中国医药城 China Medical City

ASIA-PACIFIC DIABETES AND OBESITY STUDY GROUP

3rd Scientific Meeting of the Asia-Pacific Diabetes and Obesity Study Group

Program & Abstracts

Saturday,August 25 – Sunday,August 26,2007
China Medical City
Taizhou,China

The 3rd Scientific Meeting of
Asia-Pacific Diabetes and Obesity
Study Group (APDO)

Program & Abstracts

August 25th-26th, 2007

Taizhou, China

General Information

Dates: Saturday, August 25-Sunday, August 26, 2007

Venue/Hotel Accommodation:

Petrel Hotel: www.hyhotel.cn
No.1 South Yangtze River Road, Taizhou City, Jiangsu Province, China
TEL: +86-523-6978588 +86-523-6978585
FAX: +86-523-6978580

Organized by:

Institute of Biophysics, Chinese Academy of Sciences
National Pharmaceutical & Medical Key Laboratory, School of Life Science, Nanjing University, China
Shanghai Institutes For Biological Sciences, Chinese Academy of Sciences
Administration Committee of Taizhou Medical Hi-Tech Development Zone, Taizhou City, Jiangsu Province, China

Sponsored by:

Administration Committee of Taizhou Medical Hi-Tech Development Zone ,Taizhou City, Jiangsu Province, China

Secretariat:

If you have any further requirements during the meeting, please contact the Secretariat by cell phone below:

Ms. Le Yu: 13852670399

Official Language:

The official language of APDO 2007 will be English.

Registration:

The registration desk will be located in the lobby of the Petrel Hotel and open from 8:00-22:00 on August 24th.

Attention:

1. The participants are advised to wear the name badge during APDO Meeting.
2. Please note that all participants are requested to present the meal ticket for the Meeting meals, Welcome Reception and Banquet.

Scientific Program

(August 25-26, 2007)

Saturday, August 25th, 2007

Time	Speaker	Title
08:30-09:00	**Opening Remarks:** Prof. David James (Australia) Prof. Jiarui Wu (China) Leader of Taizhou Municipal Government (China)	
09:15-09:30	**Photo**	
09:30-10:10	**Session 1: APDO Study Group Lecture 1** **Chair:** Masato Kasuga (Japan)	
	David James (Australia)	A major node of insulin resistance occurs downstream of IRS1
10:10-11:10	**Session 2: Oral Presentation** (10 min. Presentation, 5 min. Q &A) **Chair:** Peter Shepherd (New Zealand)	
Oral 2-1	Cong-Rong Wang (China)	Study on Chromosome 1q21-q25 in Chinese: The LD Pattern and The Association of ATF6 Gene with Type 2 Diabetes
Oral 2-2	Shun Ishibashi (Japan)	Adipocyte lipolysis and metabolic syndrome
Oral 2-3	Kun-Ho Yoon (Korea)	Searching the candidate miRNAs related with glucolipotoxicity-induced beta-cell dysfunction by miRNA chip analysis
Oral 2-4	Hui-Ru Tang (China)	Metabonomic changes reveal the underlying biochemical processes of the Streptozotocin (STZ) Induced Diabetes mellitus
11:10-11:20	**Coffee Break**	
11:20-12:35	**Session 3: Oral Presentation** (10 min. Presentation, 5 min. Q &A) **Chair:** Jia-Rui Wu (China)	
Oral 3-1	Wei-Ping Zhang (China)	Tissue-specific ablation of zinc finger protein gene DPZF in pancreatic β cells leads to the defects of glucose sensing and insulin secretion
Oral 3-2	Greg Steinberg (Australia)	AMPK independent pathways regulate contraction stimulated fatty acid metabolism
Oral 3-3	Kyong Soo Park (Korea)	Effect of sumoylation on PPARγ function
Oral 3-4	Sasha H. Anagnostou (New Zealand)	Glucose Regulation of the Wnt signaling pathway: A novel mechanism for the regulation of glucose metabolism?
Oral 3-5	Tony Tiganis (Australia)	Regulation of Insulin Signaling by THE Protein Tyrosine Phosphatase TCPTP
12:35-13:30	**Lunch**	
13:30-14:20	**Session 4: Invited Plenary Lecture** **Chair:** Chenyu Zhang (China)	
	Domenica Accili (USA)	Metabolic regulation by forkhead proteins
14:20-15:50	**Session 5: Oral Presentation** (10 min. Presentation, 5 min. Q &A) **Chair:** Ai-Min Xu (Hongkong, China)	
Oral 5-1	Greg Cooney (Australia)	Muscle fatty acid oxidative capacity and PGC-1 protein expression are increased in genetic and dietary models of insulin resistance and type 2 diabetes in rodent
Oral 5-2	Carsten Schmitz-Peiffer (Australia)	Dilinoleoyl-phosphatidic acid – a novel lipid mediator of muscle insulin resistance
Oral 5-3	Yukio Tanizawa (Japan)	Myosin motor Myo1c and its receptor NEMO/IKK-γ promote TNF-a induced insulin resistance via phosphorylation of IRS-1 at serine[307]
Oral 5-4	Hong-Yuan Yang (Australia)	Cellular dynamics of lipid droplets
Oral 5-5	Nan-Ping Wang (China)	Role of PPARδ in Hepatic Lipogenesis
Oral 5-6	Seung-Hoi Koo (Korea)	Roles of adipokines for the regulation of hepatic glucose metabolism
15:50-16:00	**Coffee Break**	
16:00-17:30	**Session 6: Oral Presentation** (10 min. Presentation, 5 min. Q &A) **Chair:** Greg Cooney (Australia)	
Oral 6-1	Roger Daly (Australia)	Identification of the adapter protein Grb10 as a physiological regulator of glucose homeostasis, body composition and IR/IGF-1R signalling
Oral 6-2	Kerry Loomes (New Zealand)	*Myo*-inositol; a snapshot of its demise by the enzyme *myo*-inositol oxygenase
Oral 6-3	Dong-Hai Wu (China)	The construction and primarily study of cpt1c knock-out mouse
Oral 6-4	Sheng-Cai Lin (China)	Axin is a master scaffold involved in metabolism, tumor suppression, and dorsoventral patterning
Oral 6-5	Wataru Ogawa (Japan)	Role of Kru ppel-like factor 15 in hepatic glucose metabolism
Oral 6-6	Peng Li (China)	Cide Proteins and the Development of Obesity

17:30-18:15	**Session 7: Poster Presentation and Refreshments**	
18:30-20:00	**Welcome Reception**	

Sunday, August 26, 2007

Time	Speaker	Title
08:00-08:30	**Morning Session:** TBA	
08:30-09:10	**Session 8: APDO Study Group Lecture 2** **Chair:** David James (Australia)	
	Chen-Yu Zhang (China)	Genipin inhibits UCP2-mediated proton leak and acutely reverses obesity- and high glucose-induced β cell dysfunction in isolated pancreatic islets
09:10-10:25	**Session 9: Oral Presentation** (10 min. Presentation, 5 min. Q &A) **Chair:** Tetsuro Izumi (Japan)	
Oral 9-1	Mitsuru Hashiramoto (Japan)	Ablation of PDK-1 in vascular endothelial cells enhances insulin sensitivity by reducing visceral fat
Oral 9-2	You-Fei Guan (China)	Tesaglitazar, a peroxisome proliferator activated receptor alpha/gamma dual agonist, attenuates glycemic control and diabetic nephropathy in db/db mice
Oral 9-3	Ai-Min Xu (Hongkong, China)	Rosiglitazone improves vascular dysfunction through inducing the production of high molecular weight oligomeric adiponectin in db/db diabetic mice
Oral 9-4	Mitsuhisa Komatsu (Japan)	Quantitative assessment of diabetic neuropathy using a newly developed device, PainVision®
Oral 9-5	Dave Grattan (New Zealand)	Hormonal induction of leptin and melanocortin resistance in the hypothalamus of pregnant rats
10:25-10:35	**Coffee Break**	
10:35-12:05	**Session 10: Oral Presentation** (10 min. Presentation, 5 min. Q &A) **Chair:** Kun-Ho Yoon (Korea)	
Oral 10-1	Yan Chen (China)	Apolipoprotein A-I stimulates AMP-activated protein kinase and improves glucose metabolism
Oral 10-2	Tetsuro Izumi (Japan)	Roles of Rab27a and its effector granuphilin in insulin exocytosis

Oral 10-3	Moon Kyu Lee (Korea)	Multiple Roles of Insulin Receptor in β--cells: Insulin Secretion, Proliferation and Cell Survival
Oral 10-4	Shao-Ping Zhang (New Zealand)	Fas-associated Death Receptor Signaling is induced in Islet β-cells Following Human Amylin Treatment
Oral 10-5	Sof Andrikopoulos (Australia)	The mechanism by which insulin hypersecretion may be detrimental to islet-cell function
Oral 10-6	Xiao Han (China)	Dexamethasone Inhibits PDX-1 in Pancreatic β -Cell Partially Through Foxo1 Transcription Factor
12:05-13:30	**Lunch & Closing Remarks**	
14:00-18:00	**Visit Taizhou Medical Hi-Tech Development Zone and make discussion &Tour Yangzhou**	

A major node of insulin resistance occurs downstream of IRS1

Kyle L. Hoehn[1], Cordula Hohnen-Behrens[1], Anna Cederberg[1], Lindsay Wu[1], Nigel Turner[1], Tomoyuki Yuasa[2], Yousuke Ebina[2], & David E. James[1]

[1]*Diabetes and Obesity Program, Garvan Institute of Medical Research, 384 Victoria St., Darlinghurst, NSW, Australia. (d.james@garvan.org.au)*

[2]*Division of Molecular Genetics, Institute for Enzyme Research, University of Tokushima, Tokushima, Japan.*

Insulin resistance is a major contributor to a number of metabolic diseases including Type 2 Diabetes and cardiovascular disease. It can be triggered by an array of physiological insults including glucocorticoids, inflammation, hyperlipidemia or hyperinsulinemia but it is unclear if these insults converge upon a common molecular endpoint. The insulin receptor substrate IRS1 is considered a major target of such insults. Degradation and Ser/Thr phosphorylation of IRS1 is often observed during insulin resistance and this is thought to uncouple transmission through the insulin signalling cascade to Akt disrupting glucose uptake and metabolism. However, since only a fraction of Akt activity is required for robust insulin-stimulated GLUT4 translocation and glucose uptake we have revisited this hypothesis. Here we describe an extensive analysis of 6 diverse models of insulin resistance in L6 myotubes, 3T3-L1 adipocytes, and murine skeletal muscle and show that a major node of insulin resistance occurs downstream of IRS1 and in most cases does not involve defective Akt signalling. Reversal of insulin resistance with the antioxidant MnTBAP implicates an important role for oxidative stress in many forms of insulin resistance. Studies with the ceramide biosynthesis inhibitor cycloserine indicate that intracellular ceramide accumulation is likely one major cause of oxidative stress leading to a reversible post-Akt defect. These findings indicate a distinct non-linearity between upstream insulin signalling elements and insulin action and point toward the existence of a more sensitive transducer downstream of IRS1/Akt that can both amplify and/or dampen the entry of nutrients such as glucose into the cell in a readily reversible manner.

