 0.03 (N=12). These results support that an increase in SGLT3 activity enhances arterial vessel contraction.

Gamma-L-glutamyl-L-cysteine Inhibits Oxidative Injury to Cultured Embryonic Cardiomyocytes

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Myocardial ischemia-reperfusion (IR) injury in the new-born is associated with oxidative stress involving apoptosis and represents a vital complication in neonates. The purpose of the current study was to evaluate the effectiveness of the antioxidant gamma-L-glutamyl-L-cysteine (γ Glu-Cys) in inhibiting reperfusion injury to embryonic cardiomyocytes (H9c2 cells) in culture, as a potential therapeutic. Its efficacy was compared to that of the analog gamma-L-glutamyl-D-cysteine (γ Glu-D-Cys), or the amino acid L-cysteine (Cys). To simulate ischemia and reperfusion, cells were incubated in a culture medium at 37°C in sealed chambers under the following conditions: 1) 95% N₂, 5% CO₂, 0 mM glucose, pH 6.8 (ischemia), or 2) room air, 5% CO₂, 25 mM glucose, pH 7.4 (reperfusion). Embryonic cardiomyocytes incubated for 6 hours under ischemic conditions followed by 2 hours of simulated reperfusion showed a significant increase in: 1) the release of lactic dehydrogenase (LDH, a marker of cell membrane integrity) to the incubation medium, and 2) the release of cytochrome c (a trigger of apoptosis) from mitochondria into the cytosol, when compared to cells incubated for 8 hours under control conditions. Co-incubation with 0.5 mM γ Glu-Cys significantly inhibited release of both LDH to the incubation medium and cytochrome c to the cytosol. Under the same experimental conditions, neither γ Glu-D-Cys nor Cys showed significant inhibitory effects, and Cys enhanced LDH release under control conditions. The data indicate that the glutathione precursor γ Glu-Cys protects cultured embryonic cardiomyocytes from oxidative injury including apoptosis, under the experimental conditions. It is further suggested that Cys may be toxic to the cells under these conditions.

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Structural Equation Model of Clinical Nurses' Intention to Perform Basic Life Support (BLS)

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Clinical nurses are often the first responders to patients who go into cardiac arrest in hospitals. Although their first response is critical for the patient, for many reasons, clinical nurses tend to be hesitant to initiate BLS at the scene.

Purpose: The purpose of this study was to develop a model based on major factors that could affect a clinical nurses' intention to perform BLS (IP-BLS).

Methods: Five hundred and twenty clinical nurses in five major university hospitals in South Korea were surveyed. Authors developed self-report questionnaires for measuring the level of intention, knowledge, and self-confidence of BLS performance. The data were analyzed by the SPSS 18.8 and the Amos 18.0 version.

Results: Clinical nurses' prior experience involved in BLS (PE-BLS), knowledge of BLS guidelines (K-BLS), age, and clinical career had significant impacts on their intention (IP-BLS) ($p < .05$). Clinical nurses' knowledge (K-BLS) had a direct impact on their intention (IP-BLS). Also prior- experience (PE-BLS) showed direct impact as well. All of these variables explained 19.5% of the variance of intention (IP-BLS).

Conclusion: The developed structural equation model shows that knowledge from BLS training contributes to higher confidence and intention to perform BLS (IP-BLS). We conclude that regular BLS training of clinical nurses is important to increase their IP-BLS.

Selective Toxicity of Human Pluripotent Stem Cells by Inhibition of an NAD⁺ Salvage Pathway

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Human pluripotent stem cells (hPSC) are a leading candidate for cellular therapies as they are capable of continual self-renewal and can differentiate into nearly every cell type. In recent years, significant progress has been made in developing protocols for efficient differentiation of many cell types, including cardiomyocytes. Despite increased efficiency, the tumorigenic potential of hPSC is a major limitation to the implementation of cellular therapies as remnant hPSC in differentiation cultures form teratoma tumors after transplant. Therefore, methods to selectively eliminate remnant pluripotent stem cells from differentiation cultures are required to prevent tumor formation. Metabolic inhibition is an approach to selectively target hPSC from their differentiated progeny. hPSC are characterized by a reliance on aerobic glycolysis (the Warburg effect) for ATP production, a decreased reliance on mitochondrial oxidative phosphorylation, and an increased expression of glucose transporter I (GLUT1). This study tested the selective toxicity of STF-31, a reported GLUT1 inhibitor, on hPSC. Toxicity and metabolic studies were also compared to the irreversible GLUT1 inhibitor, WZB117, and glucose deprivation. Toxicity studies show that STF-31 is selectively toxic to hPSC across a broad range of culture conditions and that a 24 h pulse treatment is sufficient to eliminate the hPSC self-renewal. Although STF-31 was originally described as an inhibitor of glucose transporter I, these data support the reclassification of STF-31 as an inhibitor of nicotinamide adenine dinucleotide (NAD⁺) synthesis through the nicotinamide phosphoribosyltransferase (NAMPT) mediated NAD⁺ salvage pathway. Altogether, these findings demonstrate the previously unrecognized importance of NAD⁺ salvage pathways in hPSC biology and describe a novel strategy that is selectively toxic to hPSC. Future studies will define the utility of STF-31 for elimination of hPSC from differentiation cultures, which could help accelerate the development of safe, clinically relevant hPSC-derived cell therapies.

