

4th Conference of Korean Society for Mitochondrial Research and Medicine

in conjunction with
1st Symposium of the Mitochondria Section of KSMCB

Mitochondria as a Potential Therapeutic Target in Diseases

Organized by
Korean Society for Mitochondrial Research and Medicine
The Mitochondria Section of Korean Society for Molecular and Cellular Biology

Local Organizer
Research Institute for Medical Sciences CNU
Research Center for Endocrine and Metabolic Diseases CNUH

Sponsored by
Ministry of Education, Science and Technology
The Korean Society for Molecular and Cellular Biology
National Research Laboratory for Mitochondrial Signaling, Inje University
Cardiovascular and Metabolic Disease Center, Inje University



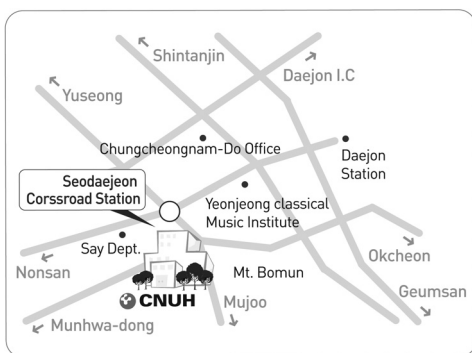
KSMRM

Mitochondrial dawn, Odra Noel

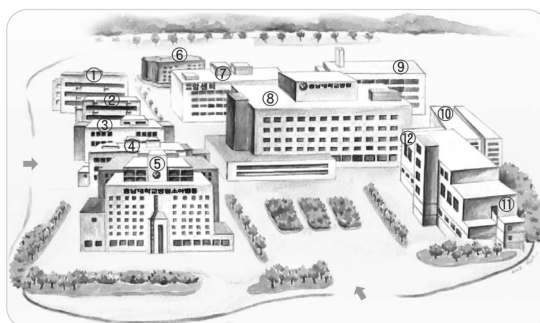


Venue Guide

| Date | **June 19, 2010**
| Venue | **Geriatric Medical Center Lecture Hall, 5F**
Chungnam National University Hospital, Daejeon



Chungnam National University
Hospital



①Funeral hall ⑤Children's Hospital ⑨Power plant
②Parking lot building ⑥Dormitory ⑩Parking lot building
③Clinical Research Building ⑦Daejeon Regional Cancer Center ⑪Emergency Medical Center
④Administration building ⑧Main Building ⑫Daejeon Geriatric Medical Center

#12, Daejeon Geriatric Medical Center
Lecture Hall, 5F

| How to get there? |

- **Subway**
Exit 1 of Seodaeseon Crossroad Station

- **Bus Number**
30, 313, 315, 317, 615, 619

- **Taxi**
It takes about 20 minutes and costs about KRW 4,000 to reach CNUH from Daejeon Station by taxi.
It takes about 10 minutes and costs about KRW 3,000 to reach CNUH from Seodaeseon Station by taxi.

- **High Way**
Daejeon IC → Dongseolo Crossroad → Seodaeseon Crossroad
Geumsan Country, Muju Country → Anyeong IC (it takes 10 minutes)





Invitation

Mitochondrion produces energy, essential to power life process. While it is an intracellular organelle, it plays major role in the question of "to live or to die" for an organism. These features make this organelle the center of life activity, master of life process and thus cornerstone of understanding life itself. Biology of life aside, mitochondrion received attention because it is associated with pathogenesis of ageing, diabetes, and metabolic syndrome, various neurodegenerative diseases including Parkinson's disease and Alzheimer disease, and cancer. It is indeed more than half century ago, when Otto Warburg insisted mitochondrial dysfunction is the prime cause of cancer. Interest on mitochondrion is growing day by day around the world, and Korea is no exception.

Many Korean mitochondriologists formed the Korean Society of Mitochondrial Research and Medicine (KSMRM) 4 years ago, after many years of various personal activities. This year it has become an official body under the auspice of Korean Society Molecular and Cellular Biology and organized its inaugural meeting. I know many die-hard mitochondriacs, who devoted to the understanding of mitochondrion. By forming KSMRM, Korean mitochondriologists finally have a legal body through which they could communicate each other more easily and effectively. Furthermore it would facilitate to communicate and collaborate with members of Asian Society of Mitochondrial Research and Medicine and other international bodies, already established many years ago.

Fourth meeting of KSMRM is organized by professor Shong MH at ChoongNam National University Medical School, who runs "Mitochondrial Function and Metabolic Syndrome Research Group", sponsored by a government program. KSMRM is widely supported not only by Korean experts, but several cooperations bodies including Han Wha Pharma Co., Ltd., Choongwae Pharma Corporation, AmorePacific Co., Ltd., LeeBaeg Science Co., Ltd. and Dalim Biotech.

I am very much delighted to have Professor Yasutoshi Koga, Kurume University, President of Japanese Society of Mitochondria Research (J-Mit) with us. He will give us his valuable experience on the treatment of the MELAS in plenary lecture. Other programs include various aspects of mitochondrial function, mitochondrial dysfunctions in disease processes, and an introduction to mitochondrial medicine. There are sessions to help people who just started mitochondrial research; methods used in mitochondrial research, diagnosis and treatment of mitochondrial diseases and an example, targeted study of a molecule, sirtuin.

I hope this society plays pivotal role in letting people interested in mitochondrial research and its application to the health problems exchanging their latest knowledge and sharing information. I also members encourage young researchers to enter this rather challenging field, which is very old and new, changing very rapidly and becoming very sophisticated. Please register as a new member, join the society, and empowered to become a future leader. With you we are making history.

Hong Kyu Lee, MD, Ph.D.
President, Korean Society for Mitochondrial Research and Medicine
Chair, Organizing Committee of 4th Conference of KSMRM





□ Korean Society for Mitochondrial Research and Medicine

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Conference Information

❑ Preparation of Abstracts

Submit all abstracts in English. Proofread abstracts carefully to avoid errors before submission. The abstract will be published exactly as it has been submitted.

There is no limit to the number of abstracts an investigator may submit. If selected, the presenter must be one of the co-authors listed. To ensure that the abstract receives proper scientific consideration, please make sure that the abstract is submitted to the appropriate category. Abstracts containing identical or nearly identical data submitted from the same institution and/or individuals will be disqualified.

Abstract Title

An abstract must have a brief, descriptive, and clear title (containing no abbreviations) that indicates the nature of the investigation. Avoid non-specific phrases such as "A study of..." or "The effects of...".

Author Name(s)

The submitting author is designated as the primary/presenting author. If an author's name appears on more than one abstract, it must be identical on each abstract. Additions or deletions of author names are not permitted after the submission deadline.

Main text

Abstracts are limited to about 250 words. This includes the text plus any graphic/table, but not the title or authors. Do not include paragraph breaks and the references. Standard abbreviations may be used without definition. Nonstandard abbreviations must be placed in parentheses after the first use of the word or phrase abbreviated.

Describe briefly the objectives of the study and include a brief statement of methods if pertinent. State findings in detail sufficient to support conclusions.

❑ Submission of Abstracts

Abstracts should be submitted via email, phyhanj@inje.ac.kr; phyhanj@gmail.com

Abstract presenters are required to register and pay a registration fee to attend the conference and present their abstract. All other expenses (e.g., lodging) associated with the submission and presentation of an abstract are the responsibility of the presenter.



Conference Information

☐ Abstract Revisions

After the deadline, abstracts may not be revised in any way or resubmitted. Proofread abstracts carefully to avoid errors before submission. If accepted, your abstract will be published as submitted.

☐ Abstract Withdrawal

Submit abstract withdrawal requests in writing by June 5, 2010 to avoid publication.

☐ Abstract Acceptance

Abstracts will be reviewed by The Abstract grading committee. Abstract grading is blinded and abstracts are selected on the basis of scientific merit. Abstract acceptance/non-acceptance status will be announced on June 10, 2010.



□ How to Register

1. Email or Fax registration is highly recommended.
Fax: 051-894-5714
E-mail: phyhanj@inje.ac.kr; phyhanj@gmail.com
2. Registration without appropriate payment will not be honored until the full payment is received.
The confirmation of registration will be sent via e-mail.
3. Pre-registration will be available by June 5, 2010.
After June 5, 2010, you will be required to register on-site.