STUDY ON CHROMOSOME 1q21-q25 IN CHINESE: THE LD PATTERN AND THE ASSOCIATION OF ATF6 GENE WITH TYPE 2 DIABETES

HU C, JIA W, ZHANG W, WANG C, ZHANG R, FANG Q, MA X, XIANG K

Shanghai Diabetes Institute, Department of Endocrine & Metabolism, Shanghai Jiaotong University Affiliated Sixth People's Hospital, Shanghai Clinical Center for Diabetes, China

(wcr601@hotmail.com)

Previous studies showed that chromosome 1q21-q25 was a susceptible region of type 2 diabetes in several populations, including Shanghai Chinese. As part of International Type 2 Diabetes 1q Consortium, more than 4,000 SNPs in a 21Mb region on chromosome 1q21-q25 were genotyped in 160 Chinese Hans, including 80 probands from early-onset diabetes families and 80 'super-normal' controls. The linkage disequilibrium (LD) pattern and tagging SNP (tagSNP) transferability were compared among our control samples and four HapMap samples. We found the LD pattern of HapMap Chinese Hans in Beijing (CHB) samples were highly consistent of our Shanghai samples. And the tagSNPs selected from CHB samples (1,287 out of 2,591 SNPs) performed well in our Shanghai samples; approximately 95% of the genetic variants were captured by these tagSNPs. Also similar results were obtained from Japanese samples. However, the Caucasian and African samples differed dramatically from our Shanghai samples. We then applied the evolutionary model of Malecot and composite likelihood to our data. The expected association was predicted by the Malecot model, which is a function of the distance in LDU or kb between the hypothesized disease variant and the marker and a few other parameters. Composite likelihood was applied to combine association at multiple loci, substantially reducing the burden of multiple testing encountered in single SNP tests. By fitting the Malecot model and maximizing the composite likelihood, we can detect association and estimate the location of the disease variant. By analyzing the 1q Consortium Shanghai data, we found several genes may be associated with diabetes, including ATF6. Then the variant Ala145Pro (GCG145CCG, rs2070150) of ATF6 gene was genotyped in 689 samples, in order to test the hypothesis whether it was associated with type 2 diabetes. The C allele frequencies in the probands of early-onset diabetic pedigrees, newly diagnosed diabetic patients and normal controls were 0.224, 0.278 and 0.306 respectively. The minor allele C was significantly higher in the controls, compared with the probands of early-onset diabetic pedigrees. Our results confirmed the applicability of HapMap SNP data to the study of complex disease among the Shanghai Chinese population. We detected several susceptible loci to diabetes. The Ala145Pro variant of ATF6 gene, which is one of the susceptible loci, showed nominal significance association with type 2 diabetes.

Adipocyte lipolysis and metabolic syndrome

Shun Ishibashi /Shu-ichi Nagashima, Hiroaki Okazaki, Motohiro Sekiya, Masaki Igarashi, Hiroaki Yagyu, Jun-ichi Osuga and others

Division of Endocrinology and Metabolism, Department of Medicine, Jichi Medical University, Tochigi, Japan

(ishibash@jichi.c.jp)

In obese people, triacylglycerol accumulated in adipose tissue contributes to the development of many features associated with metabolic syndrome. For example, breakdown of triacylglycerol in the adipose liberates fatty acids into the circulation, which stimulate production of lipoproteins rich in triacylglycerol. Similarly, cholesterol ester accumulated in arterial wall cells is the culprit of atherosclerosis. Thus, dysregulated breakdown of neutral lipid may underlie the development of metabolic syndrome. Genetic ablation of hormone-sensitive lipase (HSL) in mice has unraveled new roles of this classical enzyme in adipocyte differentiation, food intake, and reproduction. In murine models of obesity, the absence of HSL attenuates the development of obesity probably by antagonizing the orexigenic signaling in the hypothalamus. Unexpectedly, these mice retain a substantial neutral lipid hydrolytic activity in adipose tissue as well as in macrophages, suggesting the presence of a lipase(s) that is distinct from HSL. We have recently identified a novel triacylglycerol lipase (TGH-2) with 70% homology to triacylglycerol hydrolase that was identified in the liver as an enzyme involved in lipoprotein assembly. Since TGH-2 is robustly induced in the adipose tissue during fasting, a condition known to be associated with increased adipocyte lipolysis, it may take part in adipocyte lipolysis in concert with HSL and ATGL. The mRNA expression of these genes in human adipose tissues is also linked to obesity and/or insulin resistance. This presentation will highlight the recent advances in our understanding of the emerging lipid breakdown system and its implication to metabolic syndrome.

Searching the candidate miRNAs related with glucolipotoxicity-induced beta-cell dysfunction by miRNA chip analysis

Kun-Ho Yoon / Ji-Won Kim, Young-Hye You, Chenglin Sun

Department of Endocrinology and Metabolism, The Catholic University of Korea

(yoonk@catholic.ac.kr)

MicroRNAs (miRNAs) are small noncoding RNAs that function as endogenous post-transcriptional silencers of target genes. miRNAs are expressed in a tissue specific manner and play important roles in cell proliferation, apoptosis and differentiation. Very few miRNAs have actually been characterized and most of their functions remain unknown. It recently reported that miR375, a pancreatic islet-specific miRNA, inhibits insulin secretion in mouse pancreatic beta cell (Poy et. al.). To search functional miRNAs in the beta-cells especially related with the diabetic status such as glucolipotoxicty, glucolipotoxicity was induced by 33.3 mM glucose and 0.6 mM (palmitate and oleate) for three days in isolated rat islets. We isolated 30 g of total RNA and performed the miRNA chip array using an Ncode miRNA Chip (invitrogen) with primary rat islets.

miRNA chip array data were the most representative of three independent experiments and selected the candidate miRNAs which were showed statistically significant expression changes ($p < 0.05$). In miRNA chip experiments, 25 (including miRNA375) were significantly upregulated and 11 were downregulated in glucolipotoxicity induced islets and were identified functionally using www.targetscan.org and http://pictar.bio.nyu.edu. Among 36 selected miRNA, 16 might related with glucolipotoxicity-induced beta-cell dysfunction. Future studies should determine the key miRNA of 16 identified miRNA and will be required to explore the effects of changes of miRNA expression using a Northern blot and real-time PCR in glucolipotoxicity induced islets and INS-1 cell line.

Metabonomic changes reveal the underlying biochemical processes of the Streptozotocin (STZ) Induced Diabetes mellitus

Wenxin Xu, Huiru Tang*

Biospectroscopy and Metabonomic Group, State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematics

The Chinese Academy of Sciences, China

(Huiru.tang@wipm.ac.cn)

Diabetes mellitus is a common multifactorial metabolism disorder for which the aetiology remains largely undefined. The effects of Streptozotocin (STZ) induced metabolism dysfunction on rat model have been investigated using an NMR-based metabonomic approach. A total of 30 rats were divided into three groups: a control group and two Streptozotocin-induced groups, one as induced control and the other group treated with dimethylbiguanide which started at day 4 after the administration of STZ. The results showed that STZ induced blood plasma metabonomic changes were mainly related to dysfunction of energy metabolism, disruption of amino acid, carbohydrate, and lipid metabolism. The work presented here showed that metabonomics is a useful tool for understanding the early stage biochemistry changes in the STZ-induced diabetes mellitus models.

Tissue-specific ablation of zinc finger protein gene *DPZF* in pancreatic β cells leads to the defects of glucose sensing and insulin secretion

Weiping Zhang, Ye Zhang, Zhifang Xie

Department of Pathophysiology, Second Military Medical University. 800 Xiangyin Road

Shanghai 200433, China

(Zhangwp68@yahoo.com.cn)

β cell dysfunction is a central component of the pathogenesis of type 2 diabetes. The glucose-sensing pathway plays important roles in β cell dysfunction, but its regulatory network is still poorly defined. Here we show that novel transcription factor DPZF is highly expressed by pancreatic β cells and acts as a critical regulator for the glucose-sensing and insulin secretion of β cells. DPZF gene was specifically ablated in β cells by LoxP/Cre approach, and the β cell-specific DPZF knockout mice exhibit hyperglycemia, impaired glucose tolerance, and the loss of first-phase insulin secretion after glucose stimulation. The isolated islets from the β cell-specific DPZF knockout mice display defective glucose-stimulated insulin release in vitro. However, the β cell mass, total insulin content in β cells, or the KCl-stimulated insulin release doesn't differ between the knockouts and control mice. The expression patterns of the genes which are potentially involved in glucose-sensing of β cells are carefully examined. Taken together, these data suggest an important role for DPZF in β cell function and a therapeutic target for diabetes.

AMPK independent pathways regulate contraction stimulated fatty acid metabolism

Steinberg GR, Dzamko NL, Schertzer JD, Kemp BE

St. Vincent's Institute of Medical Research, 9 Princes St. Fitzroy, Victoria, 3065, AUSTRALIA

(gsteinberg@svi.edu.au)

Skeletal muscle predominantly uses fatty acids for fuel, both at rest and during exercise. The importance of skeletal muscle in energy homeostasis is becoming increasingly apparent as impairment of skeletal muscle lipid metabolism is associated with increased levels of plasma lipids as seen in insulin resistant and obese states. In skeletal muscle, the AMP-dependent protein kinase (AMPK) is thought to regulate fatty acid oxidation in response to increasing energy demand by direct phosphorylation and inactivation of acetyl-CoA carboxylase (ACC). In turn ACC produces less malonyl-CoA, relieving the malonyl-CoA mediated inhibition of CPT1. Numerous studies have demonstrated increased phosphorylation of ACC and fatty acid oxidation during exercise, or treatment with the pharmacological AMPK activator AICAR. In the present study we examined fatty acid metabolism in transgenic mice over-expressing kinase dead AMPKα2 (KD).

