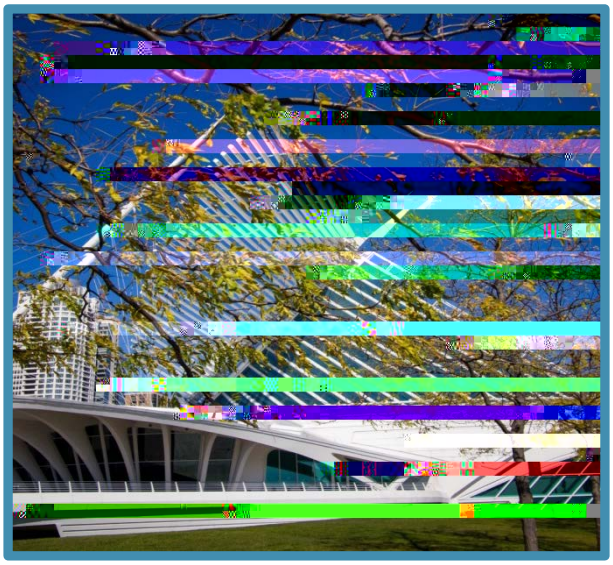
book of abstracts

Proceedings of the First Redox Biology Program Meeting

Cardiovascular Redox Signaling Symposium



Medical College of Wisconsin

Milwaukee, Wis.

November 14, 2014

mcw.edu/redoxbiologyprogram

Acknowledgements

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First Redox Biology Program

Cardiovascular Redox Signaling Symposium

Medical College of Wisconsin, Milwaukee, Wis.

November 14, 2014

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 on 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.

Symposium Chairs

Neil Hogg, PhD

Session Chairs

Morning Proceedings

Part I:
Presentation Abstracts

The Reductive Stress Hypothesis and the Antioxidant Treatment Paradox

I. J. Benjamin[1,3], M. Thao[1,3], S. Squires[1,3], M. Riedel[1,3], J. Zielonka[2,3], and B. Kalyanaraman[2,3]

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The reductive stress hypothesis in disease pathology was recently revisited by Benjamin and colleagues who have demonstrated that a protein

Role of NOXs in Hypertension Dissected in Rat Knockouts

A.W. Cowley Jr[1,2], D. Feng[4], C. Yang[1], T. Kurth[1], N. Zheleznova[1], A.M. Geurts[1,3].

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Evidence will be summarized showing that redox imbalance of NO_2^-, and H_2O_2 occurs in the renal

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

A. C. Chadwick

Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI

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 buiis14.85(m)-0.8395(r)46-2417

Myeloperoxidase and the Generation of Dysfunctional HDL

B. Shao, J.W. Heinecke

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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.

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

R.L. Silverstein, MD

Division of Hematology and Oncology, Department of Medicine, Medical College of Wisconsin, Milwau
WI

Sirtuins: From Mitochondrial Redox and Metabolism to Chromatin Regulation

J.M. Denu, PhD

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Nitric Oxide Signaling Through S-nitrosoglutathione Transnitrosation

B.C. Smith[1], S. Wynia-Smith[1], Y. Zhou[2], C. Knutson[3], S. Couvertier[2], N. Fernhoff[4], J.S. Wishnok[3], S.R. Tannenbaum[3], E. Weerapana[2], M. Marletta[5]

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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 nitrosation of only a subset of cellular cysteines remain largely unknown. Solution chemistry of nitrosation includes NO oxidation to N_2O_3 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 nitrosothi(f)-2()-1.3(d)-2.8(d)- .6(t)-4(r)4.-2.3(d)-trn inclu-4(r0.5(f)10.d

Superoxide as a Regulator of Nitric Oxide S L J Q D O L Q Cells L Q Ã-

K.A. Broniowska

Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI

Inflammatory cytokines impair pancreatic cell function by stimulating the expression of inducible nitric

Critical Ro

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

J. Zielonka[1], M. Zielonka[1], L. Verplank[2], T. Ganesh[3], A. Sun[4], G. Cheng[1], C. Communal[5], W.J. O'Brien[4], D. Lambeth[4], B. Kalyanaraman[1]

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[2]Therapeutics Platform, Broad Institute, Cambridge, MA
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NADPH oxidases (NOX) have been implicated as a major source of superoxide ($O_2^{\bullet-}$) 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 sta(r)4 O-nh21 Tf 0.5 0.5 Td <0087>Tj /TT2 1 Tf 0

Endothelial NOS Uncoupling: New Biochemical and Biophysical Developments

J. Whitsett[1], P. Martasek[2], L.J. Roman[2], K. Moon[3], J. Vasquez-Vivar[1,4]

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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 genngenaiai.8>DC 12 alT /

Part II:
Poster Abstracts

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

W.E. Antholine

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

S. Bhattacharya[1], M. Waas[1], E. Kropp[1], S. Chuppa[2], P. Goldspink[2,3], RL. Gundry[1]

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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 therapeutic generation of cell types valuable for drug

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

G. Coulis[1,2], Y. Shi[1], M. Yang[3], D. Labbé[4], G. Karsenty, PhD[5], N.K. Tonks[3], M.L. Tremblay[4], J.C. Tardif[1], B. Boivin[1,2]