□ Registration Fees

Category	Registration Fee	Membership Fee
Professor	KRW 80,000	KRW 50,000 (Regular)/ 500,000 (Premium)
Student	KRW 20,000	KRW 20,000

□ Payment Method

Wire Transfer

- EAll bank remittance charges are to be paid by registrants.
- Sender's name should be the registrant's name.
- Registration confirmation will be sent to you via e-mail within 2 weeks upon clearing up appropriate payment.
- Please note that payments must be received by December 27, 2008, in order for participants to qualify for the early registration

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Program

June 19

**Daejeon Geriatric Medical Center Lecture Hall (5th Floor)
Chungnam National University Hospital**

08:30-09:00 Registration

09:00-09:10 Opening Ceremony

Welcome Address:

Hong Kyu Lee (President of KSMRM)

Symposium 1. Mitochondrial Dysfunctions in Diseases

Chair: Minho Shong (Chungnam National University)

**09:10-09:40 Roles of GSK3 in Mitochondrial Dysfunction during TGF
 β 1-induced Cell Senescence**

Gyesoon Yoon (Ajou University)

**09:40-10:10 The Role of Beta Amyloid-induced Mitochondrial Damage in
Alzheimer's Disease Pathogenesis**

Inhee Mook-Jung (Seoul National University)

**10:10-10:40 Downregulation of OXPHOS I Enhances Myogenesis by Inducing
IGF-II Secretion**

Young-Gyu Ko (Korea University)

10:40-11:10 Coffee Break

Symposium 2. Mitochondrial Medicine: Clinical Aspect

Chair: In Kyu Lee (Kyungpook National University)

**11:10-11:40 On the Pathogenesis of Metabolic Syndrome and the Need of
Mitochondrial Medicine**

Hong Kyu Lee (Eulji University)

**11:40-12:10 The Essential Roles of mtCRIF1 in Mitochondrial Function and
Adipogenesis**

Minho Shong (Chungnam National University)

12:10-14:00 Lunch





Program

Plenary Lecture

Chair: Ki-Up Lee (University of Ulsan)

14:00- 14:50 **Novel Therapeutic Approach of L-arginine on MELAS, A Major Subtype of Mitochondrial Myopathy**
Yasutoshi Koga (Kurume University)

Symposium 3. Workshop: Methods in Mitochondrial Research
(1st Symposium of the Mitochondria Section of the KSMCB)
Chair: Gyesoon Yoon (Ajou University)

14:50-15:10 **Cell-based Mitochondrial Activity Profiling System (CMAPS)**
Youngmi Kim Pak (Kyung Hee University)

15:10-15:30 **mtDNA Expression and Cellular Energy Production**
Chan Bae Park (Ajou University)

15:30-15:50 **Non-destructive Measurements for Profiling Metabolic Pathways: How XF Analyzer Works**
Gi Ryang Kweon (Chungnam National University)

15:50-16:10 **Modeling Parkinson's Disease Using *Drosophila* Genetics**
Hyongjong Koh (Dong-A University)

16:10-16:40 **Coffee Break**

Symposium 4. Therapeutic and Diagnostics Targets for Mitochondrial Diseases
Chair: Young Myung Kim (Kangwon National University)

16:40-17:10 **Regulation of SIRT1 Function by Cellular Interacting Partners**
Eun Joo Kim (Dankook University)

17:10-17:40 **NecroX as a Novel Small-Molecule Mitochondria Modulator**
Bong-Hyun Ahn (Drug Discovery, LG Life Sciences)



Program

**17:40-18:10 C1q Tumor Necrosis Factor α -related Protein Isoform 5 (C1QTNF5)
as a Novel Diagnostic and Therapeutic Target for Insulin
Resistance and Diabetes**

Wan Lee (Dongguk University)

Presentation of KSMRM Certificate of Appreciation to

Dr. Yong-Kyung Choe (Korea Research Institute of Bioscience and Biotechnology)

Closing Remark

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¹Department of Internal Medicine, University of Ulsan College of Medicine, Seoul, Korea,
²Asan Institute for Life Sciences, Seoul, Korea,
³Department of Anatomy, College of Medicine, Inha University, Incheon, Korea, and
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¹*Department of Biochemistry and Molecular Biology, School of Medicine and*

²*Department of Molecular Science & Technology, Ajou University, Suwon,*

³*Lab. of Molecular Biochemistry, Department of Chemistry,
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*National Research Laboratory for Mitochondrial Signaling,
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¹*National Research Laboratory for Mitochondrial Signaling, Department of Physiology and
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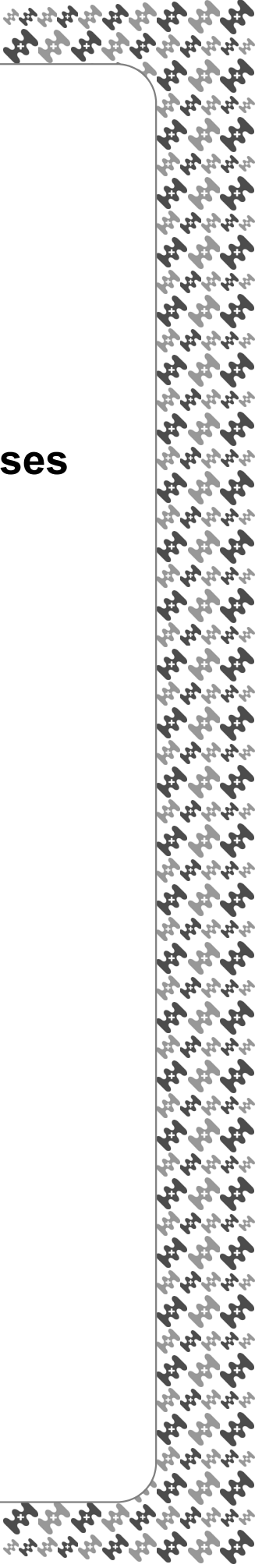
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¹*Department of Biochemistry, school of Medicine;*
²*Cancer Research Institute, ³Infection Signaling Network Research Center, Chungnam National University, Daejeon, Korea*
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¹*Department of Biochemistry, School of Medicine;*
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Symposium 1

Mitochondrial Dysfunctions in Diseases



Roles of GSK3 in Mitochondrial Dysfunction during TGF β 1-induced Cell Senescence

Gyesoon Yoon

Department of Biochemistry & Molecular Biology,
Ajou University School of Medicine and
Department of Molecular Science & Technology, The Graduate School,
Ajou University, Suwon, Korea

Transforming growth factor β 1 (TGF β 1) is one of the well-characterized cytokines that regulates cell proliferation. Recently, we reported that TGF β 1 induces senescence of Mv1Lu cells through persistently generating mitochondrial ROS, mediated by decreased complex IV activity. Therefore, we further investigated the molecular mechanism of how mitochondrial complex IV is decreased by TGF β 1. During TGF β 1-induced senescence, no significant changes of protein expression of complex IV subunits were observed despite its attenuated activity. However, TGF β 1 immediately phosphorylated both glycogen synthase kinase (GSK) 3 α and β on their negative regulatory sites and the phosphorylation levels were continuously maintained, well corresponding to the intracellular reactive oxygen species (ROS) profile. GSK3 inactivation by TGF β 1 was an upstream event of ROS generation and independent of AKT. Interestingly, GSK3 β inhibition using SB415286 significantly decreased complex IV activity, and then induced senescence. Similar results were obtained by siRNA-mediated knockdown of GSK3. Finally, we found that active GSK3 exists within mitochondria and binds subunit VIb (of complex IV) which is topologically located in mitochondrial intermembrane space. Taken together, our results suggest that GSK3 β inactivation is importantly involved in the TGF β 1-induced complex IV defects through binding subunit VIb, thereby persistently generating senescence-associated ROS.



☼ 인적사항

성 명 윤계순
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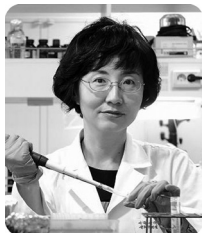
☼ 학력 · 경력

연도	학교/기관	전공/직위	학위/비고
1984	경희대학교 약학대학	약학	학사
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The Role of Beta Amyloid-induced Mitochondrial Damage in Alzheimer's Disease Pathogenesis

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Alzheimer's disease (AD) is an age-related neurodegenerative disorder affecting cognitive function including learning and memory process. The brain of AD patients shows distinct pathological hallmarks such as senile plaques, neurofibrillary tangles and neuronal loss. One of major causative factors in AD pathogenesis is beta amyloid peptide which is major component of senile plaques. Several lines of evidences showed mitochondrial function was attenuated in AD patients as well as AD animal models. In the present study, beta amyloid-induced mitochondrial dysfunction is systematically examined including JC-1 staining to see the changes of membrane potentials, MTT assay, Calcein assay, ROS measurement and quantitation of ATP synthesis using hippocampal cell line as well as primary cortical cultures. We will discuss the role of mitochondria in AD pathogenesis based on cellular mechanism study and AD animal model study.



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2. Kim I, Lee J, Hong HJ, Jung ES, Ku YH, Jeong IK, Cho YM, So I, Park KS, Mook-Jung I. (2009) A relationship between Alzheimer's disease and type 2 Diabetes Mellitus through the measurement of serum Abeta autoantibody. Journal of Alzheimer's Disease. 19(4):1371-6
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Downregulation of OXPHOS I Enhances Myogenesis by Inducing IGF-II Secretion

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Although mitochondrial biogenesis has been known to be accompanied with myogenesis, it is still remained to be solved whether mitochondrial function is essential for myogenesis. In order to address the issue, we investigated C2C12 myogenesis after downregulating NDUF57, a 20 kDa subunit of oxidative phosphorylation (OxPhos) complex I, by using small RNA interference. Unexpectedly, NDUF57 downregulation enhanced C2C12 myogenesis, as determined by cellular morphology and the expression level of myogenic marker proteins such as caveolin-3, myosin heavy chain, and myogenin. NDUF57 downregulation induced the activation of myogenic transcription factors such as MyoD and Myogenin, and MEF2A as well as Akt and S6K in C2C12 confluent myoblasts. Moreover, NDUF57-downregulated myoblasts produced and secreted much more IGF-II than control cells did. Taken together all these data, we conclude that NDUF57 downregulation enhances myogenesis via IGF-II secretion.



