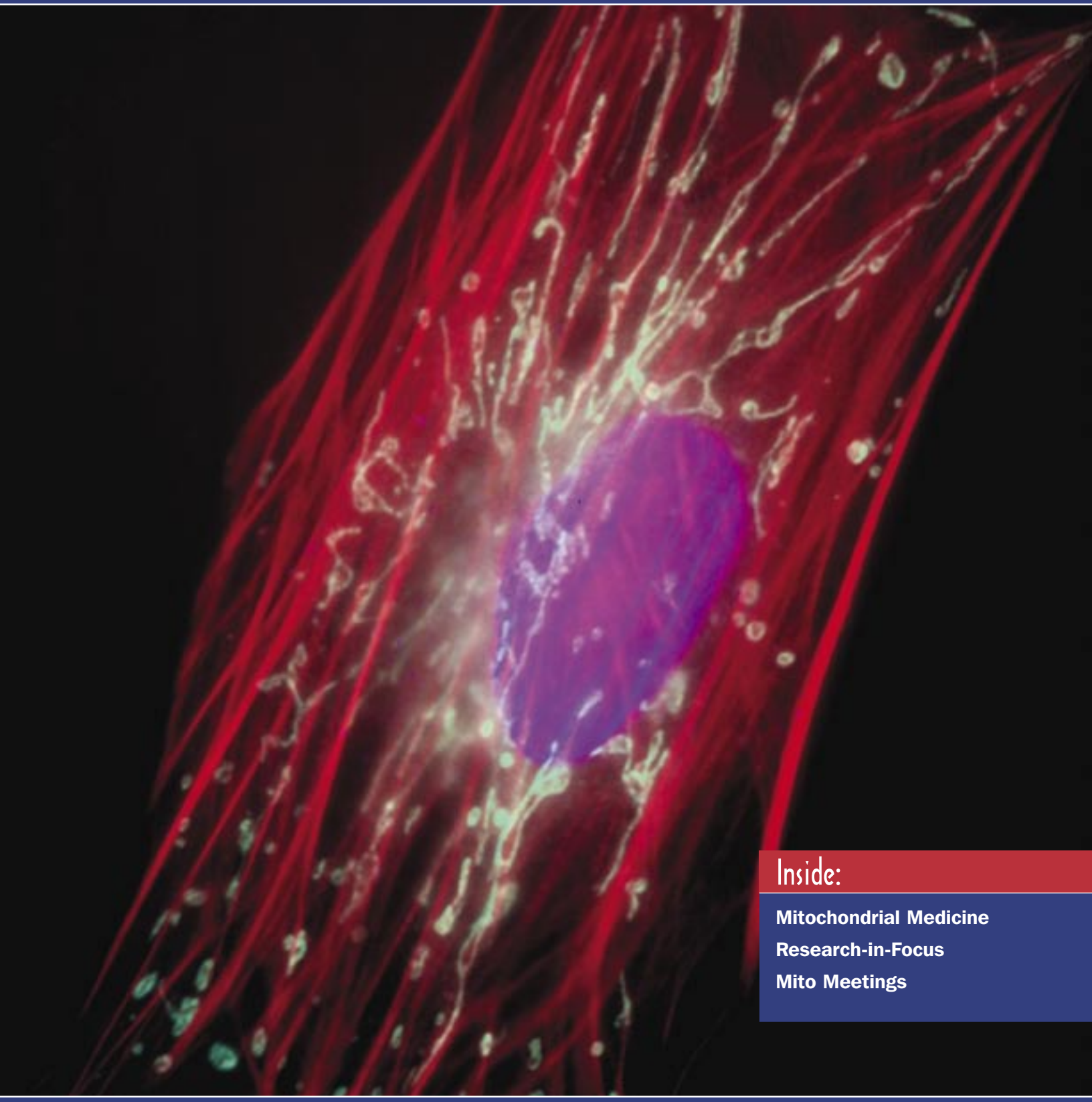
MitoMatters

The Official Newsletter of the Mitochondria Research Society



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Volume 3, Issue 2, 2004

MitoMatters

Mitochondrial Medicine 2004, 'Streams of Energy,' Pittsburgh, August 4–7

There was a lot in store for basic scientists and clinicians attending the Mitochondrial Medicine 2004 conference held in Pittsburgh on August 4–6, with special clinical sessions and a standards workshop held on Saturday, August 7.