Impairment of Macrophage Reverse Cholesterol Transport by StAR-dependent Cholesterol Hydroperoxide Trafficking: Implications for Arterogenesis Under Oxidative Stress

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Objective – Oxidative stress associated with cardiovascular disease can produce a large variety of oxidized lipids, including cholesterol ring oxides such as 7-hydroperoxide (7-OOH), 7-hydroxide (7-OH), and 7-ketone (7=O). Unlike 7=O and 7-OH, 7-OOH is redox-active, giving rise to the others via potentially toxic free radical reactions. Consequently, 7=O and 7-OH are usually found at much higher levels than 7-OOH in vascular lesions and have garnered more interest than the latter *vis-a-vis* involvement in atherogenesis. Our objective in this study was to test the novel hypothesis that under oxidative stress conditions, StAR family proteins not only deliver cholesterol to/into mitochondria of vascular macrophages but also 7-hydroperoxycholesterol (7-OOH), which induces peroxidative damage that impairs early stage reverse cholesterol transport (RCT).

Methods and Results – Stimulation of human monocyte-derived THP-1 macrophages with dibutyl-*c*AMP resulted in substantial upregulation of Western-determined StarD1 and ABCA1 proteins but not StarD4 or Cyp27A1. siRNA-based StarD1 knockdown (kd) prior to stimulation had no effect on StarD4, but it enhanced Cyp27A1 and strongly reduced ABCA1 upregulation, linking the latter to StarD1 functionality. Mitochondria in stimulated StarD1-kd cells internalized 7-OOH more rapidly than non-stimulated controls and underwent greater 7-OOH-induced lipid peroxidation and loss of membrane potential, as detected with the fluorescent probes C11-BODIPY and JC-1, respectively. A major functional consequence was significantly reduced expression of cholesterol-exporting ABCA1 and synthesis of 27-hydroxycholesterol, which signals for ABCA1 transcription. Consistent with this, stimulated THP-1 cells that were loaded with [¹⁴C] cholesterol in the presence of 7-OOH exported cholesterol to HDL (as assessed by scintillation counting) more slowly than control cells not exposed to 7-OOH. Thus, RCT at the macrophage level was significantly impaired by 7-OOH delivery to mitochondria via a natural trafficking pathway.

Conclusion – This study describes a previously unrecognized mechanism by which RCT can be incapacitated under oxidative stress-linked disorders, such as chronic obesity and hypertension. Our findings provide new insights into the role of macrophage redox damage/dysfunction in atherogenesis.

Novel Functions of Small GTPase Rap1 in Regulating Endothelial Homeostasis: Control of Nitric Oxide Release, Vascular Function, and Blood Pressure

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Endothelial dysfunction, resulting from decreased nitric oxide (NO) bioavailability, is a pathology linked to endothelial vasomotor dysfunction and hypertension, inflammation and atherosclerosis, perturbed endothelial barrier, and progression of diabetes. In blood vessels, NO is produced by the endothelial NO synthase (eNOS), the activity of which is regulated by Ca²⁺/calmodulin, binding of regulatory cofactors, and posttranslational modifications, including phosphorylation events on Ser1177, which stimulate NO production. Rap1 is a ubiquitously expressed small GTPase implicated in promoting vascular barrier. We have shown that endothelial cell (EC)-specific Rap1 deletion leads to defective angiogenesis *in vivo* due to faulty VEGFR2 activation and signaling. Importantly, EC-specific Rap1 knockout mice developed hypertension and pathological left ventricular hypertrophy.

The objective of the study was to determine the role of small G protein Rap1 in regulating endothelial NO production and endothelial-dependent vasorelaxation *in vivo* and *ex vivo*.

Using *ex vivo* myography and tamoxifen-inducible, endothelial-specific Rap1-knockout mice (Cdh5-CreERT2⁺¹⁰;Rap1^{fl/fl}), we demonstrate that Rap1 deficiency completely abrogates NO-dependent vasodilation and attenuates NO production. Mechanistically, we show that Rap1 is rapidly activated in response to receptor agonists that activate eNOS via Ca²⁺/calmodulin-dependent pathway and in response to shear flow, which modulates eNOS activity by its phosphorylation. Rap1 deletion in human ECs, *in vitro*, leads to deficient NO release in response to both these stimuli, and interferes with PI3K/Akt pathway and eNOS Ser1177 phosphorylation. Further, we demonstrate Rap1 is required for transducing signals from the endothelial mechanosensing complex comprising PECAM-1, VE-cadherin and VEGFR2 in response to shear flow, leading to ligand-independent VEGFR2 activation and signaling to stimulate NO production.

We conclude that Rap1 in endothelium is critically required for endothelial homeostasis and NO production, thereby affecting vascular tone and regulation of blood pressure. Furthermore, this study establishes Rap1 as a novel regulator of mechanotransduction in response to shear flow.

Deletion Mutation of Nrf2 Gene in the Rat — Role of NRF2 in Salt-Induced Oxidant Stress