☼ 인적사항

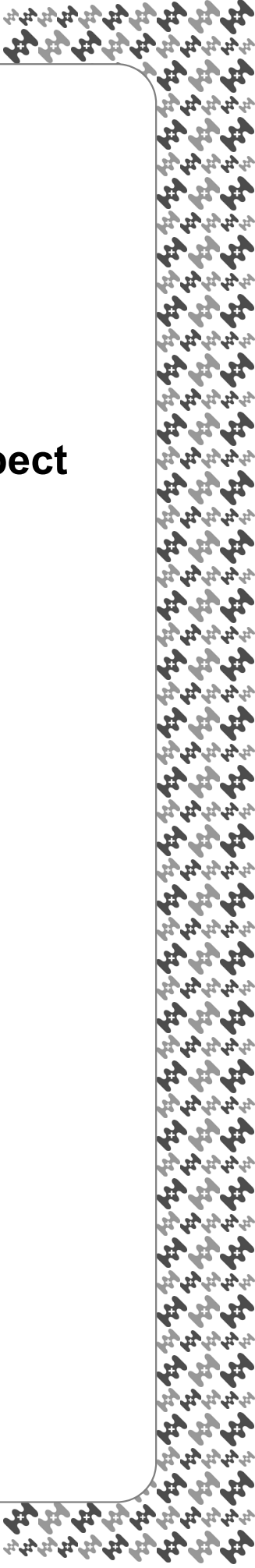
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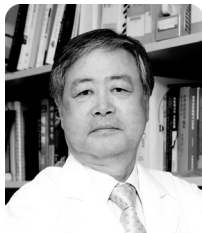
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Symposium 2

Mitochondrial Medicine: Clinical Aspect



On the Pathogenesis of Metabolic Syndrome and the Need of Mitochondrial Medicine

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It is becoming clear that the epidemic of metabolic syndrome (MS), (diabetes, obesity and many non-communicable diseases including some cancers) is caused by increased use of industrial chemicals and their slow (bio-)accumulation in human body over years. These persistent organic pollutants (POPs), including dioxins and PCBs, were shown to cause mitochondrial dysfunction and insulin resistance, forerunner of MS. One might argue that these POPs is the cause of MS, as in case of influenza is caused by influenza virus or malaria by a parasite (plasmodium). In other words, MS is a manifestation of chronic POPs toxicity.

I have been arguing that MS is a state of mitochondrial dysfunction or MDS. Once MS is defined as such, one can see that why fetal malnutrition predispose to MS, why some mitochondrial genome are susceptible to MS, why many people with MS should become obese and many others. Definition of a disease is better based on the etiology than on the mechanisms. However this principle is difficult to apply to MS as POPs toxicity. There are huge diversity in POPs, and establishing cause-effect relationship between individual POP exposure and its clinical manifestation will be impossible. One would also ask if there is anything common among POPs for their toxicity.

I will discuss why MDS is better concept than MS or chronic POPs toxicity from these considerations.



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The Essential Roles of mtCRIF1 in Mitochondrial Function and Adipogenesis

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We isolated a mitochondrial protein, called mtCRIF1, which is localized in mitochondria. In the subsequent studies, we found that mtCRIF1-deficient cells showed marked deterioration of mitochondrial function resulted from loss of assembly in oxidative phosphorylation (OXPHOS) I, III and IV complexes. In addition, we show that human or mouse cells lacking exhibit markedly destabilized supercomplexes. CRIF1-deficient cells shows high lactate production and enhanced dependency on glycolytic ATP generation, due to severe reduction of respiratory chain complex activity. Although CRIF1 itself seems not a part of oxphos complex, CRIF1-deficient cells exhibit a reduced contents of complex I, III, IV and of its components, suggesting that CRIF1 plays key roles in the mitochondrial biogenesis and/or the maintenance of mitochondrial oxphos supercomplex. Reduced CRIF1 expression in white adipose tissue, liver and muscle are observed in Ob/Ob mice.

Adipose tissue plays central role in the control of whole body energy homeostasis. The therapeutic effects of widely used thiazolidinediones on glucose intolerance rely on PPAR agonism in adipose tissues. Although both adipogenesis and therapeutic actions of thiazolidinediones appear to require the mitochondrial function, the responsible factors which link therapeutic PPAR agonism in the mitochondria of white adipocytes are not completely understood. Here, we characterized the novel mitochondria imported protein CRIF1 required for mitochondrial translation and intact OXPHOS functions in white adipocytes. mtCRIF1-null preadipocytes showed failure to differentiate into mature adipocytes by hormones and thiazolidinedione.



These observations were associated with markedly impaired mitochondrial translation stimulated by thiazolidinedione in CRIF1-null preadipocytes. The failure of differentiation of mtCRIF1-null preadipocytes was fully recovered by reexpressing mtCRIF1. The adipose tissue-specific mtCRIF1 heterozygote knockout mice showed delayed weight gain but demonstrated insulin resistances. Consistently, CRIF1 heterozygote mice demonstrated lack of responses on weight gain and insulin sensitivities by thiazolidinedione administration. These data indicate CRIF1-regulated mitochondrial translation has crucial roles in adipogenesis and improvement of insulin sensitivity on thiazolidinedione treatment.

In summary, mitochondrial CRIF1 (mtCRIF1) is essential for the maintenance of normal mitochondrial oxphos functions and its dysregulation may be possibly linked to disorders related to cellular energy metabolism.

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주요연구실적 또는 대표논문

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Plenary Lecture



Novel Therapeutic Approach of L-arginine on MELAS, A Major Subtype of Mitochondrial Myopathy

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Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) is a maternally inherited multi-system mitochondrial disorder. The primary cause for stroke-like episodes in young MELAS patients remains unknown. Based on a hypothesis that the stroke-like episodes in MELAS is caused by impaired vasodilatation in an intracerebral arterial segment, we investigated the therapeutic effects of L-arginine. Concentrations of L-arginine, citrulline, and nitric oxide metabolites (NOx) during the acute phase of MELAS were significantly lower than those in control subjects. Using FMD (flow mediated dilation) analysis, MELAS patients showed severe endothelial dysfunction. Patients were administered L-arginine intravenously at acute phase, or per orally at interictal phase. In 34 stroke-like episodes, patients were infused with either 0.5 g/kg of L-arginine or a placebo. We also studied the oral L-arginine prophylaxis in 6 interictal patients who frequently had stroke-like episodes. At 6 hours after L-arginine infusion, all symptoms suggesting stroke were significantly improved, in association with significantly increased L-arginine, citrulline, NOx, and cGMP. Oral administration significantly decreased frequency and severity of stroke-like episodes. L-arginine therapy showed promise in treating stroke-like episodes in MELAS. We are now running the investigator-mediated clinical trial of L-arginine on MELAS in Japan to get the approval of therapeutic indication for MELAS.



Biography

Dr Koga completed the doctorate in Biochemical medicine in the inherited metabolic disorders at graduate school of Kurume University in 1984. After his molecular genetic study of mitochondrial myopathy in the ultrastructural research division at National Center of Neurology and Psychiatry (NCNP), Japan, he joined the Mitochondrial Research Group in 1990 as a post doctoral research fellow granted by Muscular Dystrophy Association (USA) at the Department of Neurology, College of Physicians and Surgeons of Columbia University, where he directed his research to mitochondrial genetics especially pathogenic mechanism of MELAS. This led to the development of rho-zero cybrid system in mitochondrial research in 1992. Dr. Koga received the David Warfield fellowship Award from New York Academy of Medicine, and become assistant professor at the Department of Neurology, College of Physicians and Surgeons of Columbia University in 1994. Dr. Koga served his vice-presidency of Asian Society of Mitochondrial Research and Medicine in 2009 and is organizing the Joint Symposium of 7th Asian Society of Mitochondrial Research and Medicine, and 10th Japanese Society for Mitochondrial Research and Medicine in 2010 at Fukuoka, Japan. Dr. Koga pioneered the development of a novel therapeutic procedure for MELAS and, as a principle investigator, he is now running the investigator-mediated clinical trial of L-arginine on MELAS, which may be the first approved drug for therapeutic use of MELAS, a mitochondrial myopathy. He received the Kelsey Wright Award (the best paper award) from United Mitochondrial Disease Association (USA) in 2008, and received the best presentation award from J-mit in 2009, and from Fukuoka Medical Association in 2009.