This year's meeting was a refreshing mix of basic sciences, translational approaches, and clinical sciences focusing on issues in mitochondrial biology, genetics, and disease pathogenesis. The invited platform presentations were intermixed with submitted abstract communications. One hundred thirty registered participants attended the meeting, and 70 abstracts were submitted. Abstracts 1–41 were published in *Mitochondrion*, no. 4 (2004):1–24, while the others were published in the meeting abstract book.

The first two days of the conference focused on basic science investigations, and the last two days on clinical presentations, including a standards workshop.

Highlights of the basic science research include:

Dr. Richard Scarpulla, who initially identified the nuclear regulatory factors (NRF-) 1 and 2, discussed his latest findings on the identification of regulatory factors in mitochondrial transcription and biogenesis. Dr. Nadja Souza-Pinto discussed her research on mtDNA repair enzymes. The presentation

Front cover image: *Muntjac skin fibroblast labeled with a mouse monoclonal anti-histone antibody and visualized with green-fluorescent Alexa Fluor® 500 goat anti–mouse IgG antibody (pseudocolored blue). F-actin was labeled with biotinylated phalloidin and visualized with blue-fluorescent Alexa Fluor® 405 streptavidin (pseudocolored red), and mitochondria were stained with red-fluorescent MitoTracker® Red CMXRos (pseudocolored green). Image contributed by Michael Janes, Molecular Probes Inc.*

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covered studies of mtDNA glycosylases, with particular emphasis on the biochemistry of the human mitochondrial oxoguanine DNA glycosylase (-Ogg1).

Dr. Salvatore Alesci presented the latest developments in the generation of a mitochondrial-specific database along with a c-DNA array chip. The database will be made public in the near future. Dr. Martin Philbert presented an exciting seminar on the use of nanotechnology for single cell measurement of physiological parameters, an application that could lead to great advancement in our understanding of mitochondrial function.

Dr. Kelvin Davis discussed his studies on the characterization of the mitochondrial Lon protease, with particular emphasis on its roles in degrading oxidized proteins. These results have profound implications for our understanding of mitochondrial dysfunction in diseases of oxidative stress and aging. Dr. Dean Jones discussed the mitochondrial thyoredoxin protein. He stressed the roles of this and other thiol-containing proteins in the mitochondrial responses to oxidative stress.

Dr. David Nicholls presented research in analyzing mitochondrial function in cell culture models of neuronal dysfunction using a new method with micro-electrodes to examine mitochondrial membrane potential. The question of how to measure mitochondria permeability transition in suspension and in living cells has been intensely debated lately. Dr Nicholls' presentation addressed important points that should be considered in the search for a more physiological approach. Dr. Doug Wallace then presented a comprehensive review of his studies on the pathophysiology of mitochondrial diseases in mice and humans.

Abstracts were presented as both oral presentations and posters covering various areas of mitochondria biology, from bioenergetics to genetics. The poster sessions generated great interest and discussion. For instance, Kaleb Lund and Dr. Kendall Wallace presented an interesting abstract on the mitochondrial cardiac and metabolic effects of the nucleoside reverse transcriptase inhibitor, AZT, used to treat HIV-infected patients. Their research demonstrated that mtDNA depletion is not a prerequisite for mitochondrial dysfunction.

In the clinical sessions:

Dr. Michio Hirano presented data on the clinical aspects of complex III deficiency, specifically those due to mtDNA mutations in cytochrome b and nDNA mutations in BCS1L. Dr. David Thornburn presented data regarding genes involved in complex 1 deficiency, by far the most common respiratory chain disorder. Pathogenic mutations in 16 genes have been identified, including a supernumery subunit, NDUFS4, which plays a role in the regulation of complex 1 activity by reversible phosphorylation.