Effect of sumoylation on PPARγ function

Kyong Soo Park / Sung Soo Chung, Byung Yong Ahn, Soo Lim, Sung Hee Choi, Ho Sun Park, Young Min Cho, Hong Kyu Lee
Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea
(kspark@snu.ac.kr)

Peroxisome proliferators activated receptor gamma(PPARγ) ligands reduce insulin resistance and are currently used for the treatment of T2DM. In addition to improving insulin sensitivity, thiazolidinediones(TZD), PPARγ ligands have anti-atherogenic effect. TZDs can modulate adipokine expression which might be important in TZD's antiatherogenic effects. TZDs also showed direct effect on vessel wall. We found that in vivo transfer of the PPARγ-WT gene was found to inhibit smooth muscle proliferation, sustain apoptosis, and reduce neointima formation after balloon injury irrespective of rosiglitazone treatment while PPARγ-DN gene transfer abolish the effect of rosiglitazone on vessel wall. These results indicate that antiatherosclerotic effect of TZDs is dependent on PPARγ gene itself.

Recently it was reported that a SUMOylation dependent pathway mediates transrepression of inflammatory response genes by PPARγ. We also found that various PPARγ SUMO mutants have different effects on transcriptional activity. Among them K107R mutant showed the greatest repression of iNOS promoter activity and inhibited smooth muscle cell migration significantly. in vivo transfer of the PPARγ- K107R mutant markedly reduce neointima formation after balloon injury.

In summary, PPARγ has an important role in preventing atherosclerosis by modulating inflammatory process, direct effect on vessel wall as well as improving insulin sensitivity. Posttranslational modification of PPARγ may play an important role in modulating inflammation and insulin resistance.

Glucose Regulation of the Wnt signaling pathway: A novel mechanism for the regulation of glucose metabolism?

Sasha H. Anagnostou, Claire Chaussade, Greg C. Smith, Anassuya Ramachandran, Peter R. Shepherd.
Department of Molecular Medicine and Pathology, University of Auckland, New Zealand
(s.anagnostou@auckland.ac.nz)

High intracellular glucose levels and increased glucose metabolism are key features of diabetes and many tumours. Evidence suggests that increased intracellular glucose metabolism could play a direct role in regulating cell function, as glucose is known to modulate the levels of important regulatory proteins. A number of mechanisms have been implicated in this process, one of which is flux through the hexosamine pathway. This pathway results in the glycosylation and regulation of proteins. Here we show that glucose upregulates cellular levels of β-catenin protein in the murine macrophage cell lines J774 and RAW264.7. β-catenin is an important oncogene and deregulation of the canonical Wnt/β-catenin pathway is one of the most frequent signalling abnormalities found in human cancers. The glucose effect on β-catenin requires glutamine, so we hypothesised that increased flux through the hexosamine pathway, due to high intracellular glucose levels, is mediating this effect. Further evidence in support of this hypothesis comes from the finding that inhibiting GFAT, the rate-limiting enzyme of the hexosamine pathway, attenuates the glucose effect on β-catenin. Glucosamine enters the hexosamine pathway downstream of GFAT, thereby increasing flux through, while avoiding endogenous regulation of this pathway. Treatment of J774 cells with low levels of glucosamine significantly increases β-catenin protein levels. Thus, the findings presented here provide a novel, important link between deranged glucose metabolism and tumour development and/or progression.

Regulation of Insulin Signaling by THE Protein Tyrosine Phosphatase TCPTP

Tony Tiganis

Department of Biochemistry and Molecular Biology, Monash University, Victoria 3800, Australia

(Tony.Tiganis@med.monash.edu.au)

Type 2 diabetes mellitus has reached epidemic proportions afflicting roughly 6% of the adult population in Western society. Although the underlying genetic causes and the associated pathological symptoms are heterogenous, a common feature is high blood glucose due to peripheral insulin resistance. The molecular basis of insulin resistance is thought to be attributable to defects in insulin receptor (IR) signalling. The IR is a protein tyrosine kinase that phosphorylates itself and downstream protein substrates on tyrosine in response to insulin. Protein tyrosine phosphatases (PTPs) that dephosphorylate the IR and its substrates might be important targets for therapeutic intervention in type 2 diabetes; inhibition of specific PTPs may allow for enhanced insulin-induced signalling to alleviate insulin resistance. PTP1B is a physiological regulator of IR activation and glucose homeostasis and a validated therapeutic target for the treatment of type 2 diabetes. Previously we identified the tyrosine-specific phosphatase TCPTP as another important negative regulator of insulin signalling. We now examine TCPTP's potential to regulate glucose homeostasis *in vivo*.

Metabolic regulation by forkhead proteins

Domenico Accili

Columbia University, 1150 St. Nicholas Av., New York, NY 10032

(da230@columbia.edu)

Type 2 diabetes arises from a combination of impaired insulin action and defective pancreatic β cell function. Genetic studies of the insulin signaling pathway have led to a critical reappraisal of the integrated physiology of insulin action. These studies indicate that insulin signaling affects β cell function and regulation of β cell mass, thus raising the possibility that insulin resistance may be the overarching feature of diabetes in all target tissues. Studies in our laboratory have focused on the mechanism by which insulin affects regulation of gene expression. We have shown that the forkhead protein Foxo1 is an insulin-regulated transcription factor. Insulin-dependent phosphorylation inhibits the ability of Foxo1 to stimulate transcription of prototypic insulin-responsive genes in liver. We have shown that Foxo1 is a key effector of insulin action in several tissues: liver, where it controls insulin inhibition of glucose production, apolipoprotein turnover and lipid synthesis; pancreatic β-cells, where it controls proliferation and differentiation; pre-adipocytes, where it controls insulin-dependent adipose differentiation; myoblasts, where it controls myotube formation and fiber type specification; and brain, where it controls expression of hypothalamic neuropeptides. Thus, regulation of Foxo1 function can potentially affect insulin sensitivity *in vivo* by regulating various aspects of fuel metabolism. Inhibition of Foxo1 holds promise as a therapeutic approach to insulin resistance and diabetes.

Muscle fatty acid oxidative capacity and PGC-1 protein expression are increased in genetic and dietary models of insulin resistance and type 2 diabetes in rodents

Nigel Turner, Clinton R Bruce, Susan M Beale, Kyle L Hoehn & Gregory J Cooney

Diabetes and Obesity Research Program, Garvan Institute of Medical Research, 384 Victoria St, Darlinghurst, Sydney, NSW 2010. AUSTRALIA

(g.cooney@garvan.org.au)

A reduction in muscle mitochondrial function and fatty acid oxidative capacity have been suggested as key factors in the development of insulin resistance, obesity and type 2 diabetes, however it is unclear whether these defects are innate or acquired (i.e a result of dietary lipid excess). We have investigated mitochondrial function and fatty acid metabolism in skeletal muscle of C57Bl/6J mice fed a high fat diet for 5 or 20 wk and compared the results to those obtained from genetic rodent models of obesity and type 2 diabetes.

The results show a highly significant ($P<0.001$) increase in epididymal fat mass as a result of the high-fat diet after both 5 wk (150%) and 20 wk (350%). Glucose tolerance (2 g/kg, i.p.) was impaired after both 5 wk and 20 wk of high-fat feeding. The activity of key enzymes involved in fatty acid metabolism were assessed and in both 5 wk and 20 wk high-fat fed mice there was a significant increase (25-100%; $P<0.01$) in citrate synthase (CS), -hydroxyacyl-CoA dehydrogenase (HAD) and medium chain acyl-CoA dehydrogenase (MCAD) activities. At both time points fat–feeding also resulted in a significant ($P < 0.05$) increase in the expression of PGC-1 (100 - 250%), UCP3 (80 - 240%), and subunits of the mitochondrial respiratory chain Complex III (65 - 110%) and Complex IV (50 - 140%). Compared with chow controls, muscle from fat-fed mice displayed elevated palmitate oxidation rate (5 wk +23%, $p<0.05$ and 20 wk +29%, $p<0.05$) and increased palmitoyl-CoA oxidation in isolated mitochondria (20 wk +49%, $p<0.01$). A similar pattern was present in muscle of fat-fed rats, obese Zucker rats and db/db mice, with increases observed for oxidative enzyme activity and expression of PGC-1alpha, UCP3 and subunits of the mitochondrial respiratory chain. These findings suggest that high lipid availability does not lead to intramuscular lipid accumulation and insulin resistance in rodents by decreasing mitochondrial fatty acid oxidative capacity in muscle.

Dilinoleoyl-phosphatidic acid – a novel lipid mediator of muscle insulin resistance

Carsten Schmitz-Peiffer

Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010, Australia

(c.schmitz-peiffer@garvan.org.au)

Skeletal muscle insulin resistance is strongly associated with increased lipid availability. While the mechanisms involved are not fully understood, it is likely that specific lipid intermediates can activate signalling pathways that interfere with normal insulin action. Using cultured myotubes treated with different fatty acids (FAs), we have previously shown that the saturated FA palmitate inhibits insulin signalling at the level of protein kinase B through elevation of ceramide. In contrast, the unsaturated FA linoleate inhibits IRS-1 tyrosine phosphorylation but does not induce ceramide formation. To address the involvement of other lipid species and protein kinase C (PKC) activation, we overexpressed diacylglycerol kinase ε, which preferentially converts unsaturated diacylglycerol into phosphatidic acid (PA). This reversed the activation of PKC isoforms by linoleate, but paradoxically further diminished IRS-1 tyrosine phosphorylation. Conversely, inhibition of lysophosphatidic acid acyl transferase with lisofylline, which reduces PA synthesis, restored IRS-1 phosphorylation. PA species in linoleate-treated cells or muscle from insulin-resistant mice fed a safflower oil-based high-fat diet that was rich in linoleate were analysed by mass spectrometry (MS). MS indicated that the dilinoleoyl-PA content increased from undetectable levels to almost 20% of total PA in L6 cells and to 8% of total in the muscle of fat-fed mice. Micelles containing dilinoleoyl-PA specifically inhibited IRS-1 tyrosine phosphorylation and glycogen synthesis in L6 cells. These data indicate that linoleate-derived PA is a novel lipid species that contributes independently of protein kinase C to IRS-1 signalling defects in muscle cells in response to lipid oversupply.