Symposium 3

Workshop: Methods in Mitochondrial Research



Cell-based Mitochondrial Activity Profiling System (CMAPS)

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Mitochondrial function is critical to maintain energy homeostasis which is under the control of both mitochondrial (mtDNA) and nuclear (nDNA) genomes. Oxidative phosphorylation (OXPHOS) complex consists of approximately 92 proteins including mtDNA-encoded 13 subunits and nDNA-encoded ~79 components. To achieve metabolic adaptation, the replication, transcription, and translation of OXPHOS genes should be tightly regulated in cells separately from the transient ATP synthesis using available substrates. Tfam, NRF-1, and PGC-1 α are the well-known direct regulators for OXPHOS genes despite other novel genes have been being discovered. In a recent decade, quantitative decline of mitochondrial OXPHOS activity has been associated with nearly all age-related degenerative diseases such as type 2 diabetes, insulin resistance, neurodegenerative diseases, and ageing. Therefore, monitoring the function and regulation of OXPHOS complex within the cell, not isolated mitochondria, has important implications to understand many human diseases and to investigate novel therapeutics. In 2008, VK Mootha group reported a system (mitochondrial screening compendium) to probe OXPHOS function and regulations in response to small molecules using C2C12 cells in 384 well plates. Although this system is powerful to assay the cellular mitochondrial functions, the most critical parameter of oxygen consumption rate (OCR) was not included and a robot should be used for assay. In this workshop, I will describe the cell-based mitochondrial activity profiling system (CMAPS) using 96 well plates with C2C12 myocytes or SH-SY5Y neuroblastoma cells. CMAPS consists of MTT, calcein viability, TMRE, intracellular ATP, and DCFDA-ROS assays as well as semiquantitative RT-PCR of ~ 30 OXPHOS genes and regulators.



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mtDNA Expression and Cellular Energy Production

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Most of the energy needed in mammalian cells comes from the process of oxidative phosphorylation, OXPHOS. In fact, more than 80% of the energy needed by the normal human adult is generated through OXPHOS. The remaining 20% is generated through glycolysis. The respiratory chain (RC) is a set of five multi-subunit enzyme complexes (complex I to complex V) that resides embedded in the inner mitochondrial membrane. It carries out the process of oxidative phosphorylation, which uses oxygen and sugars to generate water and the cells' main energy source, adenosine-tri-phosphate, ATP. The respiratory chain consists of over 100 different protein species, 13 essentials of which are encoded by mtDNA. All complexes but complex II contain mtDNA-encoded subunits and are therefore dependent on mtDNA being present and decoded for their function. All proteins necessary for the replication and transcription of mtDNA are encoded by the nucleus, transcribed and translated in the cytoplasm, and imported into the mitochondrion. Initiation of mtDNA replication requires an RNA primer produced by mtDNA transcription. Therefore, defective mtDNA transcription will also affect mtDNA replication.



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Non-destructive Measurements for Profiling Metabolic Pathways: How XF Analyzer Works

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Oxygen consumption rate (OCR) is an indicator of mitochondrial respiration, and extracellular acidification rate (ECAR) is predominately the result of glycolysis. The XF analyzer measures OCR and ECAR at intervals of approximately 2-5 minutes. As cells shift metabolic pathways, the relationship between OCR and ECAR changes. Because XF measurements are non-destructive, cells can be profiled over a period of minutes, hours, or days and also can be re-used for additional assay after the metabolic testing. Dissolved oxygen and pH levels within the microenvironment are measured every few seconds by inert optical biosensors that reside approximately 200 microns above the cell monolayer. Over a period of minutes, cells consume oxygen and secrete protons until the dissolved oxygen concentration drops approximately 10%, and extracellular acid level changes approximately 0.2 mpH units. OCR and ECAR are determined simultaneously from the rate of change of oxygen and pH levels within the microenvironment to provide insight into metabolic pathways. Various experimental applications of XF analyzer will be discussed.



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Modeling Parkinson's Disease Using *Drosophila* Genetics

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Mitochondrial dysfunction has been implicated in the etiology of Parkinson's disease (PD), one of the most common neurodegenerative disorders worldwide. Although in most cases sporadic cases outnumber familial forms of PD, studies of such inherited forms provide significant insights into investigating patho-physiology of not only familial PD, but also sporadic PD. One powerful approach to analyzing disease mechanisms is the development of transgenic animal models, most notably in the mouse. However, generation and analysis of mouse models can be costly and time consuming, and mouse genetic models of PD genes could not successfully recapitulate human PD symptoms. In contrast, deletion or over-expression of PD associated genes in *Drosophila* markedly reproduces dopaminergic neuron degeneration and locomotor defects, the hallmark features of human PD patients. Moreover, genetic screens using *Drosophila* PD models provide valuable clues for understanding molecular mechanisms of diseases and molecular targets in the development of novel therapy.



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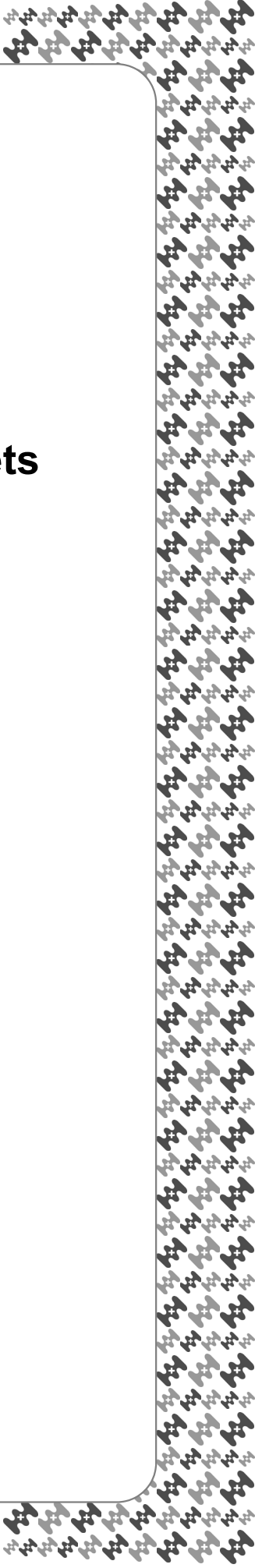
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Symposium 4

Therapeutic and Diagnostics Targets for Mitochondrial Diseases



Regulation of SIRT1 Function by Cellular Interacting Partners

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Human SIRT1 is an NAD⁺-dependent deacetylase protein that plays a role in cell death/ survival, senescence, and endocrine signaling. While its substrates, including p53, have been well characterized, its direct regulators are poorly identified. Recently, we isolated several potential regulators of SIRT1 using a yeast two-hybrid strategy. One of isolated proteins, active regulator of SIRT1 (AROS) directly regulates SIRT1 function. AROS enhanced SIRT1 -mediated deacetylation of p53 both in vitro and in vivo, and it inhibited p53-mediated transcriptional activity. Knockdown of endogenous AROS enhanced *p21WAF1* expression and increased both the G0/G1 population and apoptosis in response to DNA damage, while AROS overexpression improved cell survival. AROS is the first direct SIRT1 regulator to be identified that modulates p53-mediated growth regulation. The other associating cellular factor is a ski-interacting protein (SKIP) that reciprocally regulates RAR activity. SKIP interacts with RAR through an overlapping region that binds to SIRT1, forming a ternary complex. While SKIP augmented RAR activity by cooperating with SRC-1, SIRT1 suppressed SKIP/SRC-1-enhanced RAR transactivation. The treatment of resveratrol, a SIRT1 activator, inhibited RA-induced neuronal differentiation of P19 cells. Such inhibition was released either by SIRT1 knockdown or SKIP overexpression. These data suggest that SIRT1 and SKIP play reciprocal roles in the regulation of RAR activity. Finally, a novel protein SIP2 (SIRT1 Interacting Protein) inhibits PPAR γ -mediated transcriptional activity and suppresses rosiglitazone-enhanced adipogenesis in 3T3L-1 cells. The physiological significance of the interaction is currently being investigated. Our results provide clues to understand diverse role of SIRT1 in cancer, differentiation, and metabolism.



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NecroX as a Novel Small-Molecule Mitochondria Modulator

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There are extensive evidences that mitochondrial ROS and RNS, the major source of oxidative stresses in the cell, play a prominent role in a wide range of human disorders regarding non-apoptotic cell death. The aim of this study is to examine the cytoprotective effect of NecroX series against harmful stresses including pro-oxidant (t-BHP), doxorubicin, CCl₄, and hypoxic injury. We demonstrate that these novel chemical molecules inhibited the caspase-independent cell death with necrotic morphology, which is distinctly different from apoptosis and autophagy as well as necroptosis. In addition, these molecules displayed strong mitochondrial ROS & RNS scavenging activity. Further, oral administration of these molecules in C57BL/6 mice attenuated STZ-induced pancreatic islet beta cell destruction as well as CCl₄-induced hepatotoxicity *in vivo*. Taken together, these results demonstrate that NecroX series are involved in blockade of the non-apoptotic cell death against mitochondrial oxidative stresses. This indicates that these chemical molecules should be promising therapeutic agents in the mitochondria-related human diseases involving the necrotic tissue injury.