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Nuclear factor (erythroid-derived 2)-like-2 (Nrf2) is a master antioxidant and cell protective transcription factor that upregulates antioxidant defenses. In this study, we developed a strain of Nrf2 null mutant rats using TALEN technology, which produced a 41 base pair deletion in the first exon of the Nrf2 gene that included the start codon for Nrf2. The deletion was verified by PCR, and absence of the Nrf2 protein was verified by immunohistochemistry. Expression of mRNA for the Nrf2-regulated indicator enzymes catalase, H0-1, SOD1, SOD2 and GSR was significantly lower in livers of Nrf2(-/-) rats fed high salt (HS; 4% NaCl) for 3 days compared to wild-type controls. In Sprague-Dawley (S-D) parental rats, HS diet down regulated the expression of mRNA for the Nrf2-regulated indicator enzymes Nqo1, catalase, SOD2, and GSR in the liver. Down regulation of those enzymes was prevented by continual infusion of a subpressor dose of angiotensin II (ANG II) to prevent salt-induced ANG II suppression. ANG II infusion also increased Nrf2/KEAPI ratio and upregulated the expression of heme oxygenase-1, catalase, and SOD1 enzyme proteins in arteries of HS-fed S-D rats. Low dose ANG II infusion restored endothelium dependent dilation to acetylcholine that was lost in middle cerebral arteries (MCA) of HS-fed wild-type rats, but this protective effect of ANG II infusion to restore acetylcholine-induced dilation was absent in MCA of HS-fed Nrf2(-/-) rats. ANG II (100 nM) caused translocation of Nrf2 to the nucleus in cultured aortic endothelial cells from S-D rats, but Nrf2 fluorescence was absent in endothelial cells from Nrf2(-/-) mutant rats. These findings indicate that inhibition of the NRrf2 antioxidant defense system plays an essential role in the development of salt-induced oxidant stress and endothelial dysfunction in normotensive rats.

Sarcomere Protein Mutation Exacerbates Contractile Dysfunction Generating Inflammation and Oxidative Stress in Cardiomyopathy

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Dilated cardiomyopathy (DCM) is a leading cause of heart failure and can result from mutations in genes encoding contractile proteins. Inflammation and oxidative stress have been associated with the development of DCM and chronic heart failure. However, it is unclear whether inflammation and oxidative stress directly associate with contractile dysfunction in sarcomere protein-mutated DCM.

Hypothesis: Contractile dysfunction resulting from cardiac protein-associated mutation can cause inflammation and oxidative stress that exacerbate the severity of DCM.

Methods and Results: A previously characterized mouse model of DCM expressing a homozygous cardiac myosin binding protein-C (MYBPC3) gene mutation (t/t) was used to determine the presence of inflammation and oxidative stress markers at 3 months of age, compared to wild-type (WT) mice. Echocardiographic analysis of the hearts of DCM animals confirmed increased dilation and decreased contractile function, characteristic of DCM. Compared to WT animals, histopathological analysis of DCM hearts revealed profuse myocardial disarray and fibrosis, while second harmonic generation imaging depicted disorganized sarcomeric structure and elevated collagen content. Intriguingly, immunohistochemical analysis showed significant infiltration of CD68+ macrophages in DCM hearts particularly in areas of disarray. Fluorescence activated cell sorting indicated increased levels of monocytes in DCM spleens confirming systemic inflammation. Cardiomyopathic hearts from mice and patients had a decreased glutathione ratio and increased protein carbonyl and lipid malondialdehyde content compared to healthy control hearts, confirming elevated oxidative stress. This was supported by reduced signals for mitochondrial semiquinone radicals and Fe-S clusters in DCM hearts compared to WT, as measured by electron paramagnetic resonance spectroscopy.

Conclusion: These results demonstrate that inflammation and oxidative stress are highly elevated in cardiomyopathies produced by sarcomere protein mutation and may represent novel therapeutic targets to attenuate the progression toward heart failure.

Minute Cholesterol Crystals Can Form Under Oxidative Stress From High Membrane Cholesterol: New Signaling Pathway for Initiation and Promotion of Atherosclerosis

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Cholesterol (Chol) is asymmetrically distributed within typical eukaryotic cells, with more than 90% of cellular Chol associated with the plasma membrane, where the Chol to phospholipid molar ratio is typically from 0.5 to 1.0. At high-saturating and over-saturating Chol content, pure cholesterol bilayer domains (CBDs) are formed in model and biological membranes. Formation of CBDs precedes formation of cholesterol crystals (CHCs) [1], and it is likely that CBDs are a precursor of monohydrate CHCs.

The latest finding that the deposition of the minute CHCs found in the cells of arteries can initiate and promote atherosclerosis by activating inflammasomes [2] indicates that the formation of CHCs is an early cause rather than a late consequence of inflammation. This indicates a new direction for research which should clarify pathways leading to the formation of CHCs. The first investigated pathway is through the uptake of oxidized LDL by macrophages, which promotes the intracellular accumulation of free Chol and formation of intracellular CHCs. We hypothesize that the alternative pathway of the intracellular CHCs formation involves plasma membranes. An intracellular excess of free Chol should increase Chol content in plasma membranes, which should promote formation of CBDs and CHCs, especially under oxidative stress. This pathway of CHC formation from CBDs has not been investigated in depth. This pathway should be universal in any tissue where high Chol content in membranes and oxidative stress conditions can induce formation of CHCs, inflammation, and disease.

Acknowledgements: Supported by NIH grants EY015526, EB002052, and EB001980.

Literature: [1] Mainali, L. et al. Formation of cholesterol bilayer domains precedes formation of cholesterol crystals in cholesterol/dimyristoylphosphatidylcholine membranes: EPR and DSC studies. *J. Phys. Chem. B.* 117:8994-9003, 2013.

[2] Duewell, P. et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 464:1357–1361, 2010.