Myosin motor Myo1c and its receptor NEMO/IKK-γ promote TNF-α induced insulin resistance via phosphorylation of IRS-1 at serine[307]

Yukio Tanizawa, Yoshitaka Nakamori, Masahiro Emoto, Naofumi Fukuda, Akihiko Taguchi, Shigeru Okuya

Division of Endocrinology, Metabolism, Hematological Sciences and Therapeutics
Yamaguchi University Graduate School of Medicine,
Ube, Yamaguchi, Japan
(tanizawa@yamaguchi-u.ac.jp)

The IKK (IκB kinase) complex has been focused as a critical molecule for development of insulin resistance. This complex consists of two catalytic subunits, IKK-α and IKK-β and one scaffold subunit NEMO (NF-κB essential modulator/IKK-γ). Recent reports showed that TNF (tumor necrosis factor)-α signaling through the IKK-β attenuates insulin action via phosphorylation of IRS (insulin receptor substrate)-1 at Ser^{307}. However the precise molecular mechanism by which the IKK complex phosphorylates IRS-1 is unknown. Here we identify two NEMO binding proteins, an unconventional myosin Myo1c and an actin, that appears to connect actin cytoskeleton dynamics with TNF–α signaling. Immunofluorescence microscopy analysis revealed that NEMO localized to cytoplasmic punctate structures in 3T3-L1 adipocytes. Insulin treatment of culture adipocytes causes rapid movement of cytoplasmic NEMO to membrane ruffles or vicinity of IRS-1. This intracellular trafficking of NEMO requires insulin, an intact actin cytoskeletal network, and the motor protein Myo1c. Increased Myo1c expression enhanced the NEMO-IRS-1 interaction, which is essential for TNF-α induced phosphorylation of Ser^{307}-IRS-1. In contrast, dominant inhibitory Myo1c cargo domain expression diminished this interaction and inhibited IRS-1 phosphorylation, suggesting a role for Myo1c in TNF-α induced down-regulation of IRS-1. NEMO over-expression also enhanced TNF-α induced Ser^{307}-IRS-1 phosphorylation and inhibited glucose uptake. In contrast, a deletion mutant of NEMO, lacking the IKK-β binding domain, or a silencing NEMO blocked the TNF-α signal. Taken together, Myo1c and its receptor NEMO act co-operatively to form the IKKs–IRS-1 complex and function in TNF-α induced insulin resistance via phosphorylation of IRS-1 at $serine^{307}$.

Cellular dynamics of lipid droplets

Hongyuan Yang, Weihua Fei and Han Wang
University of New South Wales, Sydney, Australia
(h.rob.yang@unsw.edu.au)

The hallmark of human obesity is the accumulation of lipids in the form of cellular lipid droplets (LDs). LDs can be found in nearly all types of eukaryotic cells. Despite the emerging role of LDs in cell biology and their potential impact on many devastating diseases, little is known about the mechanisms governing the biogenesis, growth and degradation of LDs. Here we report that conditions of endoplasmic reticulum (ER) stress stimulate LD formation in *Saccharomyces Cerevisiae*. We discovered in this study that LDs accumulate in yeast mutants that have compromised protein glycosylatoin or ER associated protein degradation. In addition, we isolated a novel mutant which demonstrated enhanced fusion of the LDs. The possible mechanisms will be discussed.

Role of PPARδ in Hepatic Lipogenesis

Xiaomei Qin, Xuefeng Xie, Yanbo Fan, Youfei Guan, Xian Wang, Yi Zhu and Nanping Wang

Institute of Cardiovascular Institute and Departments of Physiology and Pathophysiology, Peking University, Beijing, China

(npwang@bjmu.edu.cn)

Recent work has revealed potential implications of peroxisome proliferator-activated receptor-δ (PPARδ) in lipid homeostasis and insulin resistance. In this study, we aimed to examine the effect of PPARδ on sterol regulatory element-binding protein-1 (SREBP-1), a pivotal transcription factor controlling lipogenesis, in cultured human hepatocytes. We found that PPARδ agonist as well as overexpression of PPARδ in hepatocytes induced the gene expression of Insig-1, a regulatory endoplasmic reticulum protein braking SREBP activation. PPARδ inhibited the proteolytic processing of the SREBP-1 into the mature active form, thereby suppressing the expression of the lipogenic genes fatty acid synthase, stearyl CoA desaturase-1 and glycerol-3-phosphate acyltransferase. Furthermore, chromatin immunoprecipitation, electrophoresis mobility shift assay and site-directed mutagenesis demonstrated Insig-1 as a PPARδ target gene in hepatocytes. Adenovirus-mediated overexpression of PPARδ also induced Insig-1 expression, suppressed SREBP-1 activation and, consequently, ameliorated hepatic steatosis in obese diabetic *db/db* mice. Thus, our study reveals a novel mechanism by which PPARδ regulates lipogenesis, which suggests potential therapeutic applications of PPARδ modulators in obesity and type 2 diabetes, as well as related fatty liver diseases.

Roles of adipokines for the regulation of hepatic glucose metabolism

Seung-Hoi Koo/ Young-Sil Yoon, Woo- Young Seo, Min-Woo Lee

Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Korea

(shkoo@med.skku.ac.kr)

Mammalian liver plays a major role in modulating energy homeostasis. During fasting periods, hepatic glucose production is enhanced in response pancreatic glucagon and adrenal catecholamines to provide enough fuels for other organs. This process is in part mediated via activation of cAMP dependent induction of the CREB coactivator TORC2, which in turn activates hepatic gluconeogenesis at the level of transcription. We have shown previously that TORC2 activity is tightly regulated by AMPK family members. However, the relevant endogenous signals that are directly responsible for such regulation in liver have not been identified. Here we show that the adipocyte derived cytokine hormones resistin and adiponectin regulate the hepatic gluconeogenesis by modulating TORC2 activity. Adenoviral expression of adiponectin lowered fasting glucose levels by enhancing the phosphorylation of the AMPK regulated coactivator TORC2 in liver. By contrast, resistin acutely enhanced TORC2 dephosphorylation and gluconeogenic gene expression in vivo. The regulation of TORC2 by adipokines requires the presence of active LKB1 kinase, as adenovirus-mediated knockdown of LKB1 in hepatocytes completely abolishes their effects on gluconeogenic gene expression. Taken together, we are proposing that resisitin and adiponectin might represent the endogenous signals for the modulation of AMPK-dependent TORC2 activity to influence hepatic gluconeogenesis.

Identification of the adapter protein Grb10 as a physiological regulator of glucose homeostasis, body composition and IR/IGF-1R signalling

Roger J Daly, Lowenna J Holt, Bronwyn D Hegarty, Ruth J Lyons, Gregory J Cooney and Andrew Ward

Cancer Research and Diabetes and Obesity Research Programs, Garvan Institute of Medical Research, 384 Victoria St, Sydney, NSW 2010, Australia; Department of Biology and Biochemistry, University of Bath, BA2 7AY, UK

(r.daly@garvan.org.au)

Growth factor receptor bound (Grb)10 is a member of a family of src homology (SH)2 domain-containing adapter proteins that also includes Grb7 and Grb14. These proteins possess a similar molecular architecture, containing a conserved proline-rich motif at the N-terminus, centrally-located Ras-association and pleckstrin homology (PH) domains, and a C-terminal src homology (SH)2 domain. In addition, the BPS region, located between the PH and SH2 domains, mediates binding of these proteins to the insulin receptor (IR). We have previously characterized Grb14 as a tissue-specific negative regulator of insulin signalling, a role explained by its function as a pseudosubstrate inhibitor of the IR kinase. We have now used gene-targeting to reveal the physiological function of Grb10. Adult Grb10-deficient mice exhibit improved whole-body glucose tolerance and insulin sensitivity, as well as increased muscle mass and reduced adiposity. In addition, these animals exhibit tissue-selective effects on insulin signalling. IR tyrosine phosphorylation is markedly reduced in skeletal muscle and white adipose tissue, consistent with a model in which Grb10, like Grb14, protects the activation loop phosphotyrosines from dephosphorylation by specific protein tyrosine phosphatases. In addition, insulin-induced IRS-1 tyrosine phosphorylation is enhanced in these tissues, supporting a role for Grb10 in attenuation of signal transmission from the IR to this docking protein. In order to determine the mechanistic basis for the observed alterations in body composition, we have characterized the effects of Grb10 ablation on candidate signalling pathways, using murine embryonic fibroblasts (MEFs). Importantly, Grb10-deficient MEFs exhibit a striking increase in IGF-1-induced IRS-1 phosphorylation and Akt activation. Therefore the phenotype of Grb10 gene-disrupted mice likely reflects the physiological role of Grb10 as a negative modulator of receptors that regulate muscle mass and body composition, such as the IGF-1R, as well as the IR.

Myo-inositol; a snapshot of its demise by the enzyme *myo*-inositol oxygenase

Kerry M. Loomes, Peter M. Brown, Tom T. Caradoc-Davies, James M. J. Dickson, Garth J. S. Cooper, Edward N. Baker

Maurice Wilkin's Centre for Molecular Biodiscovery, School of Biological Sciences, University of Auckland, New Zealand

(k.loomes@auckland.ac.nz)

Diabetes mellitus is associated with altered metabolism of *myo*-inositol and its rarer epimeric form, D-*chiro*-inositol. *Myo*-inositol is an essential precursor of phosphoinositides, which play fundamental roles in cellular signaling, and it is also an important osmolyte in the control of intracellular osmolarity. Although the biology surrounding D-*chiro*-inositol is less well understood, it has been identified as a component of endogenous inositol phosphoglycans that act as putative insulin mediators. Consistent with a relative deficiency of these mediators, chronic administration of D-*chiro*-inositol reportedly enhances insulin action in human patients with polycystic ovary syndrome and decreases hyperglycemia in several animal models of diabetes. We are currently investigating the role of the enzyme, *myo*-inositol oxygenase (MIOX), in inositol homeostasis. Under normal physiological conditions, MIOX is expressed predominantly in the proximal tubular epithelial cells of the kidney where it catalyses the first committed step in the only known pathway for *myo*-inositol catabolism. Importantly, MIOX also acts on D-*chiro*-inositol and likely mediates its breakdown *in vivo*. Here, we review the rationale for our interest in MIOX as a regulator of inositol levels *in vivo* and its potential significance in diabetes. We also present the MIOX tertiary structure in complex with the substrate, *myo*-inositol, and discuss new scientific insights gained from its unique reaction reaction mechanism.

The construction and primarily study of cpt1c knock-out mouse

Xuefei Gao, Wei Chen, Zhugang Wang, Aimin Xu, Donghai Wu

Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, China

(Wu_donghai@gibh.ac.cn)

Carnitine palmytoyltransferase1 (cpt1) is the enzyme catalyzing the transesterification reaction between long-chain acyl-CoA and acylcarnitine esters, the committed step of fatty acid oxidation, Cpt1c is the novel homologue of cpt1a and cpt1b and is specifically expressed in brain. Howerver, unlike other cpt1s, no acyltransfer activity is detected from cpt1c although it also has malonyl-CoA binding site. Disruption of the cpt1c gene results in the mice more susceptible to obesity and insulin resistance under HFD feeding. Here we present our gene knockout strategy to produce a targeted KO of exon 3 of cpt1c mice and some preliminary results.