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[2]Department of Medicine, Université de Montréal, Montréal, QC, Canada
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[4]Goodman Cancer Research Centre, McGill University, Montréal, QC, Canada
[5]Department of Genetics and Development, Columbia University, New York, NY

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-labelling assay to detect reversible oxidation of PTPs, we have identified PTP1B as a target of ROS in cardiomyocytes and hearts undergoing hypertrophy. Since we have recently identified a novel signaling pathway linking PTP1B 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), we explored whether the RAS-ROS-PTP1B-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 PTP1B in HRASV12-expressing myocytes as well as in TAC hearts of transgenic mice expressing a PTP1B trapping mutant. We generated cardiomyocyte-specific PTP1B knockout (PTP1B cKO) mice to study the role of PTP1B in the heart while avoiding the systemic effects of a global PTP1B KO or of global PTP1B inhibition. Preliminary results of PTP1B 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 PTP1B, confirming the involvement of the PTP1B pathway in hypertrophy. We propose that ROS-mediated inhibition of Pesul.3(s)4.5-2.3(l)1.6(v1B)]TJ c 0 Tw 8.35 Tc 0.003

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

EN. Dedkova

Department of Molecular Biophysics and Physiology, Rush University, Chicago, IL

The risk of cardiovascular disease (CVD) and mortality in type-2 diabetic patients is twice as high as in agematched healthy subjects. However, the mechanisms linking type-2 diabetes with CVD remain poorly

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

JT. Eells[1], K. Bach[1], S. Maleki[2], H. Schmitt[1], P. Summerfelt[3], A. Dubis[4], M. Ranji[2], J. Carroll[3], S. Gopalakrihn(a)-3n

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

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Feinberg Cardiovascular Research Institute, Northwestern University, Chicago, IL

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 select-2.3(d)-3.9(w)]TJ -0

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

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[2]Department of Physiology, Medical College of Wisconsin, Milwaukee, WI

Uncontrolled hyperglycemia in type 2 diabetics increases their risk of developing hypertension and kidney damage. Patients with comorbidity of diabetes and hypertension are at 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% O_2/5% CO_2. Rings were equilibrated at 0.5 g passive force, and responses to deoxynojirimycin (DNJ, potent SGLT3 antagonist) were analyzed with DMT Myo 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 ± 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 Cultur ed Embryonic Cardiomyocytes

H. Huang[1], E. J. Sukowski[1], R. Vazzalwar[2], G. Stefanov[2], C. White[1], D.R. Peterson[1]

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[2]Department of Pediatrics, Advocate Children's Hospital, Park Ridge, IL

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

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

E. Kropp

Impairment of Macrophage Reverse Cholesterol Transport by StAR-dependent Cholesterol Hydroperoxide Trafficking: Implications for Artherogenesis 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-à-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

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

J.R.C. Priestley[1], B.D. Weinberg[1], K. Kautenburg[1,2], M. Casati[1], B. Endres[1,3], A.M. Guerts[1,3], J.H. Lombard[1]

[1]

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

T.L. Lynch[1], M. Sivaguru[2], M. Velayutham[3], A.J. Cardoune[3], C. Liebtrau[4], M. Michels[5], D. Barefield[1], S. Govindan[1], J. van der Velden[6], C. Troidl[4], S. Sadayappan[1]

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Dilated cardiomyopathy (DCM) is a leading cause of heart failure and can result from mutations in genes

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

L. Mainali[1], M. Raguz[1,2], W.K. Subczynski[1]

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

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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

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

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Rationale: Hydrogen peroxide (H_2O_2) 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 Ca^{2+}-activated K+ (BKCa) channels. The vasodilator effect and mechanism of action of H_2O_2 seem to vary among different vascular beds and species. In human coronary arterioles from subjects with coronary artery disease (CAD), H_2O_2 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 H_2O_2 and its underlying mechanism in human adipose arterioles.

Methods and Results: Human adipose arterioles (130-200 µm) obtained from subjects without CAD were cannulated under 60 mmHg and examined for diameter changes using video microscopy. H_2O_2 (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

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

Development of a Conditional Knockout of Cholesterol Synthesis in the Mouse

SB. Patel

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Dhcr24 (3 à-hydroxysteroid- E24 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-

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

B.M. Schickling

Department of Internal Medicine, University of Iowa, Iowa City, IA

Myocardin is a transcriptional coactivator 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-ß and thrombin, miR-9 and miR-25 are induced in human SMCs. Treatment of SMCs with miR-9 or miR-25 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-s

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

D. Vasudevan, J.R. Hickok, V. Pham, D.D. Thomas

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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) and α-ketoglutarate dependent family of dioxygenases mediate 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

M

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 type-A 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 Lamin A/C 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

Hydrogen Sulfide - Mediated Intoxication by Invasive Bacteria

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[3]Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI

Introduction: Hydrogen sulfide (H_2S) is a gasotransmitter that, with larger environmental exposures, results in fatal intoxications or permanent brain damage. Many invasive bacterial pathogens also produce H_2S 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 H_2S 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, H_2S mobilized to the headspace, and then derivatized with monobromobimane to form an H_2S-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 H_2S (80 µM) in 5 hours, in contrast to control streptococcal production (S. oralis, SO) of < 4 µM. Small molecules (PAG, AOA, Asp) partially inhibited H_2S 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 H_2S generation are planned.

Conclusion: Microbial production of H_2S 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

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 30-70%. 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 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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