Large-Scale Transcriptome-Wide Co-Expression Analysis Identifies Novel Disease-Relevant Mitochondrial Proteins and Modulators of Mitochondrial Function

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Mitochondrial dysfunction contributes to multiple monogenic and complex pathologies. Elucidating the functional properties of mitochondrial proteins and establishing gene-disease associations has been greatly aided by MitoCarta, a compendium of proteins with strong evidence of mitochondrial localization. MitoCarta is estimated to include approximately 80% of all mitochondrial proteins, meaning a substantial quantity remains undiscovered. We sought to identify novel physiologically and pathologically significant members of the mitochondrial proteome by exploiting the fact that genes encoding such proteins are frequently coordinately regulated by a finite group of upstream factors and thus exhibit predictable expression signatures. Using a large-scale co-expression analysis approach in which 935 mitochondrial protein-encoding transcripts (MPETs) from the MitoCarta compendium were assessed in an unbiased manner for correlation with other transcripts in a pre-compiled bank of almost 10,000 unique and varied microarray datasets, we (i) find marked intra-connectivity between bona fide MPETs (Fisher's exact $P < 0.0001$), and (ii) identify a population of transcripts that correlate highly with MPETs but that are not recognized as generating mitochondrial proteins or modulating mitochondrial processes. Reanalysis and cross-referencing with additional data sources (e.g. disease-specific networks, microarray datasets, protein-protein interactions) highlight multiple genes of potential context-dependent significance. As proof of this concept, mitochondrial localization was demonstrated for select candidates including LRR2, a poorly annotated gene with a putative role in cellular bioenergetic homeostasis. The use of cellular locale as a proxy for functional significance is especially informative in the case of the mitochondrion. Our meta-analysis represents a complementary means to nominate putative mitochondrially-localized and mitochondrial effector proteins, and theoretically expands the catalogue of genes considered in cases of suspected mitochondrial disease.

Specific Mirnas Are a Crucial Determinant of Fibroblast-to-Myofibroblast Transition and Cardiac Fibrosis

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Cardiac fibrosis is the pathological consequence of stress induced fibroblast-to-myofibroblast transition (FMT) and fibroblast proliferation. In this study, we investigated the miRNA-driven mechanisms involved in the pathogenesis of cardiac fibrosis. Our results demonstrate that miR-125b is a novel regulator of cardiac FMT and cardiac fibrosis. In particular, miR-125b is induced in both fibrotic human heart and murine models of cardiac fibrosis. We further provide evidence that miR-125b is necessary and sufficient for the induction of FMT and is a potent inducer of fibroblast proliferation. In addition, we identified that miR-125b targets apelin and p53, two important repressors of fibrogenesis. Notably, *in vivo* silencing of miR-125b protects against Angiotensin II-induced cardiac fibrosis. We conclude that increased expression of endogenous miR-125b is a critical determinant of profibrotic signaling in the diseased heart. Our findings suggest that miR-125b may serve as a therapeutic target for the prevention of cardiac fibrosis.

H₂O₂-Induced Dilation in Human Adipose Arterioles: Role of Smooth Muscle K⁺ Channels

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Rationale: Hydrogen peroxide (H₂O₂) has been proposed as an endothelium-derived hyperpolarizing factor. It elicits smooth muscle relaxation by activating potassium (K⁺) channels, in particular voltage-gated K⁺ (KV) and large-conductance Ca²⁺-activated K⁺ (BKCa) channels. The vasodilator effect and mechanism of action of H₂O₂ seem to vary among different vascular beds and species. In human coronary arterioles from subjects with coronary artery disease (CAD), H₂O₂ induces potent dilation that is mediated by the activation of protein kinase G-BKCa channel pathway. It remains unknown whether this K⁺ channel-mediated dilation is conserved in other systemic vascular beds in humans.

Objective: We investigated the vasomotor effect of H₂O₂ and its underlying mechanism in human adipose arterioles.

Methods and Results: Human adipose arterioles (100-200 μm) obtained from subjects without CAD were cannulated under 60 mmHg and examined for diameter changes using video microscopy. H₂O₂ (1-100 μM) induced vasodilation in a concentration-dependent manner. The dilation was not affected by L-NAME (nitric oxide synthase inhibitor) or indomethacin (cyclooxygenase inhibitor) alone, or in combination, or by removal of the endothelium but was abolished by high K⁺. H₂O₂-induced dilation was markedly reduced by both paxilline, a BKCa channel blocker and 4-AP, a non-selective KV channel blocker. We further investigated which subtype(s) of the KV1 channels (the major KV family in the vasculature) is responsible for H₂O₂-induced dilation. Similar to non-selective KV channel blocker 4-AP, DPO-1 (KV1.5 channel blocker) reduced H₂O₂-induced dilation. In addition, the KV1.3/1.5 blocker Psora-4 caused a similar rightward shift in H₂O₂-induced dilation, but further reduced the maximal dilation to 100 μM H₂O₂.

Conclusions: H₂O₂ dilates human adipose arterioles from non-CAD subjects, and the dilation is mediated by both KV and BKCa channels in smooth muscle cells. The mechanism by which H₂O₂ activates K⁺ channels (especially KV channel) remains to be determined.