Axin is a master scaffold involved in metabolism, tumor suppression, and dorsoventral patterning

LIN Shengcai

School of Life Sciences, Xiamen University, China

(linsc@xmu.edu.cn)

Axin is a multi-domain protein, serving as a scaffold for many signaling pathways. Originally identified as the product of the mouse *Fused* locus, Axin acts as a negative regulator of Wnt signaling in that its mutation results in axis duplication. In this pathway, Axin binds directly to adenomatous polyposis coli (APC), glycogen synthase-3β, and β-catenin, thereby downregulating β-catenin levels. It has also been implicated in the regulation of glucose transport and mTOR signaling. In addition, we found that Axin plays a pivotal role in TGF-bet signaling and UV-induced p53 activation.

Moreover, we found that Axin also activates the JNK MAP kinase, and have since delineated several structural and biochemical elements in Axin that are important for JNK activation. However, the biological significance of this Axin/JNK signaling cascade remained obscure until our recent demonstrations that Axin depends on its ability to activate JNK to cause dorsalization during embryogenesis. The intrinsic dorsalizing activity is inhibited by a newly identified factor termed Aida for *A*xin *i*nteraction partner and *d*orsalization *a*ntagonist.

Role of Kru ppel-like factor 15 in hepatic glucose metabolism

Wataru Ogawa, Mototsugu Takashima, Hiroshi Sakaue, Yasuo Okamoto, Shinichi Kinoshita, Masato Kasuga

Division of Diabetes, Metabolism and Endocrinology, Kobe University Graduate School of Medicine, Japan

(ogawa@med.kobe-u.ac.jp)

[Aim] To identify a novel regulatory mechanism of glucose homeostasis in the liver, we analyzed the alterations of hepatic gene expression in various physiological and pathological conditions in mice with the use of Gene Chip analyses. [Methods & Results] We have found that hepatic expression of Krüppel-like factor 15(KLF15), a member of C2-H2 family of transcription factors, was increased in fasted mice and decreased in fed mice. In cultured hepatocytes, the expression of KLF15 was induced by cAMP or by glucocorticoids, and inhibited by insulin in a PI3K-dependent manner. Forced expression of KLF15 in hepatocytes increased mRNA abundance and the promoter activity of the PEPCK gene, and the inhibition of KLF15 expression with the use of a shRNA prevented cAMP-induced PEPCK expression. KLF15 and PGC-1α coordinately induced PEPCK expression in hepatocytes, and the shRNA of KLF15 inhibited PGC-1α-induced PEPCK expression. Administration of shRNA of KLF15 in the liver of C57/BL6 mice with the use of adenovirus-mediated gene transfer reduced the mRNA abundance of the genes for gluconeogenic enzymes without affecting that of PGC1α as well as for enzymes contributes to amino-acid catabolism. This treatment in C57/BL6 mice decreased the concentration of plasma insulin, but not of blood glucose. In the liver of db/db diabetic mice, the abundance of KLF15 in the liver was increased, and the inhibition of hepatic KLF15 in these mice resulted in a marked reduction of blood glucose levels. [Conclusion] KLF15 is a physiological regulator for hepatic gluconeogenesis and serves as a potential therapeutic target of diabetes mellitus.

Cide Proteins and the Development of Obesity

Peng Li

Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing, China

(li-peng@mail.tsinghua.edu.cn)

The number of obesity individuals increased rapidly in last few years not only in developed countries but also in quickly developing countries like China. Obesity develops as a result of energy intake exceeding energy expenditure and is a major risk factor for many metabolic diseases such as hypertension, stroke, liver steatosis, cancer and inflammatory diseases. The development of obesity involves functional interactions among various tissues such as brain, adipose tissue, skeletal muscle, liver and intestine. Adipose tissues can be divided into brown adipose tissue (BAT) and white adipose tissue (WAT). Both BAT and WAT contain abundant amount of lipid and can serve as energy storage organ. BAT plays a unique role in energy expenditure by uncoupling oxidative phosphorylation and dissipating energy as heat to maintain core body temperature in animals when exposed to cold. The primary role of WAT is to store energy in the form of triglycerides (TAG) in lipid droplets and immobilize the energy in time of needs such as starvation. Adipose tissue can also serve as an endocrine organ to secret crucial hormones such as Leptin and Adiponectin for the control of whole-body energy homeostasis. Liver plays a central role in energy homeostasis as it is the main organ for lipid *de novo* synthesis, lipid uptake and secretion, fatty acid oxidation, as well as the production of ketone bodies. Cide proteins, including Cidea, Cideb and Fsp27 (Cidec in human), were originally identified by their sequence homology to the N-terminal region of DNA fragmentation factor DFF40/45. While Cidea is expressed at higher levels in BAT, *Cideb* mRNAs and proteins were detected in various tissues with highest levels of expression in the liver, moderate levels in kidney and lower levels in stomach and small intestine. To understand the physiological role of Cide proteins, we generated *Cidea* and *Cideb*-null mice by homologues recombination. Interestingly, we observed that mice deficient in *Cidea* or *Cideb* exhibited higher energy expenditure and were resistant to high-fat-diet induced obesity and diabetes. Here, we will compare the detail phenotype of *Cidea* and *Cideb* null mice and analyze the underlying mechanism of Cide proteins in the regulation of energy homeostasis and the development of obesity and diabetes.

Genipin inhibits UCP2-mediated proton leak and acutely reverses obesity- and high glucose-induced β cell dysfunction in isolated pancreatic islets

Chenyu Zhang

State Key Laboratory of Pharmaceutial Biotechnology, School of Life Sciences, Nanjing University, 22 Hankou Road, Nanjing, Jiangsu, China

(cyzhang@nju.edu.cn)

Uncoupling protein (UCP) 2 negatively regulates insulin secretion. UCP2 deficiency (by means of gene knockout) improves obesity and hyperglycemia-induced β-cell dysfunction, and consequently improves type 2 diabetes. In the present study, we have identified a cell-permeable inhibitor of UCP2, genipin. In isolated mitochondria, genipin specifically inhibits UCP2- and UCP3-mediated proton leak, which is induced by exogenously added superoxide. In pancreatic islets, genipin increases mitochondrial membrane potential and stimulates insulin secretion in a UCP2-dependent manner. Importantly, acute addition of genipin to isolated islets reverses hyperglycemia-and obesity-induced β-cell dysfunction. Therefore, genipin (or derivatives) represent a new class of compounds that might be useful for the treatment of β-cell dysfunction and type 2 diabetes.

Ablation of PDK-1 in vascular endothelial cells enhances insulin sensitivity by reducing visceral fat

Mitsuru Hashiramoto, Kazuhito Tawaramoto, Ko Kotani and Kohei Kaku

Department of Internal Medicine; Division of Diabetes and Endocrinology, Kawasaki Medical School, Okayama, Japan

(hashira@med.kawasaki-m.ac.jp)

The phosophoinositide-dependent protein kinese 1 (PDK1) signaling pathway is involved in a broad range of cellular processes governed by insulin. We investigated the role of PDK1 in vascular endothelial cells by generating tissue-specific knockout mice that lack PDK1 in vascular endothelial cells (VEPDK1KO). VEPDK1KO mice manifested enhanced glucose tolerance and whole body insulin sensitivity due to suppression of hepatic glucose production without any change in peripheral glucose disposal. Circulating adiponectin levels were higher and the activities of hepatic gluconeogenic enzymes were lower in VEPDK1KO mice than in control mice. When VEPDK1KO and control mice were fed a high-fat diet, adiponectin mRNA abundance was higher and MCP1, leptin and TNF . mRNA levels lower in white adipose tissue of VEPDK1KO compared with control mice. As a result, hepatic AMP kinase was significantly activated, subsequently enhancing whole body insulin sensitivity in VEPDK1KO mice. High-fat-diet-induced obesity and adipocyte hypertrophy were attenuated in VEPDK1KO mice, due, at least in part, to suppression of angiogenesis in white adipose tissue, in association with a reduction in visceral fat area. These results suggest that angiogenesis and adipogenesis are closely related and that PDK1 signaling in endothelial cells plays an important role in maintaining proper glucose homeostasis, primarily through regulation of adipocyte development.

Tesaglitazar, a peroxisome proliferator activated receptor alpha/gamma dual agonist, attenuates glycemic control and diabetic nephropathy in db/db mice

Zhang XY, Wu J, Chen L, Zhang D, Pu D, Guan Y.

Department of Physiology and Pathophysiology, Peking (Beijing) University Diabetes Center, Peking (Beijing) University Health Science Center, Beijing 100083, China

(youfeiguan@bjmu.edu.cn)

Peroxisome proliferator-activated receptors (PPARs) are nuclear transcription factors and play a central role in insulin sensitivity, lipid metabolism, and inflammation. Both PPARalpha and -gamma are expressed in the kidney, and their agonists exhibit beneficial metabolic effect and renoprotective effect in type 2 diabetes. In the present studies, we determined the effect of the PPARalpha/gamma dual agonist tesaglitazar on glucose homeostasis regulation and diabetic nephropathy in type 2 diabetic db/db mice. Treatment of db/db mice with tesaglitazar for 3 months significantly lowered fasting plasma glucose and homeostasis model assessment of insulin resistance levels but had little effect on body weight, adiposity, or cardiac function. Treatment with tesaglitazar was associated with reduced plasma insulin and total triglyceride levels and increased plasma adiponectin levels. In addition, tesaglitazar markedly attenuated albuminuria and significantly lowered glomerulofibrosis, collagen deposition, and transforming growth factor-beta1 expression in renal tissues of db/db mice. In cultured mesangial cells and proximal tubule cells, where both PPARalpha and -gamma were expressed, tesaglitazar treatment abolished high glucose-induced total collagen protein production and type I and IV collagen gene expression. Collectively, tesaglitazar treatment not only improved insulin resistance, glycemic control, and lipid profile but also markedly attenuated albuminuria and renal glomerular fibrosis in db/db mice. These findings support the utility of dual PPARalpha/gamma agonists in treating type 2 diabetes and diabetic nephropathy.