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C1q Tumor Necrosis Factor α -related Protein Isoform 5 (C1QTNF5) as a Novel Diagnostic and Therapeutic Target for Insulin Resistance and Diabetes

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Depletion of mitochondrial DNA (mtDNA) in myocytes causes insulin resistance and alters nuclear gene expression that may be involved in rescuing processes against cellular stress. Here, we show that the expression of C1q tumor necrosis factor α -related protein isoform 5 (C1QTNF5) is drastically increased following depletion of mtDNA in myocytes. C1QTNF5 is homologous to adiponectin in respect of domain structure, and its expression and secretion from myocytes correlated negatively with the cellular mtDNA content. Similar to adiponectin, C1QTNF5 induced the phosphorylation of AMP-activated protein kinase (AMPK), leading to increased cell surface recruitment of GLUT4 and increased glucose uptake. Treatment of cells with purified recombinant C1QTNF5 increased the phosphorylation of acetyl-CoA carboxylase (ACC), and stimulated fatty acid oxidation. C1QTNF5-mediated phosphorylation of AMPK or ACC was unaffected by depletion of adiponectin receptors such as AdipoR1 or AdipoR2, which indicated that adiponectin receptors do not participate in C1QTNF5-induced activation of AMPK. Serum C1QTNF5 levels were significantly higher in obese/diabetic animals (OLETF rats, *ob/ob* mice and *db/db* mice). These results highlight C1QTNF5 as a putative biomarker for mitochondrial dysfunction and a potent activator of the AMPK signaling pathway.



☼ 인적사항

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☼ 주요연구실적 또는 대표논문

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POSTER

eNOS Plays a Major Role in Adiponectin Synthesis in Adipocytes

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Background: Nitric oxide (NO) stimulates mitochondrial biogenesis. We recently reported that adiponectin synthesis is regulated by mitochondrial function in adipocytes. This study was undertaken to test the hypothesis that endothelial NO synthase (eNOS) plays an important role in adiponectin synthesis by producing NO and enhancing mitochondrial function in adipocytes.

Methods: We examined the effects of exogenous NO supply and eNOS knockdown on adiponectin synthesis in 3T3L1 adipocytes. We also examined plasma adiponectin levels and the mitochondria in adipose tissue of eNOS knockout (eNOS^{-/-}) mice with and without chronic administration of a NO donor.

Results: In cultured 3T3L1 adipocytes, the NO donor 3-morpholinosydnonimine (SIN) increased adiponectin secretion, and eNOS siRNA decreased rosiglitazone-induced adiponectin secretion, which was associated with increases and decreases, respectively, in mitochondrial proteins and biogenesis factors. Plasma adiponectin concentrations were reduced in 30-week-old eNOS^{-/-} mice, compared to age-matched wild-type mice. Mitochondrial contents in adipose tissue were reduced in eNOS^{-/-} mice, and this was associated with decreased expression of mitochondrial biogenesis factors, increased levels of 8-hydroxyguanosine, a biomarker of oxidative stress, and morphological abnormalities in mitochondria. Rosiglitazone-induced increases in adiponectin expression and mitochondrial content were also significantly reduced in eNOS^{-/-} mice. Chronic administration of SIN reversed mitochondrial abnormalities and increased adiponectin expression in adipose tissue of eNOS^{-/-} mice.

Conclusion: eNOS plays an important role in adiponectin synthesis in adipocytes by increasing mitochondrial biogenesis and enhancing mitochondrial function.



Role of 11 β -hydroxysteroid Dehydrogenase Type 1 on the Mitochondrial Function and Adiponectin Synthesis in Aging-related Adipocyte Hypertrophy

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Background: 11-beta-hydroxysteroid dehydrogenase type 1 (11 β -HSD1) is an enzyme that converts inactive cortisone into the active glucocorticoid cortisol. Recent studies have shown that 11 β -HSD1 in adipose tissue plays a pivotal role in the development of obesity and metabolic syndrome. Activation of 11 β -HSD1 leads to metabolic deterioration including inhibition of mitochondrial respiration. TZD decreases 11 β -HSD1 in adipocytes, and plasma adiponectin concentration is increased in 11 β -HSD1 knock-out mice. From these results, we hypothesized that rosiglitazone increases adiponectin synthesis by modulating 11 β -HSD1 in adipocytes.

Methods: We examined the effects of rosiglitazone and dexamethasone on ageing-associated adiponectin synthesis in 3T3L1 adipocytes. We also examined adiponectin synthesis and the mitochondrial biogenesis in adipocyte with and without 11 β -HSD1 selective inhibitor.

Results: In cultured adipocytes, 11 β -HSD1 mRNA expression increased with increasing duration of culture. Rosiglitazone attenuated ageing-dependent increase in 11 β -HSD1 and glucocorticoid receptor expression concomitantly with the increase in adiponectin level. Treatment with carbenoxolone, selective inhibitors of 11 β -HSD1, increased adiponectin synthesis as well as NRF-1 protein expression and mitochondrial DNA contents. Conversely, prolonged culture of differentiated adipocytes with dexamethasone treatment decreased adiponectin synthesis and NRF-1 protein levels and mitochondrial DNA contents.

Conclusion: These results suggest that endogenous activity of 11 β -HSD1 is critical in determining the fate of adipocyte, i.e., differentiation vs. hypertrophy. Increased 11 β -HSD1 activity in adipocytes associated with ageing impairs mitochondrial biogenesis and consequent decrease in mitochondria in adipocytes make the cells metabolically quiescent, large adipocytes that make less adiponectin.

Alpha-lipoic Acid Attenuates Vascular Calcification via Reversal of Mitochondrial Dysfunction and Restoration of Gas6-Axl Survival Pathway

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Objective: Vascular calcification (VC), an independent risk factor for cardiovascular diseases (CVD) is prevalent in patients with atherosclerosis, diabetes and chronic kidney disease (CKD). Elevated levels of inorganic phosphate (Pi), commonly observed in CKD patients on dialysis stimulate calcification by inducing apoptosis of vascular smooth muscle cells (VSMCs). The purposes of this study were to explore 1) the potential role of mitochondrial dysfunction in Pi-induced VSMC calcification and 2) the anti-calcific effect of alpha-lipoic acid (ALA), a naturally occurring antioxidant that improves mitochondrial function.

Methods: We evaluated the alterations in mitochondrial function and structure during Pi-induced VSMC calcification. We also examined the effect of ALA on Pi-induced mitochondrial defects, apoptosis and calcification, and on aortic calcification induced by vitamin D3 in mice.

Results: Treatment of VSMCs with Pi caused mitochondrial dysfunction, as evidenced by decreased mitochondrial membrane potential and ATP production, and increased ROS production. Structural integrity of mitochondria was also disrupted in Pi-treated VSMCs. These Pi-induced mitochondrial defects were accompanied by mitochondria-dependent apoptosis and calcification. ALA rescued this Pi-induced mitochondrial dysfunction and reduced ROS levels, leading to the inhibition of VSMC apoptosis and calcification. Moreover, ALA abolished Pi-induced downregulation of survival genes such as growth arrest-specific gene 6 (Gas6) and its receptor Axl, and activated their downstream AKT phosphorylation. Finally, ALA significantly ameliorated vitamin D3-induced aortic calcification and mitochondrial damage of aortic SMCs in mice.

Conclusion: Our findings suggest ALA attenuates VC by inhibiting VSMC apoptosis through two distinct mechanisms; preservation of mitochondrial function and restoration of the Gas6/Axl/Akt survival pathway.



Glutathione Peroxidase 1 Protects Mitochondria Against Hypoxia-reoxygenation Damage in Mouse Hearts

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Glutathione peroxidase 1 (GPx1) plays an important role in preventing cardiac dysfunction following ischemia-reperfusion injury. However, its role in protecting cardiac mitochondria against reoxygenation-induced reactive oxygen species (ROS) generation *in vivo* is unclear. We examined the role of GPx1 in protecting cardiac mitochondria against hypoxia-reoxygenation (HR) damage by testing for alterations in cardiac mitochondrial function. We used a two-dimensional gel electrophoresis proteomics analysis to examine the effects of reoxygenation on cardiac protein in wild-type (GPx1^{+/+}) and GPx1 knockout (GPx1^{-/-}) mouse hearts. We identified 42 protein spots showing differential expression in the two groups. Sixteen of the proteins identified were located in mitochondria and were involved in a number of key metabolic pathways. To verify our proteomics findings functionally, we performed NADH autofluorescence measurements and ATP production assays. The reduced expression of oxidative phosphorylation proteins in GPx1^{-/-} mice following HR treatment resulted in loss of the mitochondrial membrane potential and decreased mitochondrial respiration. Mitochondrial ROS production and oxidative mtDNA damage were increased markedly during reoxygenation in GPx1^{-/-} hearts. We also found morphological abnormalities in cardiac mitochondria and myocytes in HR-treated GPx1^{-/-}. This is the first report of the role of GPx1 in protecting cardiac mitochondria against reoxygenation damage *in vivo*. These findings will help clarify the mechanisms of HR injury and will aid in the development of antioxidant therapies to prevent cardiac mitochondrial dysfunction associated with reoxygenation.