Mechanistic Characterization of the Thioredoxin System in the Removal of Hydrogen Peroxide

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The thioredoxin system plays a critical role in the defense against oxidative stress by removing harmful hydrogen peroxide (H_2O_2). Specifically, thioredoxin (Trx) donates electrons to peroxiredoxin (Prx) to remove H_2O_2 , and then thioredoxin reductase (TrxR) maintains the reduced Trx concentration with NADPH as the cofactor. Despite a great deal of kinetic information gathered on the removal of H_2O_2 by the Trx system from various sources/species, a mechanistic understanding of the associated enzymes is still not available. We address this issue by developing a thermodynamically-consistent mathematical model of the Trx system, which entails mechanistic details and provides quantitative insights into the kinetics of the TrxR and Prx enzymes. Consistent with experimental studies, the model analyses of the available data show that both enzymes operate by a ping-pong mechanism. The proposed mechanism for TrxR, which incorporates substrate inhibition by NADPH and intermediate protonation states, describes the available data and accurately predicts the bell-shaped behavior of the effect of pH on the TrxR activity. Most importantly, the model also predicts the inhibitory effects of the reaction products (NADP⁺ and Trx(SH)₂) on the TrxR activity for which suitable experimental data are not available. The model analyses of the available data on the kinetics of Prx from mammalian sources reveal that Prx operates at very low H_2O_2 concentrations compared to their human parasite counterparts. Furthermore, the model is able to predict the dynamic overoxidation of Prx at high H_2O_2 concentrations, consistent with the available data. The integrated Prx-TrxR model simulations show that the coupling of TrxR- and Prx-dependent reduction of H_2O_2 allowed ultrasensitive changes in the Trx concentration in response to changes in the TrxR concentration at high Prx concentrations.

Development of a Conditional Knockout of Cholesterol Synthesis in the Mouse

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Dhcr24 (3 β -hydroxysteroid- Δ 24 reductase) is responsible for reducing the C24-C25 double bond in sterol intermediates of cholesterol, and this step is necessary to synthesize cholesterol. In humans, mutations in the Dhcr24 gene cause desmosterolosis (OMIM #602398), characterized by severe developmental abnormalities and elevated levels of desmosterol in plasma and tissue. The first case was reported in 1998, when a premature infant who died shortly after birth had elevated levels of desmosterol and phenotypic abnormalities. Although initially reported as a 'cholesterol-free mouse' in Science, the global Dhcr24 knockout mouse shows neonatal lethality within 10 h of birth, a finding reproduced in many different laboratories. We now report a conditional knockout model using the LoxP-cre deletion system and show proof-of-principle by studying the effects of Dhcr24 deletion in the adult liver.

Nitric Oxide is an Epigenetic Regulator in MDA-MB-231 by Mediating Changes in the Modifications of Histones and DNA

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Methylation and acetylation of Lysine 9 on Histone 3 (H3K9me2/ac) has been shown to play an important role in the epigenetic regulation of gene expression. The methylation status of H3K9 is regulated by methyltransferases (HMT) and demethylases. Demethylation of these epigenetic marks is performed predominantly by a family of JMJC domain Fe(II)-dependent enzymes which use alpha-ketoglutarate and O₂ as substrates. Nitric oxide (•NO), a biological free radical with a similar chemical structure as O₂, has been shown by our lab to inhibit the JMJC class of lysine-specific demethylases (KDM) and affect global histone methylation. Here, we will demonstrate that the removal of •NO on MDA-MB-231 can reverse the expression of HMT and KDM caused by •NO exposure. Also, levels of H3K9me2/ac returned to basal level after •NO had been removed from the cellular environment. Furthermore, H3K4mono/di/tri methylation are all shown to be affected by •NO. This study provides important insights on how this endogenously-produced molecule can possibly regulate transcription of genes by changing global methylation and acetylation levels of histones. TETs are dioxygenase enzymes that convert 5-methylcytosine (5mC) containing DNA to 5-hydroxymethylcytosine (5hmC) containing DNA. 5mC has been associated with transcription repression, whereas elevated 5hmC levels are associated with gene activation. TET-mediated conversion of 5mC to 5hmC is considered an important epigenetic component of transcriptional regulation. Similar to JMJC domain-containing demethylases, TETs are oxygen-dependent and require α-ketoglutarate and Fe(II) for their catalytic activities. In addition to determining the modifications of histones by •NO, we will demonstrate the ability of •NO to inhibit TETs functions and to decrease the overall conversion of 5mC to 5hmC. Overall, our study reveals novel roles of •NO in regulating the epigenetics landscape via altering DNA modifications and histone modifications.

Peroxynitrite and Epigenetics: A Potential New Connection Through KRAB-KAP1

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Peroxynitrite, nitrite, and nitroprusside are formed through redox-active complexes and are involved in the oxidation and nitration of amino acids such as tyrosines. In a high-throughput screen for tyrosine nitrations in human pituitary adenoma, ZN432 was identified to be nitrated in the Krüppel associated box (KRAB) domain when cells had an elevated peroxynitrite. The human genome contains at least 423 Krüppel-type zinc finger genes yielding a staggering 742 distinct predicted proteins all sharing a KRAB domain that regulates chromatic state through interaction with the KAP1 (TRIM28/TIF1B) protein. We performed in silico analysis of the KRAB domain proteins, elucidating 119 additional KRAB domains that are predicted to have a nitratable tyrosine. Molecular modeling of the KRAB-KAP1 complex suggested that the nitration event could potentially disturb the protein interactions. Therefore, we purified four KRAB domain proteins, in addition to the KAP1 RBCC domain and showed all four of these predicted proteins could be nitrated by peroxynitrite. To our surprise the KAP1 protein was also nitrated. Cysteine nitrosylation assays confirmed that none of the proteins had modified cysteines. Amino acid mutations revealed Y30 of the domain to be nitrated, in which the nitration event also required E36, N37, and N40. Binding assays revealed that the nitration of either the KRAB domain or the KAP1 protein results in a loss of protein-protein interaction. Additionally, preformed complexes were dissociated rapidly by peroxynitrite treatment. This data reveals a potential of peroxynitrite to alter epigenetic regulation and warrants further molecular characterization.