Rosiglitazone improves vascular dysfunction through inducing the production of high molecular weight oligomeric adiponectin in db/db diabetic mice

Aimin Xu / Ruby C L Hoo, Yu Huang, Lawrance Chan and Karen S L Lam

Department of Medicine and Research Center for Heart, Brain, Hormone and Healthy ageing, University of Hong Kong & Dept of Physiology, The Chinese University of Hong Kong

(amxu@hkucc.hku.hk)

The PPARγ agonists Thiazolidinediones (TZDs), such as rosiglitazone and pioglitazone, are widely used for the treatment of type 2 diabetes. In addition to the insulin-sensitizing activities, TZDs has beneficial effects on vascular disorders associated with diabetes. However, the mechanisms underlying the vasculo-protective actions of TZDs remain poorly understood. In both animal models and human subjects, TZDs have been shown to induce the production of adiponectin, an insulin-sensitizing adipokine with anti-inflammatory and anti-atherogenic activities. In this study, we investigated the role of adiponectin in mediating the vasculo-protective activity of TZDs in db/db diabetic mice. To this end, we have crossed db/+ mice with adiponectin knockout mice (ADN (-/-) to generate db/db diabetic mice without or with adiponectin (d*b/db/ADN(+/+) and db/db/ADN(-/-)*) respectively. The body weight, food intake, lipid and glucose profiles were comparable between these two groups of mice. Treatment with Rosiglitazone (20 mg/kg body weight/day) for 2 weeks resulted in a marked alleviation of hyperglycemia even in the db/db/ADN(-/-) mice, suggesting that the glucose-lowering effects of Rosiglitazone, at least at this dosage, is independent of adiponectin. In *db/db/ADN(+/+)* mice, rosiglitazone treatment caused a selective elevation of high molecular weight (HMW) oligomeric form of adiponectin, and also markedly enhanced acetylcholine-induced vaso-dilation of the aorta rings. However, the beneficial effect of Rosiglitazone on vascular reactivity was largely abrogated in *db/db/ADN(-/-)* mice. Our pulse-chase experiment in human adipocytes showed that Rosiglitazone selectively increased the secretion of the HMW oligomeric adiponectin, but had no effect on the hexameric and trimeric adiponectin. In conclusion, our results suggest that the vasculo-protective functions of TZDs are mediated by induction of the HMW adiponectin, independent of its effects on hyperglycemia control.

Quantitative assessment of diabetic neuropathy using a newly developed device, PainVision®

Mitsuhisa Komatsu, Kunihide Hiramatsu and Kiyoshi Hashizume
Department of Aging Medicine and Geriatrics, Shinshu University, Japan
(mitsuk@hsp.md.shinshu-u.ac.jp)

Aim: The Painvision® (Osachi, Inc.) is a newly developed device to quantitatively evaluate the degree of pain. This portable and battery-operated device also provides a sensitive quantitative measure of sensory function, and is quick and easy to use. To determine the standard value of minimum perceived current (MPC) obtained from the device, and to explore the usefulness of MPC in detecting early diabetic neuropathy, we examined the MPC with PainVision in 1012 patients in our hospital.

Methods: Study subjects were 248 diabetic patients without neuropathy (DN-), 240 diabetic patients with neuropathy (DN+), and 524 non-diabetic subjects (non-DM). We applied the electrodes (14mm in diameter) to the medial forearms of the subjects and measured MPC by increasing electrical stimulus (50 Hz, 0-150 μA, pulse width 0.5 ms).

Results: MPC were higher in male (11.8±0.3μA) than in female (9.2±0.2μA). Diabetic subjects (DN- and DN+) exhibited significantly higher MPC than non-DM. Age-related increases in MPC were found in both genders. In male subjects, MPC was significantly higher in DN+ than in other groups. In females, it was significantly higher in DN- than in non-DM and no difference between DN- and DN+ was fond. Longer duration of diabetes tended to increase MPC.

Conclusion: We established the standard values of MPC in non-diabetic subjects and found that a quantitative evaluation of MPC is a sensitive test for the detection of diabetic neuropathy. In particular, MPC is a useful marker for clinically silent diabetic neuropathy in females.

Hormonal induction of leptin and melanocortin resistance in the hypothalamus of pregnant rats

David R. Grattan, Sharon R. Ladyman and Rachael A. Augustine

DR GRATTAN, RA AUGUSTINE, SR LADYMAN
Centre for Neuroendocrinology and Department of Anatomy and Structural Biology, University of Otago, P.O. Box 913, Dunedin, New Zealand
(dave.grattan@anatomy.otago.ac.nz)

Despite elevated plasma leptin concentrations, food intake and fat deposition is increased during pregnancy. We have demonstrated that intracerebroventricular (i.c.v.) leptin administration is unable to suppress food intake in pregnant rats, as it does in non-pregnant animals. Hence, a state of leptin resistance develops during pregnancy. These changes are physiologically appropriate, serving to provide increased energy reserves to help meet the high metabolic demands of fetal development and lactation. We have observed a significant reduction of Ob-Rb mRNA levels in the ventromedial hypothalamic nucleus (VMH) during pregnancy compared to non-pregnant animals, with no changes detected in other hypothalamic nuclei. Levels of leptin-induced pSTAT3 were also specifically suppressed in the VMH of pregnant rats compared to non-pregnant rats. Levels of NPY and AGRP mRNA in the arcuate nucleus were not significantly different during pregnancy, despite elevated leptin which would be expected to suppress expression of these proteins. Leptin-induction of pSTAT in these neurons was normal, suggesting that signalling through other pathways, such as the Pi3-kinase pathway, might be impaired during pregnancy. Finally, we have also shown that pregnant rats are resistant to the appetite suppressing effects of aMSH, suggesting that pathways downstream of the 1st-order leptin-responsive neurons might also be less responsive during pregnancy. To investigate the mechanism underlying pregnancy-induced leptin resistance, we have investigated effects of hormone treatments on hypothalamic responses to leptin in a pseudopregnant rat model. Pseudopregnant (PSP) rats were hyperphagic but did not become leptin resistant, even when given progesterone implants to extend PSP beyond the time that resistance develops during pregnancy. Chronic i.c.v. infusion of ovine prolactin to mimic patterns of placental lactogen secretion characteristic of pregnancy, however, completing block the ability of leptin to suppress food intake. These data demonstrate that multiple different adaptations occur to induce leptin resistance during pregnancy, and suggest that placental lactogen secretion may be a key mediator of the hormone-induced loss of response to leptin.

Apolipoprotein A-I stimulates AMP-activated protein kinase and improves glucose metabolism

Ruijun Han, Riyong Lai, Qiurong Ding, Zhenzhen Wang, Xiaolin Luo, Yixuan Zhang, Guoliang Cui, Jing He, Weizhong Liu, and Yan Chen

Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences, Shanghai 200031, China

(ychen3@sibs.ac.cn)

Aims/hypothesis In humans, one of the hallmarks of type 2 diabetes is reduced plasma concentration of high-density lipoprotein and its major protein component apolipoprotein A-I (APOA-I). However, it is unknown whether APOA-I has a direct protective role in the pathogenesis of diabetes. The aim of this study was to characterize the functional role of APOA-I in glucose homeostasis.

Materials and methods The effects of APOA-I on phosphorylation of AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC), glucose uptake and endocytosis were analyzed in C2C12 myocytes. Glucose metabolism was investigated in *apoA-I* knockout mice.

Results APOA-I was able to stimulate the phosphorylation of AMPK and ACC, and elevated glucose uptake in C2C12 myocytes. APOA-I could be endocytosed into C2C12 myotubes through a clathrin-dependent endocytotic process. Inhibition of endocytosis abrogated AMPK phosphorylation stimulated by APOA-I. *ApoA-I*-deficient mice had reduced AMPK phosphorylation in the skeletal muscle and liver with increased expression of gluconeogenic enzymes in the liver. In addition, the *apoA-I*-deleted mice had increased fat content and compromised glucose tolerance.

Conclusions/interpretation Our data indicate that APOA-I has an anti-diabetic effect via activation of AMPK. *ApoA-I* deletion in mouse led to increased fat mass and impaired glucose tolerance.

Roles of Rab27 and its effectors in regulated exocytosis

Tetsuro Izumi, Kazuo Kasai, and Hiroshi Gomi

Department of Molecular Medicine, Institute for Molecular and Cellular Regulation, Gunma University, Japan

(tizumi@showa.gunma-u.ac.jp)

Since the identification of granuphilin and its partner Rab27a in pancreatic beta cells, we have studied the function of the protein complex both in vitro and in vivo and found that granuphilin is essential for the docking of insulin granules to the plasma membrane. Furthermore, in pancreatic alpha cells, another Rab27a effector, exophilin4, functions in the docking of glucagon granules. Therefore, even developmentally close cells utilize distinct machinery for granule docking. We also found that Rab27b, another Rab27 subfamily member, is expressed in a wide range of exocytic cells and involved in the delivery of secretory granules near the plasma membrane. The property of these docking machinery suggests that stable predocking of granules to the plasma membrane is not necessarily required for a subsequent membrane fusion reaction. To examine dynamic exocytic profiles in living beta cells, we recently performed total internal reflection fluorescence, TIRF, microscopy, which detects fluorescence in the proximity of the plasma membrane, and would like to discuss the molecular mechanism and functional significance of secretory vesicle docking in regulated exocytosis.

Multiple Roles of Insulin Receptor in β-cells: Insulin Secretion, Proliferation and Cell Survival

Moon-Kyu, Lee / Seung Hyun Hong / Yucheol Hwang

Division of Endocrinology & Metabolism, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

(mklee@smc.samsung.co.kr)

Insulin receptor (IR) mediated signaling pathway in pancreatic β-cell plays a critical role in the maintenance of β-cell mass and normal insulin secretion. However, the role of IR during insulin signaling in pancreatic β-cells is not clearly understood. In this study, we introduced the human IR into INS-1 cells with plasmid vector to evaluate the biological role of IR in β-cells.

Insulin receptor overexpressed INS-1 (INS-IR) cells showed higher levels of insulin-stimulated tyrosine kinase activity and glucose-induced insulin secretion (GIIS) and which was associated with a significant increase in insulin mRNA expression and glucose metabolism. Thus INS-IR cells might be expected to have more ATP channels closed causing subsequent Ca^{2+} influx and insulin secretion. In addition, INS-IR cells also exhibited a high rate of proliferation and a decrease in apoptosis induced either by high glucose or free fatty acid. This result was associated with the changes in mitogenic and apoptotic signaling pathways of INS-IR cells. INS-IR cells exhibited increased Erk1/2 phosphorylation in the unstimulated state that was involved in the phosphorylation and activation of cyclin dependent kinases (CDKs) progressed to S phase. INS-IR cells showed higher levels of protein kinase B (PKB) phosphorylation, the latter of which is related to effectively blocked caspase 3 initial cleavage with a consequent reduction in apoptosis in hyperglycemic and hyperlipidemic condition. Our findings suggest that the insulin receptor has multiple roles in pancreatic β-cells and the model might be used for the treatment of type 2 diabetes mellitus by preventing β-cell dysfunction.