Key words: mitochondria, Glutathione peroxidase 1 (GPx1), hypoxia-reoxygenation, reactive oxygen species

Regional Differences in Mitochondrial Anti-oxidant State During Ischemic Preconditioning in Rat Heart

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Ischemic preconditioning (IPC) is known to protect the heart against ischemia/ reperfusion (IR)-induced injuries, and regional differences in the mitochondrial antioxidant state during IR and IPC may promote the death or survival of viable and infarcted cardiac tissues under oxidative stress. To date, however, the interplay between the mitochondrial antioxidant enzyme system and the level of reactive oxygen species (ROS) in the body has not yet been resolved. In the present study, we examined the effects of IR- and IPC-induced oxidative stresses on mitochondrial function in viable and infarcted cardiac tissues. Our results showed that the mitochondria from viable areas in the IR-induced group were swollen and fused, whereas those in the infarcted area were heavily damaged. IPC protected the mitochondria, thus reducing cardiac injury. We also found that the activity of the mitochondrial antioxidant enzyme system, which includes manganese superoxide dismutase (Mn-SOD), was enhanced in the viable areas compared to the infarcted areas in proportion with decreasing levels of ROS and mitochondrial DNA (mtDNA) damage. These changes were also present between the IPC and IR groups. Regional differences in Mn-SOD expression were shown to be related to a reduction in mtDNA damage as well as to the release of mitochondrial cytochrome c (Cyt C). To the best of our knowledge, this might be the first study to explore the regional mitochondrial changes during IPC. The present findings are expected to help elucidate the molecular mechanism involved in IPC and helpful in the development of new clinical strategies against ischemic heart disease.

Key words: DNA damage, ischemic preconditioning, mitochondria, oxidative stress



MPP⁺-mediated Mitochondrial Dysfunction Suppresses Akt Phosphorylation in Parkinson's Disease Cellular and Animal Models

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Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder that typically manifests with impairments of motor function and is characterized by the loss of dopaminergic neurons (DN) of the midbrain. The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its toxic metabolite 1-methyl-4-phenylpyridinium ion (MPP⁺) are neurotoxins which are used widely to induce DN cell death in *animal* and *cellular* models of PD, respectively. MPP⁺ has been known as an inhibitor of complex I in mitochondrial respiratory chain, but the precise molecular mechanism of mitochondrial damage remains unclear. Insulin signaling pathway may also be implicated in PD because 50-80% of PD patients exhibited metabolic syndrome and insulin resistance. Previously, we have reported that mitochondrial dysfunction blocked Akt phosphorylation in insulin signaling in several cell types. In this study, we demonstrated that MPP⁺ indeed suppressed mitochondrial activity and NRF-1 and/or Tfam-mediated mitochondrial gene expressions using SH-SY5Y cells. Interestingly, MPP⁺ fragmented mitochondria and suppressed Akt phosphorylations at both Ser473 and Thr308 sites. In MPTP-injected mouse PD models, western blot revealed that ND9 (complex 1), NRF-1, and Tfam as well as tyrosine hydroxylase (TH) were reduced in substantia nigra (SN) and striatum (ST). Akt phosphorylations were not observed in MPTP-treated SN and ST. It should be noted that COXIV (complex 4) expressions were not altered in SN and ST differently from MPP⁺-treated cell models. Overexpression of Tfam or NRF-1 by transient transfection reversed all MPP⁺-mediated changes including neuronal cell death, mitochondrial membrane potential, intracellular ATP, ROS generation and Akt phosphorylations. This study provides the potential roles of defective Akt and mitochondria in PD neurodegeneration and the possibility that activation of Akt and/or mitochondria may be the critical points of therapeutic intervention for PD.

ER Stress Impairs the Insulin Signaling Pathway Through Mitochondrial Damage in Human Neuroblastoma Cells

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Both ER-stress and mitochondrial stress are considered as causal factors to induce several neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease and Huntington's disease although the relationship between two stresses is still not understood. To investigate the molecular mechanism underlying the crosstalk between ER and mitochondria in neuronal cell death, we used thapsigargin (Thaps, an inhibitor of Ca^{2+} -ATPase) and tunicamycin (Tuni, an inhibitor of N-linked protein glycosylation) as ER stressors and atrazine (ATZ, a triazine-based herbicide) as a mitochondrial stressor. All three stressors induced the mitochondrial damages at the dose to induce apoptosis in SH-SY5Y neuroblastoma cells: decreases of intracellular ATP, mitochondrial membrane potential and endogenous cellular respirations and increase of oxidative stresses. Oligonucleotide microarray analysis using Illumina Sentrix human-8 bead chip (24K) and following validation assays revealed that ER-stresses, Thap and Tuni, induced mitochondrial dysfunction and down-regulated the expressions of most mitochondria-related genes. In contrast, the mitochondrial stress, ATZ, did not change ER stress marker gene expressions and the pattern of gene expressions by 3D Principal Component Analysis was separated from those of ER stresses. All three stresses impaired Akt phosphorylation in insulin signaling pathway. Our study suggests that ER stress impairs the insulin signaling pathway through mitochondrial damage in SH-SY5Y human neuroblastoma cells.



Depletion of Mitochondrial DNA Induces IL-6 Expression Through the Activation of NF- κ B and Causes Insulin Resistance

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Reduction of oxidative enzyme activities impairs regulation of glucose and lipid metabolism in skeletal muscle and is closely associated with the development of insulin resistance in metabolic diseases. To elucidate the association of cellular mitochondrial DNA (mtDNA) content and insulin resistance, we produced L6 GLUT4myc myocytes depleted of mtDNA by long-term treatment with ethidium bromide (EtBr). The mtDNA-depleted myocytes showed significant decreases in basal and insulin-stimulated glucose uptake, indicating that L6 GLUT4myc cells developed insulin resistance and impaired glucose utilization. The results from DNA microarray, qRT-PCR and ELISA revealed that the depletion of mtDNA drastically increased expression and secretion of interleukin-6 (IL-6) as compared to control, whereas the expression and secretion of IL-6 in the reverted cells returned to near control. Upon mtDNA depletion, the intracellular steady-state Ca^{2+} level and expression of calcineurin (Cn) were significantly increased. In addition, mtDNA depletion reduced inhibitor of nuclear factor- κ B, concomitant with the nuclear translocation of nuclear factor- κ B (NF- κ B) that leads to the induction of IL-6 expression. Treatment of IL-6 to myocytes provoked reductions in the expression and insulin-stimulated phosphorylation of IRS-1. In the depleted cells, the expression of IRS-1 was also decreased by 85% and the insulin-stimulated phosphorylation of IRS-1 and Akt were hardly observed, indicating that increased IL-6 expression resulted in insulin resistance by IRS-1 reduction. Taken together, our data suggest that insulin signaling pathway intermediates are modulated by the alteration of cellular mtDNA content, and that IL-6 production induced by NF- κ B activation is associated with the development of insulin resistance in mtDNA- depleted myocytes.

Keywords: mtDNA, insulin resistance, IL-6, NF- κ B

Mitochondrial CRIF1 Determines Adipogenesis and Whole Body Energy Metabolism

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Adipose tissue plays central role in the control of whole body energy homeostasis. Although adipogenesis and actions of thiazolidinediones appear to require not only PPAR γ but also mitochondrial function, the responsible factors which link therapeutic PPAR γ agonism in the mitochondria of white adipocytes are not completely understood. So we investigate the role of mitochondria in adipogenesis and therapeutic actions of thiazolidinediones with CRIF1, a protein for maintaining intact OXPHOS functions.

CRIF1 expression is upregulated during early phase of adipocyte differentiation process and treatment of rosiglitazone. Knock down of CRIF1 with siRNA in 3T3-L1 cells showed inhibition of differentiation by hormone and rosiglitazone. And CRIF1-deficient preadipocytes prepared from CRIF1^{loxP/loxP} mice by infecting adenovirus Cre recombinase, differentiated poorly into mature adipocytes. These observations were associated with markedly impaired mitochondrial OXPHOS complex.

To analyze the function of CRIF1 in vivo, we produced the adipose tissue-specific CRIF1 knockout mice by breeding with mice expressing cre-recombinase from the FABP4-promoter. These animals had a dramatically shortened life span with a maximal longevity of 3 weeks. Histological examinations of adipose tissue-specific CRIF1 knockout mice showed abnormal structural, smaller adipocyte. At this stage, white and brown adipose tissues had abnormal mitochondria structure. The adipose tissue-specific CRIF1 heterozygote knockout mice were resistance to diet induced obesity but demonstrated insulin resistances.

These data indicate that differentiation of white adipocytes requires CRIF1 which maintains integrity of mitochondrial OXPHOS complex and CRIF1-regulated mitochondrial OXPHOS in adipocytes has crucial roles in improvement of insulin sensitivity.



Beta Cell-specific Deletion of Mitochondrial CRIF1 Causes Marked β -cell Dysfunction and Impaired Glucose Homeostasis

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Aims: Beta cell dysfunctions characterized with decreased cell mass and compromised insulin secretory capacity are hallmark feature of diabetes. Although several studies have been suggested that mitochondrial dysfunction is accompanied with impaired glucose-stimulated insulin secretion, the in vivo effects of Oxphos defect in beta cells are limited. To investigate the beta cell specific roles of mitochondrial Oxphos dysfunction, we have developed and analyzed the mice which have mitochondrial dysfunction in beta cells by deleting mitochondrial CRIF1, an essential protein for mitochondrial translation.