Dystrophic iPSC-Derived Cardiomyocytes Have Mislocalization of eNOS and Increased Susceptibility to Cell Death, Which is Reversed by the Nitrate-Like Properties of Nicorandil

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Background: Cardiomyopathy is a leading cause of death in Duchenne and Becker muscular dystrophy (D/BMD) patients, and specific therapies are lacking. Dystrophin deficiency is associated with mislocalization of nitric oxide synthase (NOS) whose dysregulation contributes to the pathogenesis of dystrophic cardiomyopathy. We have modeled dystrophic cardiomyopathy using patient-specific induced pluripotent stem cells (iPSCs) and show that DMD- and BMD- iPSC derived cardiomyocytes have mislocalization of endothelial (eNOS) but not neuronal NOS (nNOS). In this study, we determined whether nicorandil, a drug with nitrate-like properties, protects against stress-induced dystrophic cardiomyocyte injury.

Methods/Results: DMD-iPSC (exon 3-6 deletion), BMD-iPSC (exon 45-53 deletion), and non-dystrophic (N)-iPSC lines were differentiated into cardiomyocytes (iCMs) and matured for 35-38 days. Immunofluorescence revealed eNOS co-localized with dystrophin along the sarcolemma in N-iCMs but not in DMD- or BMD-iCMs. eNOS also co-localized with caveolin-1 in all groups. nNOS did not co-localize with dystrophin but with the ryanodine receptor 2 in all groups. Both DMD- and BMD-iCMs have increased cell death, as determined by TUNEL + staining when subject to 2 hours of metabolic stress and 4 hours of recovery (DMD: 66±3%, BMD: 29±4% vs. 1.5±0.04%; n=3-6). This was associated with dissipation of the mitochondrial membrane potential as monitored by laser-scanning confocal microscopy (DMD: complete loss, BMD: 441±168 vs. N: 891±183 AU; n=3-5). Nicorandil pretreatment was able to restore mitochondrial membrane potential and decrease cell death. The protective effects of nicorandil were abolished by ODQ, a selective inhibitor of NO-sensitive guanylyl cyclase.

Conclusion: Nicorandil protects against stress-induced cell death and preserves mitochondrial function in dystrophic cardiomyocytes through its nitrite-like properties. This suggests a potential therapeutic role of nicorandil in the treatment of dystrophic cardiomyopathy.

Epigenetic Modulation of Cellular Redox State and Differentiation in Vascular Smooth Muscle Cells

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Myocardin is a transcriptional co-activator of serum response factor (SRF) responsible for vascular smooth muscle cell (SMC) differentiation. Changes in Nox4 NADPH oxidase expression are associated with myocardin and SRF levels and have been implicated in the phenotypic switching of SMCs. However, the mechanisms underlying this molecular switch in response to vascular injury are poorly understood. We examined the role of microRNAs (miRs) in response to vascular injury and found that miR-25 was induced after carotid ligation. Additionally, we found that expression of both miR-9 and miR-25 are increased in SMCs derived from neointima as compared to the medial layer. Upon injury, a variety of inflammatory cytokines and platelet activators are known to infiltrate the vessel wall. We found that in response to TNF- α and thrombin, miR-9 and miR-25 are induced in human SMCs. Treatment of SMCs with miR-25 or miR-9 silenced myocardin mRNA expression and 3'UTR luciferase, but the effects of miR-25 were prevented with transfection of anti-miR-9. The transfection of mir-9 and miR-25 mimics in spindle-shaped SMCs converted them to an epitheloid morphology similar to SMCs derived from the neointima and a reduction in protein expression of myosin heavy chain. MiR-9 and miR-25 interacted with the Nox4 3'UTR, silencing its expression and causing global changes in cellular thiol redox state. However, only miR-25 was sufficient to decrease Nox4 protein levels. Finally, miR-25 was shown to increase miR-9 expression through hypomethylation of its promoter. Preventing miR-9 induction after miR-25 transfection prevented Nox 4 silencing, suggesting cooperative binding of these two miRs to Nox4. Based on our findings, we propose that miR-25-dependent silencing of Nox4 regulates SMC differentiation, in part via miR-9-dependent regulation of myocardin involving a mechanism dependent on changes in the cellular redox state.

Imaging the Dynamic Redox Changes of Endoplasmic Reticulum with Green Fluorescent Protein

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Misfolded proteins contribute to a number of cardiovascular diseases. They are a result of stress that perturbs the folding of proteins during or immediately after synthesis, leading to cell and organ dysfunction. The endoplasmic reticulum (ER) plays a key role in protein trafficking and quality control, especially of newly synthesized proteins destined for the cell surface. The oxidized environment of ER facilitates the formation of disulfide bonds and prevents unfolded protein accumulation. Perturbing the redox state of ER is expected to lead to function loss and, ultimately, cell death. Therefore, it is of importance to be able to monitor in real time the ER redox status in response to various stimuli. By using redox-green fluorescent protein tagged ER (roGFP-ER), we can monitor the dynamic changes at diverse ends of the cellular redox spectrum in the ER. In H9C2 myoblasts, we confirmed robust expression after transfection of roGFP-ER and localization in the ER under normal and stress conditions. The additions of 0.4mM H₂O₂ promoted more oxidation in the ER of H9C2 cells while 5mM dithiotretol (DTT) reduced it. Cell viability greatly decreased under these conditions. The treatment with 4 mM N-acetyl-cysteine (NAC), a radical scavenger, slowly reduced the ER. To further our investigation, doxorubicin, an anthracycline known to generate high levels of oxidative stress in mitochondria, was used to determine the effects on ER redox states. Surprisingly, the ER was reduced by doxorubicin. By imaging with roGFP-ER, we gained insight into the effects of these reagents. This imaging technique can serve as a platform to study the redox effects of other drugs.