Two groups presented their data and experiences with the DCA trials, a particularly relevant discussion for those of us long awaiting the outcome of these trials. Dr. Peter Stacpoole recounted his experience with 43 patients in a trial using DCA as a lactate-lowering agent. This was a randomized, placebo-controlled trial, involving a double blind crossover design with crossover at six months and 12 months. The drug was well tolerated, with no significant adverse effects. However, there was no measurable improvement in neuromuscular or neurobehavioral measures, no reduction in either the frequency or severity of intercurrent illnesses or hospitalizations, and no difference in linear growth. Neither a lack of difference in basal blood or CSF lactate, nor a decreased blood lactate response to a carbohydrate feed was noted. Intergroup analysis suggested a possible disease-stabilizing effect for certain subpopulations of subjects with congenital lactic acidosis, possibly those with PDC E1alpha deficiency.

In general, lessons learned from this trial were that more subjects were needed to address any potential subgroup benefit. It is possible that DCA might be of benefit to certain subgroups of patients, not obvious in this study design.

Dr. Richard Haas presented his team's experience with a DCA trial. His trial enrolled a similar number of patients, with 37 undergoing an open label study, 42 undergoing a blinded crossover study design, and 41 remaining on a maintenance protocol. Patients were followed clinically and with EMG and blood chemistries and lactate measures. Of 37 patients who completed the study, 18 had clinical improvement defined as stabilization of disease, or improvement of headaches in patients with MELAS. In 10 patients, no discernable effect was seen; eight patients worsened, with outcomes including multiple episodes of sepsis, organ failure, strokes, and death. Again, a larger patient base as well as quantitative measures for clinical improvement would have been useful to understand the benefit of DCA in certain groups.

Although many mitochondrial patients present with exercise intolerance, two speakers demonstrated that moderate isotonic exercise may be useful in such patients. Dr. Ronald Haller presented a novel therapeutic approach for expanding the percent of wild type mtDNA in mitochondrial disease patients by the use of exercise. Dr. Mark Tarnopolsky spoke about the way in which changes in diet and supplements may be used to enhance mitochondrial function. For example,

in patients with complex I defects, the use of a high-fat diet may lead to enhanced free fatty acid oxidation thereby providing more reducing substrates (in the form of FADH2 and NADH) to participate in mitochondrial beta oxidation. Likewise, studies have suggested that a high-fat diet (i.e., ketogenic) with PDH deficiency in subjects who either had the diet initiated earlier in life or who were placed on greater carbohydrate restriction had increased longevity and improved mental development. Thus, he recommended explicit diet history be obtained in all patients with mitochondrial cytopathies. He also spoke of his experience with the use of compounds such as creatine monohydrate, coenzyme Q-(10), alpha lipoic acid, ketone bodies, riboflavin, and nictotinamide, neuroprotection, trauma, and oxidative damage. There is no currently recognized treatment for mitochondrial disease, and future clinical trials are needed.

Saturday Workshops

An optional Saturday session was offered with two concurrent sessions including clinical sessions and a standards workshop.

Clinical Sessions

The clinical sessions focused on an overview of mitochondrial disease features, biochemistry, genetics, and phenotypes presented by Dr. Michio Hirano, followed by sessions addressing specific aspects of mitochondrial disease including neurological involvement (Dr. Ronald Haller); gastrointestinal issues—dysmotility and pseudoobstruction (Dr. Mahmoud Sabri,); symptom management—seizures, headaches, movement disorders, attentional problems, stroke, muscle pain and weakness, neuropathy, autonomic dysfunction, and pain control (Dr. Bruce Cohen); nutrition and exercise (Dr. Mark Tarnopolsky, Canada); and lastly pain management and end-of-life issues (Dr. Regina Jakacki and Dr. Ronald Glick).

Standards Workshop

The purpose of the standards workshop was to bring together scientists and clinicians involved/interested in developing consensus about standardization of clinical and laboratory measures/methods for the diagnosis of mitochondrial diseases. A secondary aim was to develop a sample exchange program of patient diagnostic material for this venue.