Methods: We created mice lacking CRIF1 using Cre/loxP recombination in adult pancreatic beta cells. The floxed *crif1* mice were breed with transgenic *rip1* cre-recombinase mice, to generate CRIF1-deficient mice in pancreatic beta cells. The beta cell-specific CRIF1-deficient animals were investigated for mitochondrial dysfunction and glucose homeostasis.

Results: Homozygous knockout of CRIF1 in beta cells resulted hyperglycemia in 10-week-old animals. The fasting (over 16 h) plasma glucose levels were significantly elevated in beta cells CRIF1 knockout mice (homozygote knockout: 220 mg/dL, n = 3, heterozygote : 81 mg/dL, n = 3, wild type : 89 mg/dL, n = 3). Intraperitoneal glucose tolerance test revealed that beta cell CRIF1 knockout mice showed delayed glucose clearance. In addition, the postprandial (2h) plasma insulin levels were decreased in beta cell CRIF1 knockout mice (*crif1* knock-out : 2.5 ng/mL, n = 3, *crif1* heterozygote : 5 ng/mL, n = 3, wild type : 7 ng/mL, n = 3, using insulin ELISA kit). Beta cell CRIF1 knockout mice showed strikingly decreased beta cell mass in islets. The islet area of beta cell CRIF1 knockout mice displayed marked reductions compared to heterozygote or wild type mice (*crif1*



knock-out : $1,300 \mu\text{m}^2$, crif1 heterozygote : $12,713 \mu\text{m}^2$, wild type : $13,443 \mu\text{m}^2$). Interestingly, heterozygous CRIF1 knockout mice developed apparent glucose intolerance fed on high-fat diet (42.5% fat involved) for 16 weeks (start at 6-week-old female mice).

Conclusion: We have shown that beta cell-specific CRIF1 deficiency exhibited impaired beta cell functions and glucose intolerance. In addition, haploinsufficiency of CRIF1 resulted diabetogenic beta cell dysfunctions challenged on high fat diet. In conclusion, we have developed the mice model of beta cell specific mitochondrial dysfunction which results in glucose intolerance and beta cell dysfunction.



Contribution of Decreased Mitochondrial 8-oxoguanine DNA Glycosylase Expression to Delayed Cell Growth of Invasive Hepatoma Cell

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Mitochondrial respiratory function, which is essential for normal cell growth and function, strictly depends on mitochondrial genomic integrity. Mitochondrial DNA (mtDNA) is highly vulnerable to oxidative damage due to its proximity to ROS generation site and its naked structure, implying that appropriate DNA repair system is required for maintenance of the respiratory function. However, the existence of mtDNA repair system in mitochondria has not been clearly understood. Base excision repair (BER) is the best characterized mechanism in mitochondria. In this study, we focused on elucidating the relationship between mitochondrial respiration-associated cancer cell growth and expression of mitochondrial 8-oxoguanine DNA glycosylase (mtOGG1), one of the BER enzymes localized in mitochondria. Firstly, we assessed mitochondrial function and cell growth rate of SNU hepatoma cells. Decreased O₂ consumption rates accompanied by low expression levels of respiratory complexes were largely associated with increased ROS production and delayed cell growth rate of the invasive SNU cells. Surprisingly, when we monitored expression level of mtOGG1, it was clearly down-expressed in the SNU cells with mitochondrial defects. Next, we performed mtOGG1 knock-down via treatment of siRNA in Chang cells, normal immortalized human hepatocytes. As a result of mtOGG1 knock-down, cell growth rate and O₂ consumption rate were decreased, whereas ROS production was increased. Finally, over-expression of mtOGG1 in SNU423 (a SNU HCC cell with low level of mtOGG1) reversed the mitochondrial function-associated cellular growth. Taken together, our results suggest that mtDNA damage induced by down-regulated mtOGG1 contributes to the damaged mitochondrial function and delayed invasive cell growth.

Involvement of Mitochondrial Functional Loss in Oncogenic K-Ras-induced Transformation

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Although mitochondrial dysfunction has often been implicated in carcinogenesis, an important question regarding ATP generation is whether it is a causative process rather than an epiphenomenon by hypoxic environment in cancer development. When oncogenic K-RasV12 is overexpressed by viral vector in Rat2 cells, mitochondrial respiratory proteins and its activity significantly declined along with acquisition of typical tumorigenic activities, whereas the cellular ATP level was compensated via activated glycolysis. Interestingly, the mitochondrial functional defects were mediated by progressive activation of the autophagic process via beclin 1 induction. Blocking the K-Ras-induced autophagy using inhibitors (bafilomycin A or 3-methyladenine) or si-beclin 1 led to not only recover mitochondrial respiratory function, but also attenuate cell-transforming activity. Finally, we demonstrate that the class I PI3K/JNK pathway is involved in beclin 1 induction, thereby triggering oncogenic K-Ras-induced autophagy, mitochondrial impairment, and cell transformation. We propose that oncogenic K-Ras-induced autophagy provokes into mitochondrial functional loss during tumorigenesis even in the absence of hypoxia, and that class I PI3K/JNK/beclin 1 is a major signaling pathway for this process in Rat2 cells.



Exploring Skeletal Muscle Transcriptome for Signatures of Insulin Resistance

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Attempts have been made to find the characteristic transcription profile of insulin resistance in skeletal muscle of people with insulin resistance such as diabetes or impaired glucose tolerance. To find the genetic signature of insulin resistance, we performed microarray experiment with skeletal muscle tissues obtained from three groups of individuals undergoing hip joint surgery: diabetes patients, old and young people without diabetes, because diabetes and advanced age are both associated with insulin resistance. It has been reported that mitochondrial oxidative phosphorylation related genes are down-regulated in skeletal muscle of diabetes patients and elderly people. We found that genes coding for proteins localizing to mitochondria is more extensively down-regulated in skeletal muscle of diabetes patients than in aged individuals without diabetes. To find consistent transcriptional changes reflecting insulin resistance in pathologic state, meta-analysis of 4 additional microarray datasets from skeletal muscle of diabetes patients with different racial and anthropometric characteristics was carried out. Genes coding for mitochondrial proteins, apoptosis related genes, DNA damage pathway genes, and subunits of protein complexes of transcription and RNA processing machinery are differentially down-regulated in skeletal muscle of diabetes patients. Hub regulators were c-Myc, GABP α , and ELK1, the nuclear convergence point of MAPK signaling, reflecting decreased MAPK activity in the nucleus and necessitating further experiments to reveal the underlying mechanism.

The Investigation of the Role of Intracellular B7-H4 in Mitochondrial Energy Metabolism of Cancer Cell

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B7-H4 is a recently described B7 family coregulatory ligand that has been implicated as an inhibitor of T cell-mediated immunity. Although expression of B7-H4 is typically limited to lymphoid cells, aberrant B7-H4 expression has also been reported in several human malignancies. As yet, the role of intracellular B7-H4 in mitochondrial energy metabolism in cancer cell is poorly understood. To better understand the role of intracellular B7-H4 in mitochondrial function in cancer metabolism, we investigated proteomic alteration of B7-H4 Knock down (KD) Hela cell using non-labeled quantitative 1D LC-MS/MS analysis method. As a result, we identified total 121 differentially expressed proteins ($P < 0.05$ vs. non treated Hela cell) including 80 up-regulated and 41 down-regulated proteins in B7-H4 KD. Among whole proteome, 18 (14% of total) mitochondrial proteins were differently expressed in B7-H4 KD. Furthermore, systemic analysis of proteomic results revealed that B7-H4 KD extensively modified the mitochondrial energy metabolism process, including TCA cycle and enoyl-CoA hydratase activity. Based on these findings, we conclude that B7-H4 is not only act as anti-immunity molecule, but also has essential role for regulation of mitochondrial energy metabolism in cancer.



Kv Channel Expression by TGF-beta1-induced Differentiation of Mesenchymal Stem Cells to Vascular Smooth Muscle Cells

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TGFbeta-1 induces differentiation of human adipose tissue-derived mesenchymal stem cells (hADSCs) to vascular smooth muscle-like cells. In order to explore whether TGFbeta-1 induces differentiation of hADSCs to functional vascular smooth muscle cells (SMCs), we determined ion channel (Kv) properties and subtypes of the SMCs differentiated from hADSCs (hADSC-SMC) by using RT-PCR, western blot, and whole cell patch clamp. The hADSC-SMC exhibited increased expression of voltage-dependent K⁺ (Kv) channel subtypes such as Kv1.1, 1.2, 1.6, 3.2, 3.4, 6.3, and 9.2 in TGFbeta-1 induced differentiation of hADSCs. Western blot analysis also revealed that specific subtypes of Kv (Kv1.1, 1.6, 3.2) were increased the expression level. Kv currents of native vascular smooth muscle rise rapidly activated and then slowly and partially inactivated during repolarization above +10 mV. Consistent with these facts, TGFbeta-1-induced differentiation of hADSCs had strong inactivation process above 0 mV, but not observed in undifferentiated hADSCs. These results suggest that TGFbeta-1-induced differentiation of hADSCs have very similar Kv channel properties of native vascular smooth muscle cells.