Coordinated Regulation of Post-Translational Histone Modifications by Nitric Oxide via Inhibition of JumonjiC Domain Containing Demethylases

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Methylation and acetylation of histone lysine residues are important epigenetic regulators of cellular transcription. Lysine methylation is maintained by the activity of both methyltransferases and demethylases, while acetylation is regulated by the concerted effort of acetyltransferases and deacetylases. The JumonjiC (JmjC) domain containing Fe(II) alpha-ketoglutarate dependent family of dioxygenases mediates the majority of histone demethylation. We have previously shown that NO inhibits the JmjC demethylase, KDM3A, leading to increased dimethylation of Lys9 on histone 3 (H3K9me₂). NO also significantly lowers acetylation at H3K9 (H3K9ac), precluded due to increased methylation at the same residue. Changes in global histone methylation and acetylation were measured by immunoblotting. Next, we examined the ability of NO to regulate gene expression by altering histone modifications. Genes differentially expressed due to association with NO-altered posttranslational histone marks were identified by combined bioinformatic analysis of microarray and ChIP-seq data across the genome. This revealed significant effects of NO on transcription via epigenetic mechanisms. These data demonstrate a unique mechanism of NO signaling via regulation of multiple post-translational histone modifications, which ultimately dictate gene expression and cell phenotype. These results provide a novel explanation for the pleiotropic nature of NO and allow us to rethink classical NO-signaling mechanisms.

MS Based Antigen Design for Novel Antibodies Targeting the Extracellular Domain of Cell Surface Proteins in hiPSC-Derived Cardiomyocytes

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In recent years, the ability to efficiently generate hiPSC-derived cardiomyocytes (hiPSC-CM) has greatly improved, offering an unlimited supply of human cardiomyocytes. These hiPSC-CMs represent a clinically relevant model system that offers utility to drug and toxicity studies, probing mechanisms of genetic heart diseases, and investigations of very early stages of human cardiac development not otherwise accessible. The exploitation of hiPSC-CM for these applications continues to generate enthusiasm; however, this *in vitro* differentiation process is heterogeneous with regards to CM subtype. In addition, hiPSC-CMs exhibit highly dynamic expression patterns and proteins change daily, which complicates the ability to obtain homogeneous populations of desired cell types. To overcome this limitation, a system to identify and isolate hiPSC-CMs with defined functional properties is needed. Using an innovative chemoproteomic approach that enables highly specific capture and identification of extracellular domains of cell surface proteins, we have identified >800 cell surface proteins in hPSCs and hPSC-CMs. Comparative analysis of the proteins identified at the cell surface has revealed novel cell surface protein (nCSP), whose function is currently unknown and for which commercial monoclonal antibodies are unavailable. nCSP has not been previously reported as a marker in cardiac development, but our mRNA analyses reveal it is restricted to the heart during development, and its expression profile is coincident with established cardiac proteins, TNNT2 and NKX2.5. While antibody development for membrane proteins has traditionally been difficult, we illustrate how the protein structure information generated by the targeted proteomics approach facilitates antibody development. To date, we have successfully generated four monoclonal antibodies recognizing the extracellular domain of this novel protein. Application of our antibodies to human tissue has confirmed nCSP presence and revealed localization in fetal and adult hearts. Currently, efforts to functionally define the cells sorted by these antibodies, as well as knockdown models to probe its functional role in cardiomyogenesis, are underway.

Myopathic lamin Mutations Cause Reductive Stress and Activate the Nrf2/Keap-1 Pathway

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Mutations in the human LMNA gene cause muscular dystrophy and dilated cardiomyopathy by mechanisms that are not well understood. The LMNA gene encodes A-type lamins, intermediate filaments that form a network underlying the inner nuclear membrane. This network provides structural support for the nucleus and plays a role in gene regulation. To better understand how mutant lamins cause disease, we performed structural and functional analyses on mutant lamins identified in patients with muscular dystrophy. LMNA mutations that cause single amino acid substitutions in A-type lamin Ig-fold domains were found to perturb the tertiary, but not secondary structure, of the domain. To test for functional consequences of these structural perturbations, we modeled the mutations into *Drosophila* Lamin C and expressed the mutant lamins in larval body wall muscle. The mutant lamins caused larval locomotion defects and semi-lethality at the pupal stage. The muscles showed cytoplasmic aggregation of lamins and other nuclear envelope proteins. These phenotypes correlated with changes in gene expression and reductive stress. Genes regulated by the transcription factor Nrf2 were among those up-regulated. Normally Nrf2 is sequestered in the cytoplasm by Keap-1; however, the accumulation of cytoplasmic aggregates of nuclear envelope proteins caused elevated levels of the autophagy adaptor, p62/SQSTM1, which also binds Keap-1. Titration of Keap-1 by p62/SQSTM1 allowed Nrf2 to translocate into the nucleus. Both elevated levels of p62/SQSTM1 and nuclear enrichment of Nrf2 were confirmed in muscle biopsy samples from the muscular dystrophy patients, demonstrating disease relevance. Collectively these data demonstrate a novel mechanism for the regulation of gene expression by mutant lamins and suggest that regulation of protein folding, protein metabolism, and redox homeostasis are potential routes of therapy.