Fas-associated Death Receptor Signaling is induced in Islet β-cells Following Human Amylin Treatment

Shaoping Zhang, Hong Liu, Hua Yu and Garth J. S. Cooper

The School of Biological Sciences, Faculty of Science, University of Auckland, Auckland, New Zealand

(s.zhang@auckland.ac.nz)

Objective: Aggregation of human amylin (hA) into β-sheet-containing oligomers has been linked to the onset of islet β-cell dysfunction and the pathogenesis of type-2 diabetes (T2DM). Here, we investigated possible contributions of Fas-associated death-receptor signalling to hA-evoked β-cell apoptosis.

Research Design and Methods: We studied responses to hA in isolated mouse islets and two insulinoma cell lines, wherein we measured Fas/FasL and FADD expression by real-time qPCR, western blotting and immunofluorescence staining. We employed two anti-Fas/FasL blocking antibodies and a Fas/FasL antagonist, Kp7-6, to probe roles of Fas/receptor interactions in the regulation of apoptosis in hA-treated β-cells. We quantitated Kp7-6-mediated effects on β-sheet formation and aggregation by hA, by circular dichroism and thioflavin-T binding.

Results: Amylin treatment elicited Fas and FADD expression in β-cells. Both blocking antibodies suppressed hA-evoked apoptosis, but did not modify hA aggregation; therefore, the Fas/receptor interactions played a critical role in induction of this apoptotic pathway. Interestingly, hA-evoked β-cell apoptosis was suppressed by Kp7-6, which also impaired hA-β-sheet formation.

Conclusions: This is the first report linking hA-evoked induction and activation of Fas and FADD to β-cell apoptosis. We have identified a Fas/FasL antagonist Kp7-6 as a potent inhibitor of hA aggregation and associated β-cell death. These results also support an interaction between hA and Fas expressed on the β-cell surface. Thus, increased expression and activation of Fas signalling in β-cells could constitute a molecular event common to the pathogenesis of T1DM and T2DM, although its mode of initiation may differ between these two forms of diabetes.

The mechanism by which insulin hypersecretion may be detrimental to islet-cell function

Sof Andrikopoulos, Joe Proietto, Grant Morahan

University of Melbourne, 300 Waterdale Road, Heidelberg Heights Victoria 3081, Australia

(sof@unimelb.edu.au)

Type 2 diabetes is characterized by beta cell dysfunction which contributes to the ensuing hyperglycaemic state. However, in many cases this state is preceded by a period of relative increased insulin secretory function, which is not necessarily in response to insulin resistance. Indeed a number of studies have shown that in populations at increased risk of developing diabetes, insulin hypersecretion occurs independently of insulin resistance and contributes significantly to beta cell dysfunction. To understand the mechanism by which insulin hypersecretion may be associated with beta cell dysfunction, we have been studying the DBA/2 mouse, which has previously been shown to be susceptible to diabetes when stressed with obesity and insulin resistance. In contrast, mice from the C57BL/6 and 129T2 strains did not develop diabetes when rendered obese and insulin resistant. Our studies have shown that in vivo and in vitro, DBA/2 mice secreted significantly more insulin in response to glucose compared with C57BL/6 and 129T2 mice and that this trait was genetically determined. A genome-wide scan was performed and identified one QTL on chromosome 13 that was linked to the insulin hypersecretion trait in DBA/2 mice. Further analysis of this QTL showed that one gene, nicotinamide nucleotide transhydrogenase (Nnt) was increased 5-fold in islets from DBA/2 mice compared with C57BL/6 and 129T2. Furthermore, we and others identified a 5-exon deletion in the C57BL/6 but not DBA/2 or 129T2 strains, causing further dysfunction in this strain. Nnt is a mitochondrial enzyme involved in proton translocation and energy production. In agreement with this, we have shown that DBA/2 islets have increased glucose metabolism and ATP production compared with C57BL/6 islets. Of importance is that following high glucose culture DBA/2 (but not C57BL/6) islets display a selective defect in glucose-mediated insulin secretion associated with increased mitochondrial metabolism and oxidative stress. This defect in insulin secretion in the high glucose cultured DBA/2 islet can be reversed by the anti-oxidant N-acetyl-cysteine. This data suggests that genetically determined insulin hypersecretion can lead to beta cell dysfunction via increased oxidative stress. The implication of this study is that treatments that stimulate insulin secretion may be detrimental to beta cell function and survival in the long-term. This supposition is supported by the recent ADOPT study which showed that newly diagnosed patients with Type 2 diabetes progressed to a second hypoglycaemic agent to maintain adequate plasma glucose levels earlier, when initially treated with a sulfonylurea, compared with those initially treated with insulin sensitizers such as metformin or rosiglitazone.

Dexamethasone Inhibits PDX-1 in Pancreatic β-Cell Partially Through Foxo1 Transcription Factor

Xiongfei Zhang,[1] Wei Yong,[1] Jinghuan Lv,[1,2] Yunxia Zhu,[1,2] Jingjing Zhang,[1] Rihua Zhang,[1] Yujie Sun,[1,2] and Xiao Han,[1,2]

Key Laboratory of Human Functional Genomics of Jiangsu Province, Nanjing Medical University, 140 Hanzhong Road, Nanjing, 210029, China[1]; Jiangsu Diatetes Center[2]

(hanxiao@njmu.edu.cn)

Glucocorticoids can induce steroid diabetes, the underlying mechanism of which has not been clearly understood. It has been shown that dexamethasone can reduce the expression of PDX-1. In this study, we demonstrated that Foxo1 plays a role as a mediator in this process. Our research proved that dexamethasone increases Foxo1 expression accompanied by a decrease in phosphorylated Foxo1; this results in an increase in active Foxo1 in pancreatic β-cell line RINm5F and isolated rat islets. An RNA interference experiment demonstrated that dexamethasone inhibited PDX-1 transcription partially through increased active Foxo1. In addition, dexamethasone also induced nuclear exclusion of PDX-1 through increase in active Foxo1, namely, nuclear Foxo1. In conclusion, Foxo1 is involved in dexamethasone-induced inhibition of PDX-1.

Role of TSC2 in the regulation of pancreatic beta cell mass

Yoshiaki Kido

Division of Diabetes, Metabolism and Endocrinology, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan
(kido@med.kobe-u.ac.jp)

Recent study have demonstrated the importance of insulin or insulin-like growth factor (IGF)-1 regulating pancreatic β cell mass. Given the role of tuberous sclerosis complex (TSC) 2 as an upstream molecule of mammalian target of rapamycin (mTOR), we examined the effect of TSC2 deficiency on β cell function. Here we show that mice deficient in TSC2 specifically in pancreatic β cells (β*Tsc2*$^{-/-}$ mice) manifest increased IGF-1-dependent phosphorylation of p70 S6 kinase and 4E-BP1 in islets as well as an initial increased islet mass attributable in large part to an increase in the size of individual β cells. These mice also exhibit hypoglycemia and hyperinsulinemia at a young age (4 to 28 weeks). After 40 weeks of age, however, the β*Tsc2*$^{-/-}$ mice develop progressive hyperglycemia and hypoinsulinemia accompanied by a reduction in islet mass due predominantly to a decrease in the number of β cells. These results thus indicate that TSC2 regulates pancreatic β cell mass in a biphasic manner.

RhoA/Rho Kinase Blocks Muscle Differentiation via Serine Phosphorylation of Insulin Receptor Substrate-1 and 2

Sung Soo Kim

Department of Biochemistry and Molecular Biology (BK21 project), Medical Research Center for Bioreaction to Reactive Oxygen Species, Kyung Hee University, Seoul 130-701, Korea
(sgskim@khu.ac.kr)

Although the RhoA/Rho kinase (RhoA/ROK) pathway has been extensively investigated, its roles and downstream signaling pathways are still not well understood in myogenic processes. Therefore, we examined the effects of RhoA/ROK on myogenic processes and their signaling molecules using H9c2 and C2C12 cells. Increases in RhoA/ROK activities and serine phosphorylation levels of IRS-1 (Ser307 and Ser636/639) and IRS-2 were found in proliferating myoblasts, while IRS-1/2 tyrosine phosphorylation and PI 3-kinase activity increased during the differentiation process. ROK strongly bound to IRS-1/2 in proliferation medium (PM) but dissociated from them in differentiation medium (DM). ROK inactivation by a ROK inhibitor, Y27632, or a dominant negative ROK, decreased IRS-1/2 serine phosphorylation with increases in IRS-1/2 tyrosine phosphorylation and PI 3-kinase activity, which led to muscle differentiation even in PM. Inhibition of ROK also enhanced differentiation in DM. ROK activation by a constitutive active ROK blocked muscle differentiation with the increased IRS-1/2 serine phosphorylation, followed by decreases in IRS-1/2 tyrosine phosphorylation and PI 3-kinase activity in DM. Interestingly, FGF-2 added to DM also blocked muscle differentiation through RhoA/ROK activation. FGF-2 blockage of muscle differentiation was reversed by Y27632. Collectively, these results suggest that the RhoA/ROK pathway blocks muscle differentiation by phosphorylating IRS proteins at serine residues, resulting in the decreased IRS-1/2 tyrosine phosphorylation and PI 3-kinase activity. The absence of the inhibitory effects of RhoA/ROK in DM due to low concentrations of myogenic inhibitory growth factors seems to allow IRS-1/2 tyrosine phosphorylation, which stimulates muscle differentiation via transducing normal myogenic signaling.