Keywords: voltage-dependent K⁺ channel, stem cell, vascular smooth muscle

Proteomic Alterations of Fibroblast-like Synoviocytes During Lipopolysaccharides Induced Inflammation Process

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Rheumatoid arthritis (RA) is a chronic autoimmune joint disease that affects approximately 1% of the population. The disease characteristically involves the small joints of the hands and feet, although the larger joint inflammation is also frequent. In addition to disability and decreased quality of life, RA decreases life expectancy, most commonly from accelerated atherosclerosis. Fibroblast-like synoviocytes (FLS) in RA play a key role by producing cytokines that perpetuate inflammation and proteases that contribute to cartilage destruction. RA-FLS is actively involved in the matrix degradation through the production of matrix metalloproteinases (MMP) and cathepsin. Recent advances in understanding the biology of FLS, including their regulation of innate immune responses and activation of intracellular signaling mechanisms that control their behavior, provide novel insights into disease mechanisms. We investigated the proteomic alterations which occurred to response of Lipopolysaccharides (LPS) induced inflammation process, time-dependently. After treatment of LPS to FLS at 12, 24, 36 hours, FLS were harvested and total proteome were analyzed by using 2-DE and CAF (Chemical Association Fragment) -MALDI sequencing. As a result, we identified significantly altered 15 proteins. Among the result, interestingly, the expression of vimentin and Annexin I was time-dependently decreased and the expression of PDIA6 and HSP60 was decreased after 12 hour of LPS treatment.



POSTER

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Mitochondrial Function of Corneal Dystrophy

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Granular corneal dystrophy type II (GCD II) is an autosomal dominant disorder characterized by age-dependent progressive accumulation of transforming growth factor- β -induced protein (TGFB1p) deposits in the corneal stroma. Several studies have suggested that corneal fibroblasts may decline with age. Aging is frequently characterized by the accumulation of altered proteins and dysfunctional mitochondria. Mitochondria have a central role in aging-related diseases. We examined mitochondrial activity through mitotracker, cytochrome c and mitochondrial activity assay in primary corneal fibroblast. Increased mitochondrial activity was observed in homozygote cell. Mitochondrial fusion or fission and dilated mitochondria increased in homozygote cell. Comparing early or late passage cultured corneal fibroblasts, decreased cellular viability and proliferation was observed in old passage of homozygote cell. To reveal the effect of mitochondrial function during aging, specific activity of different mitochondrial enzymes were measured and significant differences in the oxidative phosphorylation (OXPHOS) enzyme cytochrome c oxidase (COX) in mitochondria were observed between early and late passage primary keratocyte cells. The expression of complex I, II and IV decreased in old mutated cells. Also mitochondrial membrane potential and Mitotracker expression changed in old passage of homozygote cell. The early aging and decreased mitochondrial activity may be one of mechanisms which can explain the pathogenesis the disease.

Mitochondrial Genome Analysis of the Cybrids Carrying Mitochondrial DNA A3243G Mutation

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Mitochondria are the intracellular organelles responsible for ATP production by oxidative phosphorylation (OXPHOS). Mitochondria are also linked to fatty acid oxidation, ROS signaling and apoptotic pathways. Mitochondrial DNA (mtDNA) 3243 A > G mutation is the most well-known and the most frequent mutation causing diabetes mellitus. It is associated with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episode (MELAS) and also associated with maternally inherited diabetes and deafness (MIDD). In the present study, we established cybrid cells carrying mtDNA 3243 A > G mutation and investigated differentially expressed genes (DEGs) in carrying this mutation. Using a microarray analysis, we identified that 2249 genes were differentially expressed in cybrid cells and mitochondrial OXPHOS genes were significantly reduced in cybrid cells with mtDNA 3243 A > G mutation. In order to identify a major regulator, we reconstructed the major set of DEGs into networks. Using a gene network analysis, we analyzed protein-protein interaction, protein-DNA binding and subcellular localization. Finally, we found that decreased expression of retinoid X receptor α (RXR α) might be an important player connecting mtDNA 3243 mutation and mitochondrial dysfunction.



Phosphate, a Regulator of Mitochondrial Function in Insulin-releasing Cells

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Cytosolic ion concentration may critically affect the mitochondrial bioenergetics. However, the functional roles of extramitochondrial ions have not been clearly understood. We investigated the effects of alterations in cytosolic phosphate concentration on mitochondrial functions of permeabilized insulin-secreting (INS-1E) cells. Fluorescence microscopic imaging system or microplate reader was used to record the mitochondrial membrane potential and the matrix pH. ATP levels in incubating solutions and lysates of permeabilized cells were measured using bioluminescence assay. Treatment with staphylococcal alpha-hemolytic toxin to INS-1E cells resulted in selective permeability of plasma membrane to small molecule including ions and ATP, but did not affect the membrane of intracellular organelles. Permeabilized cells showed mitochondrial membrane hyperpolarization and matrix alkalinization in response to metabolites such as succinate or glycerol-3-phosphate. Metabolite-induced hyperpolarization was greatly dependent on extramitochondrial phosphate concentration. Addition of phosphate itself induced mitochondrial hyperpolarization and matrix acidification in permeabilized cells. Metabolite-induced ATP increases in incubating solutions, but not in lysates, were completely blocked by an inhibitor of adenine nucleotide translocase (ANT), atractyloside, or the absence of extramitochondrial ADP, implying that ATP release from mitochondria is exclusively dependent on ANT activity. Upon stimulation with metabolite, the amount of ATP export from mitochondria was markedly accelerated by the increases in phosphate concentration contrasting to the modest elevation of ATP level in lysates. We suggest that changes in cytosolic phosphate concentration may affect the electrochemical gradient and ATP synthesis as well as ATP export from mitochondria, all of which could be important for metabolism-secretion coupling.

Synergistic Bcr/Abl Degradation Effect is Triggered by MB12066 with NQO2 Inhibitors in Chronic Myelogenous Leukemia

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Mutations of Bcr-Abl protein reduce the sensitivity of imatinib treatment for chronic myelogenous leukemia (CML). The resistance against imatinib treatment to CML patient needs to develop another target drug of Bcr-Abl through a novel mechanism. In present study, we investigated the effect of MB12066, small molecule related to quinone derivatives, on k562 cells (CML cell line) and the expression level of Bcr-Abl protein down regulated by MB12066. When k562 cells were treated with MB12066, dose- and time-dependent Bcr-Abl degradation was detected, followed by decreasing of procaspase-3 and cleavage of PARP, which represent apoptotic stimuli. Quinone oxidoreductases [NAD(P)H:quinone Oxidoreductase 1 (NQO1) and NRH:quinone oxidoreductase2 (NQO2)] are the major enzymes involved in the bio-reduction of quinone-containing drugs. We found that MB12066 was a substrate of both NQO1 and NQO2 by examining recycling assay. For the functional study of NQO1 and NQO2 in Bcr-Abl degradation, we used inhibitors for each enzyme, dicumarol, quercetin and imatinib respectively. Interestingly, Bcr-Abl degradation was inhibited by dicumarol, but it was accelerated by quercetin and imatinib. Collectively, it may show the possibility that combination therapy with NQO2 inhibitor may be beneficial for treating CML patients.

Keywords: Nqo1, Nqo2, Bcr-Abl, Apoptosis



DJ-1 Null Dopaminergic Cells are Resistant to Paraquat Toxicity : Involvement of Mitochondrial Complex 1 Dysfunction

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There are two causes of Parkinson's disease (PD): environmental toxins and genetic mutations of PD associated genes. Recently, the mitochondrial dysfunction in neuronal cells is suggested a central cause of PD. Many researchers revealed that environmental toxins affect on mitochondrial function and role of PD associated genes in mitochondria. However, it is unclear how two causes are related in the pathogenesis of PD through the mitochondria. Among the PD related genes, DJ-1 acts as a sensor for oxidative stress and has been also proposed to maintain mitochondrial complex I activity. So we generated the DJ-1 null cell in premature dopaminergic neuronal cells SN4741. To further investigate the susceptibility to environmental toxins in DJ-1 null cells, we chose paraquat and rotenone which are known to inhibit complex 1 activity. Interestingly, we found that DJ-1 null cells display resistance to paraquat-induced apoptotic cell death, clearly implicating mitochondria in the death process. Paraquat showed typical morphological and biochemical features that are consistent with apoptosis, including poly (ADP-ribose) polymerase and subsequent caspase-3 cleavage. Also DJ-1 null cells generate less superoxide than wild-type cells by paraquat, when cells are stained with Dihydroethidium (DHE). These effects appear to be specific because it was not observed in H₂O₂-induced apoptosis and generation of reactive oxygen species (ROS). These resistances result from decrease of complex I activity in DJ-1 null cells.

The finding that DJ-1 null cells are resistance to paraquat-induced neurodegeneration provides an example of how a disease-related gene can modulate susceptibility to relevant environmental insults.

Keywords: DJ-1, Paraquat, Mitochondrial complex I, Parkinson's disease

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