Hydrogen Sulfide-Mediated Intoxication by Invasive Bacteria

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Introduction: Hydrogen sulfide (H₂S) is a gasotransmitter that, with larger environmental exposures, results in fatal intoxications or permanent brain damage. Many invasive bacterial pathogens also produce H₂S in large volumes using organic substrates associated with broad-spectrum antimicrobial resistance and abscess production in an animal model. We developed a rat model of brain abscess formation with the intent to explore small molecule inhibitors of microbial sulfur metabolism as well as rescue therapies classically used for environmental intoxications. We also collected clinical isolates of *Streptococcus anginosus* group (SAG), considered non-pathogenic but associated with brain abscesses, and correlated microbial production of H₂S with clinical severity of infection.

Methods: Clinical isolates from sterile body sites were collected and the severity of the associated infection abstracted with informed consent. Isolates were grown in cysteine-supplemented BHI media, H₂S mobilized to the headspace, and then derivatized with monobromobimane to form an H₂S-specific fluorescent product, sulfide dibimane, which was quantified by HPLC with fluorescent detection. Rats were inoculated by stereotaxis, with SAG bacteria suspended in cysteine-supplemented artificial CSF. Animals were monitored serially by 9.4 T MRI before euthanasia.

Results: SAG clinical isolates universally produced large amounts of H₂S (80 μM) in 5 hours, in contrast to control streptococcal production (*S. oralis*, SO) of < 4 μM. Small molecules (PAG, AOA, Asp) partially inhibited H₂S production in a strain-specific pattern. Clinical correlation is ongoing. In the rat model, SAG strains produced brain abscesses, while control SO bacteria at similar densities did not. Inhibition of brain abscess build-up and rescue experiments using inhibitors of H₂S generation are planned.

Conclusion: Microbial production of H₂S can result in focal, infection-driven intoxications. These infections may be optimally treated with small molecule antimicrobials and inhibitors of microbial sulfur metabolism, as well as rescue therapies conventionally applied to environmental intoxications. Proof of concept is underway.

Coupling of Energy Metabolism and Synaptic Transmission at the Transcriptional Level: The Concurrent and Parallel Regulation of Specific Glutamate Receptor Subunit Genes and Cytochrome C-Oxidase by Nuclear Respiratory Factors 1 and 2

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Neuronal activity is highly dependent on energy metabolism. It has been shown that regions high in excitatory depolarizing neuronal activity contain high levels of an important energy-generating enzyme, cytochrome c oxidase (COX), and that perturbations to neuronal activity have concomitant changes in COX levels. Recently, we have found that the coupling between energy metabolism and neuronal activity extends to the molecular level in that the same transcription factor, nuclear respiratory factor 1 (NRF-1), co-regulates COX as well as critical subunits of glutamatergic receptors. Since nuclear respiratory factor 2 (NRF-2) also regulates all 13 subunits of COX, we hypothesized that it too co-regulates COX and glutamatergic neurochemicals. Three mechanisms are proposed through which NRF-2 and NRF-1 jointly regulate glutamatergic receptor subunit genes: complementary, concurrent and parallel, and a combination of the complementary and concurrent mechanisms. By means of *in silico* analysis, electrophoretic mobility shift, supershift assays, chromatin immunoprecipitation (ChIP), and promoter mutational analysis, we found that NRF-2 regulates the same glutamatergic subunits as NRF-1, namely the GluN1 and GluN2b subunits of the N-methyl D-aspartate (NMDA) receptors and the GluA2 subunit of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Moreover, we found that GluN1, GluN2b, and GluA2 receptors are up-regulated by depolarizing KCl treatment and down-regulated by TTX-mediated impulse blockade in neurons. Preliminary data showed that up-regulating NRF-2 led to an up-regulation of GluN1, GluN2b, and GluA2, and that this up-regulation partially rescued the down-regulation caused by TTX treatment. These data suggest that NRF-2 functionally co-regulates energy metabolism and neuronal activity via a parallel and concurrent mechanism with NRF-1.

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Genome-Wide Fine-Mapping of Metabolic Traits in Outbred Rats

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Obesity and overweight are major risk factors for multiple diseases, including cardiovascular disease (CVD) and type 2 diabetes (T2D). Of particular importance is visceral, or abdominal, adipose tissue, which is a known predictor of metabolic health. Although environmental factors such as poor diet and a sedentary lifestyle contribute, genetic factors are known to play a significant role with a heritability of 50-80%. Human genome-wide association studies have currently identified well over 100 genes for traits related to diabetes and adiposity. Despite this success, however, these genes explain less than 10% of the heritable variance. Heterogeneous stock (HS) rats are outbred from eight inbred strains and allow genetic fine-mapping to only a few Megabases. Similar to humans, we show that visceral fat pad weight is significantly correlated with several measures of metabolic health in HS rats. The goal of the current study was to use HS rats to fine-map multiple metabolic traits genome-wide. We measured several metabolic traits in 1090 male HS rats and genotyped all rats using the Affymetrix 10K SNP array. Linkage disequilibrium mapping by mixed model regression was used to identify significant loci. We identified one or more significant loci for all traits, with an average confidence interval of only 2 Mb. Using expression data and founder sequence, we identified potential candidate genes within several loci, including several that have previously been identified in human genome-wide association studies. These results demonstrate the power of HS rats for fine-mapping metabolic traits and rapidly identifying candidate genes and indicate that animal models can be used to identify at least some of the missing heritability for metabolic traits in humans.

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