Essential Role of RNA-Editing Enzyme ADAR2 in Regulation of Glucose-Stimulated Insulin Secretion in Pancreatic β-Cells

Yong Liu / Zhenji Gan, Liyun Zhao, Liu Yang, Wenjun Li
The Institute for Nutritional Sciences, Shanghai Institute for Biological Sciences
Chinese Academy of Sciences
(liuy@sibs.ac.cn)

We have previously demonstrated that ADAR2 deaminase and ADAR2-mediated RNA editing in pancreatic islet β-cells are metabolically up-regulated in response to elevated glucose concentrations. However, the functional importance of ADAR2 in modulating β-cell function remains completely unknown. Here we show that selective ablation by RNA interference of ADAR2 markedly inhibits glucose-stimulated insulin secretion in INS-1 β-cells. Moreover, suppression of ADAR2 leads to impairment in both the ATP-sensitive K+ channel-dependent and -independent pathways that mediate the stimulatory effect of glucose for insulin secretion. In contrast, neither IBMX-enhanced nor L-arginine-induced insulin secretion is influenced, implicating a role for ADAR2 deficiency in causing a regulatory defect specific to glucose, a key feature of β-cell dysfunction associated with type 2 diabetes. While exhibiting no impact on glucose metabolism, insulin biosynthesis or intracellular Ca^{2+} influx, abrogated ADAR2 expression correlates with an appreciable reduction in the number of insulin granules docked to the cell membrane as analyzed by electron microscopy. Our results thus have revealed a hitherto unrecognized role for ADAR2 deaminase in regulating fuel-stimulated insulin secretion in β-cells, likely through modifying the molecular components involved in exocytosis.

Identification and Mechanism Exploration of two novel Clones which could Down-regulate the Expression of PPARGC1 by Cell-based High-throughput Screening

Sun Liang*, Shi Taiping, Shi Xiaohong, Wang Xiaoxia, Yang Ze
National Institute of Geriatric Medicines, Beijing Hospital, Ministry of Health, Beijing 100730, China
(SUNL90@genetics.ac)

INTRODUCTION

As a versatile coactivator, PPARGC1 has been shown to be broadly involved in metabolic pathways such as insulin-regulated gluconeogenesis, uptake of glucose in skeletal muscles and glucose-induced secretion of insulin by beta-cells, all of which are crucial in the pathogenesis of Type 2 diabetes. In both of rodents and human-being, the abnormal expression in target tissues has been considered potentially related to the determinations of Type 2 diabetes, such as liver and skeletal muscles. Among the new technologies for functional genome research, high-throughput screening (HTS) assays with their simple, rapid and sensitive characteristics are showing promise and will play more important roles. Here, we constructed a gain-of-function HTS platform based on the full-length promoter of PPARGC1. With the dual-luciferase system, we expect to find new molecules which could benefit the therapy of Type 2 diabetes, considering PPARGC1 as a potential cut-in target.

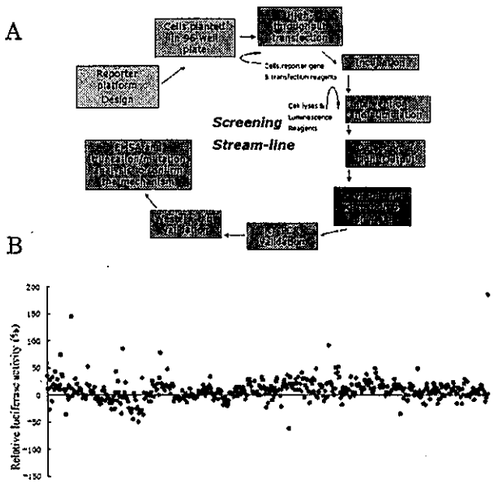
METHODS

To identify new molecules which could regulate the expression of PPARGC1, we firstly set up a HTS platform with the 3kb-promoter of PPARGC1 basing on Dual-luciferase system in HepG2 cells. Cooperated with Chinese National Human Genome Center, we have cloned a large number of human novel genes and studied their functions. Up to now, over 1000 individual sequence-validated human full-length ORFs have been subcloned into mammalian expression vectors to construct the ORFs library. Our work focused on the functional screening of 600 clones from the above library. Further, we conducted endogenous validation by RT-PCR to positive molecules in reporter assay. To test the consistence of the regulation between transcript level and translation level, we conducted Western Blot after positive molecules over-expressed in HepG2 cells. And the truncation assay and EMSA assay have been carried out to further explore the mechanism (Table A).

RESULTS AND DISCUSSION

We firstly set up the PPARGC1 HTS platform, by which functional screening has demonstrate 11 clones could regulate the promoter activity of PPARGC1, among them 7 are up-regulated (activation) and 4 are repression (repression) (Table B). After endogenous validation in over-expressed HepG2 cells, clones 766# and 285# demonstrate to consistently repress the

expression of PPARGC1 whether in mRNA level or protein level (Table C). Truncation analysis (Table D) and EMSA assay (Table E) indicated the repression by clones 766# and 285# seemed to dependent on the CRE element in PPARGC1 promoter and no effect on the phophorylation of CREB-Ser133 has been detected. The lose-of-function analysis around clones 766# and 285# is undergoing in order to clarify the real possibility of these two novel genes applied in the improvement of molecular pathogenesis of Type 2 diabetes.



Dissecting the role of PI 3-kinase isoforms in insulin signalling and glucose metabolism

Claire Chaussade[1], Hege Rui[2], Gordon Rewcastle[1], Jackie Kendle[1], Mei Ling Chong[1], Kitty Cho[1], Bill Denny[1], Jorgen Jensen[2], and Peter R. Shepherd[1]

[1] *University of Auckland, Auckland, New Zealand* [2] *National Institute of Occupational Health, Oslo, Norway (peter.shepherd@auckland.ac.nz)*

The insulin signaling pathways controlling glucose metabolism have been intensively studied and there is strong evidence that activation of PI 3-kinase is required. This conclusion is largely based on the use of LY294002 and wortmannin, two relatively specific inhibitors of PI 3-kinase. However, these cannot distinguish between the 8 different isoforms of PI 3-kinase. It was widely assumed that one of the insulin stimulated class-IA isoforms of PI 3-kinase (p110α, p110β or p110δ) would be responsible for this effect. We have used PI-103 and PIK75, two novel isoform selective inhibitors of p110α (IC_{50} 8 nM and 4 nM respectively), a p110β selective inhibitor (TGX221, IC_{50} 9 nM) and a p110δ selective inhibitor (IC87114, IC_{50} 60 nM). We find that PI103 and PIK75 cause >90% inhibition of growth of a range of cell lines including MCF7 and HEK293 cells. PI103 at 100 nM and 500 nM caused a 60% and 100% inhibition of insulin stimulated phosphorylation of ser 473 on PKB in 3T3-L1 adipocytes while the same concentrations caused only a 3% and 50% reduction in insulin stimulation of glucose uptake. At these concentrations PI103 did not reduce insulin stimulation of glycogen synthase activity in 3T3-L1 adipocytes. In rat soleus muscle we find that wortmannin inhibits insulin stimulated glucose transport but that PI103 and PIK75 were without effect, even at 1 μM (>100 x IC50), despite the fact these concentrations of inhibitor caused a >50% reduction in phosphorylation of PKB at ser473 and thr308. Inhibitors of p110β and p110δ did not affect insulin signaling or insulin stimulated glucose uptake in soleus muscle at concentrations up to 1 μM and neither did a combination of PI103, TGX221 and IC87114, each at 500 nM. Our findings suggest that the class-IA isoforms of PI 3-kinase may not be responsible for all of the observed effects that wortmannin and LY294002 have on insulin signaling pathways regulating glucose uptake and glycogen synthesis.

Mechanism Guided Discovery of New Therapeutics for Diabetes and Obesity from a Traditional Chinese Medicine: Berberine

Jiming Ye[1], / Jingya Li [1,2], Cordula Hohnen-Behrens[1], Lihong Hu [2], Jia Li [2], David E James[1] and Edward W Kraegen[1]

1. Garvan Institute of Medical Research, Sydney, Australia.

2. Shanghai Institute of Materia Medica, Shanghai, China

(j.ye@garvan.org.au)

We have recently reported that berberine (BBR), a natural product used in traditional Chinese medicine, has anti-diabetic properties via activating the AMPK pathway (*Diabetes* 55: 2256-64, 2006). To improve its therapeutic efficacy, we developed 20 derivatives from BBR by modifying its chemical structure. Based on our previous finding that BBR stimulates glucose transport and AMPK in L6 myotubes, we first screened these new compounds in these cell-based assays. Among them, the derivatives hydroxyberberine (hBBR) and dihydroxyberberine (dhBBR) were found to strongly stimulate glucose transport and/or AMPK/ACC phosphorylation. To assess their *in vi`vo* effects against diabetes and obesity, we conducted studies in high-fat fed (HF) mice. The results showed that dhBBR had greater efficacy in correcting liver steatosis and glucose intolerance compared with BBR and hBBR (see Table). We further compared the effect of dhBBR and BBR on insulin sensitivity in HF rats using a hyperinsulinaemic-euglycemic clamp technique combined with glucose tracers. As expected, dhBBR significantly improved whole-body insulin sensitivity (clamp GIR: 27.4±1.9 vs 19.0±0.4 mg/kg.min in HF-Con, $p<0.01$) by enhancing insulin-stimulated glucose disposal (30.3±0.6 vs 23.2±0.5 mg/kg.min, $p<0.01$). In comparison, the effect of BBR at this low dose (100mg/kg.day in food) was not significant.

	Ch-Con	HF-Con	HF-BBR	HF-hBBR	HF-dhBBR
Epididymal fat (% BW)	1.4±0.1	3.6±0.2*	3.7±0.7*	3.3±0.4*	1.8±0.2††
Liver TG (umol/g)	6.6±1.1	15.5±1.2*	14.4±1.6*	13.7±1.7*	10.2±1.0*††
AUC (mM.min)	493±51	873±46*	861±113*	774±79*†	511± 9††

BBR, hBBR or dhBBR was added in food at 100mg/kg.day for 2 wks. Ch: chow; TG: triglyceride; AUC: blood glucose area under the curve during ipGTT (0-120min, at 2g/kg glucose). *P<0.05 vs Ch-Con; †P<0.05, ††P<0.01 vs HF-Con (n=5-13/group).

As dhBBR exerted similar effects on AMPK and glucose uptake L6 myotubes comparable to BBR, we investigated whether the pharmacokinetic property of dhBBR may explain its superior *in vivo* efficacy. Indeed, the results showed that the bioavailability of BBR was substantially enhanced following oral gavage of dhBBR compared with oral gavage of BBR itself, whereas plasma BBR was hardly detectable after gavage of BBR. Interestingly, the plasma BBR profile (area under curve) was approximately 8 times greater than dhBBR itself after an oral gavage of dhBBR, indicating substantial *in vivo* conversion of dhBBR to BBR. These findings suggest that the improved in vivo efficacy of dhBBR results from a direct cellular effect plus an indirect effect of enhanced BBR bioavailability converted from dhBBR, both acting to stimulate the AMPK pathway. Thus we conclude that modification of BBR can significantly improve its therapeutic effectiveness for insulin resistant states.