Dr. Bob Naviaux presented the introductory comments. Dr. David Thornburn presented his findings on measuring oxidative phosphorylation enzyme activities in fresh versus frozen human tissue, followed by discussions about polarography in fresh tissue (Dr. Charles Hoppel). Dr. Mike Marusich presented research on using monoclonal antibodies for monitoring and diagnosis of mitochondrial disease. Mitochondrial proteomics was discussed by Dr. Sudhir Srivastava. Methods of quantitative mutational analysis of mtDNA used in a molecular diagnostic setting was presented by Dr. Lee Jun Wong. Histopathological diagnostic methods and their standardization for clinical diagnostics were discussed by Dr. Arthur Hays. Development of neurological mitochondrial-related clinical scales was presented by Dr. Richard Haas. Standardized neuroimaging analysis scales and multinuclear magnetic resonance spectroscopy were discussed by Dr. Rosalind Dietrich and Dr. Fernando Arias Mendoza, respectively. Standardized exercise physiology protocols for use in baseline assessments as well as intervention trials was presented by Dr. Mark Tarnopolsky.

In summary, the fresh versus frozen debate concluded with the following:

1. There are slight variations in methods used in different labs.
2. Frozen specimens were robust for single enzyme assays.
3. As this may be the only available and practical sample, it allows more flexible scheduling.
4. However, some defects will require functional assays of liver and fibroblasts.
5. Interpretation requires experience.
6. Standardized, narrow normal ranges of enzyme activity are unrealistic.
7. Results from biochemical analysis cannot be interpreted in isolation, but rather need to be seen in combination with the clinical data, physical examination, family history, and other supportive studies (i.e., blood biochemical studies, molecular, imaging, etc.)
8. A sample exchange program would provide ability to look at issues of sensitivity and sensitivity of methods, as well as to address the challenges of respiratory chain analysis methodology, sampling handling, controls, validation, and reporting.

Drs. Mike Marusich and Roderick Capaldi have developed monoclonal antibodies to several mitochondrial proteins, including OXPHOS enzyme complexes, PDH, and ANT, for use in western blot and immunocytochemistry. Immunocapture assays being developed have the potential to assist in diagnosis and treatment of patients with mitochondrial diseases.

Dr. Wong demonstrated an improved, more sensitive allele-specific oligonucleotide (ASO) radioactive dot-blot hybridization method, underscoring the importance of a sensitive mutation detection method and the need for a search for mtDNA mutations if the patient's clinical symptoms are suggestive.

Mitochondrial Medicine

It was concluded that while various centers have expertise in one or a number of aspects related to the diagnosis and/or management of mitochondrial disease patients, a more standardized approach would serve the goals of the MRS, MMS, and clinicians and investigators. Such standardization would assist with disease diagnosis and management, and establish the ability for the rigorous study of preclinical and clinical interventions as they become available.

Overall, the conference blended clinical and basic sciences successfully with an eye to the future of mitochondrial medicine. The 2005 Mitochondrial Medicine meeting will be in St. Louis at the Hyatt Union Station on June 14–18. See you there!

Research-in-Focus

1. A new mitochondrial disease: A collaborative study by German and American scientists, led by Michael Schröder and Jeffery Vance, has identified a new mutation causing Charcot-Marie-Tooth neuropathy type 2A, a frequent occurring peripheral neuropathy that seems to be genetically heterogeneous but rather uniform regarding clinical features. Their study identified a mutation in the gene encoding mitofusin 2 that segregates with all seven families studied. Mitofusin 2 localizes to the outer membrane and regulates the mitochondrial network architecture by fusion of mitochondria.

Zuchner et al., *Nat. Genet.* 36 (2004): 449–451.

2. From Abeta to mitochondria: There is a vast body of experimental evidences showing mitochondrial dysfunction in Abeta-induced neuronal toxicity in Alzheimer's disease. However, the molecular mechanisms for this effect have not been clearly established. Lustbaster and colleagues recently demonstrated that the Abeta-binding alcohol dehydrogenase (ABAD) is a direct molecular link from Abeta to mitochondrial toxicity. Abeta interacts with ABAD causing a substantial deformation of the active site that prevents nicotinamide adenine dinucleotide (NAD) binding. These results provide new mechanistic insight into the pathogenesis of Alzheimer's and may contribute to the development of new therapeutic approaches.

Lustbader et al., *Science* 304 (2004): 448–452.

3. Mitochondria and aging: The mitochondrial theory of aging proposes that aging is caused by mitochondrial dysfunction due to accumulation of DNA damage and mutations. The recent work by Larsson and colleagues has provided strong support for this theory and caused a considerable stir in the aging community. They have generated a mouse that expresses a defective DNA polymerase gamma that generates high levels of point mutations and deletions of the mtDNA. These transgenic animals have reduced life span and premature onset of associated features that closely resemble those observed in normal aging. This work brings renewed attention to the role of mitochondria in aging and aging-associated degenerations.

Trifunovic et al., *Nature* 429 (2004): 357–359.

4. The diverse roles of mitochondria in life and death: Mitochondria control cellular fate through their roles in apoptotic and necrotic cell death. In this comprehensive review, Green and Kroemer discuss the various pathways involved in mitochondria-induced apoptosis, with particular emphasis on mitochondrial outer membrane permeabilization and release of various pro-apoptotic factors. Apoptosis is a controlled procees that has ubiquitous roles in normal development but also in various pathological processes such as cancer and neurodegeneration. Understanding the molecular events that lead to cell death will, ultimately, increase our chances of efficiently treating such conditions.

Green & Kroemer, *Science* 305 (2004): 626–629.

5. Where is ROS going? Mitochondria are the main cellular site generating reactive oxygen species and oxidative stress. Superoxide anions are produced in both complex I and III of the electron transport chain, but since they are charged they do not cross membranes easily. Muller and colleagues studied the membrane sidedness of superoxide generation in intact mitochondria. They show that, while complex I releases superoxide only to the matrix side, complex III releases this species to both sides of the membrane and thus directly contribute to intracellular oxidative stress without dismutation into hydrogen peroxide.

Muller et al., *J. Biol. Chem.*, Aug. 17 (e-pub ahead of print), 2004.

Mito Meetings

MAY 28–JUNE 2, 2005

INTERNATIONAL CONFERENCE ON PLANT MITOCHONDRIAL BIOLOGY

This meeting is organized by the Departement de Biogenese desmitochondries vegetales, Institut de Biologie Moleculaire des Plantes (CNRS)
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JUNE 15–18, 2005

MITOCHONDRIAL MEDICINE 2005

This meeting is hosted by the Mitochondria Research Society and the United Mitochondrial Disease Foundation (UMDF) at St Louis, Missouri, USA
E-mail: Kara Strittmatter (kara@umdf.org)
Tel: 412-793-8077
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SEPT 16–20, 2005

4TH CONFERENCE ON MITOCHONDRIAL PHYSIOLOGY 2005

This meeting is hosted by Mitochondrial Physiology Society, Schrocken, Voralberg, Austria
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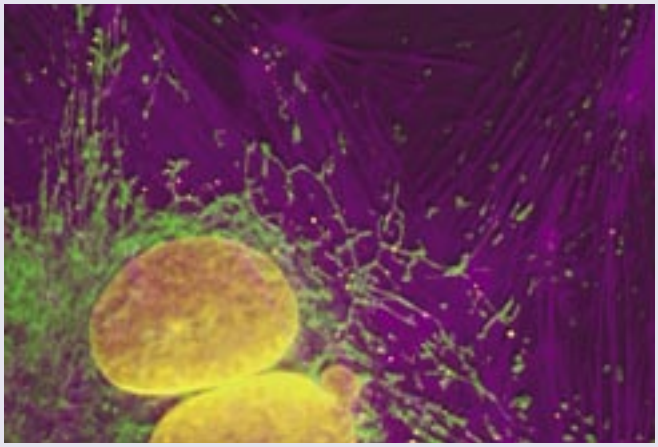

THE MITOCHONDRIA RESEARCH SOCIETY

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