**Abstracts**

**PL-1**

**Fibroblast growth factor 21: a multifarious regulator of glucose metabolism**

K. SL. Lam
Department of Medicine, The University of Hong Kong, Hong Kong

FGF21, fibroblastic growth factor 21, a hormone produced from the liver, adipose tissue, pancreas and muscles, has in recent years emerged as a major regulator of glucose, lipid and energy metabolism. It exerts pleiotropic beneficial effects in multiple target organs, mediated through endocrine, paracrine and autocrine actions. Paradoxically, circulating levels are found to be raised in obesity and type 2 diabetes, suggesting the presence of FGF21 resistance or a compensatory response to the underlying metabolic disturbance or tissue injury. Indeed, FGF21 resistance has been demonstrated in the adipose tissue of both humans and mice, consequent to the enhanced expression of pro-inflammatory cytokines and microRNA-34a. In the pancreatic islet, hyperglycemia also impairs the action of FGF21, through repression of the co-receptor protein β-Klotho, probably via reduced PPARγ expression. Epidemiologically, a raised serum FGF21 level has been shown to be as good as the oral glucose tolerance test in predicting incident type 2 diabetes. In contrast, serum FGF21 levels are reduced in autoimmune diabetes, including LADA and type 1 diabetes, with the levels correlating positively with C-peptide levels and inversely with titres of autoimmune antibodies related to β-cell destruction. The role of FGF21 in the bodily response to tissue injury has been demonstrated in the liver and the pancreas, in part through the enhancement of anti-oxidant capacity. Studies in FGF21 knockout mice suggest that FGF21 plays a physiological role in protecting against islet inflammation, hyperplasia and dysfunction, in the context of obesity and diabetes. On the other hand, rat islets treated with FGF21 are partially protected from glucolipotoxicity and cytokine-induced apoptosis, and FGF21 treatment prevents islet destruction and onset of hyperglycemia in New Zealand obese mice. Furthermore, administration of FGF21 analogs of sustained action to db/db mice has been shown to enhance pancreatic insulin content, islet number and glucose-dependent insulin secretion, while reducing plasma glucagon levels. FGF21 may also reduce lipotoxicity to the β-cells through its beneficial effects on lipid metabolism, including the suppression of lipolysis and hepatic sterol regulatory element-binding protein-2, and the enhanced expression of adiponectin. The physiological relevance of circulating FGF21 in humans is supported by its genetic regulation, its responses to meal-related fatty acid changes and treatment with PPARα/PPARγ agonists. The therapeutic potential of FGF21-targeting approaches in the treatment of diabetes and other obesity-related cardiometabolic disorders, using FGF21 mimetics or enhancers, is under active research in animal and human studies.

**PL-2**

**Role of islet produced incretins**

M. Y. Dorath
University Hospital of Basel, Switzerland

According to the classical incretin concept, GLP-1 is viewed as a hormone produced in the intestinal L cells and acting via the circulation on satiety in the brain, gut motility, and insulin and glucagon secretion in the pancreatic islet. However, in contrast to typical hormones, plasma levels of GLP-1 are relatively low with a very short half-life. Furthermore, GLP-1 is rapidly inactivated by DPP-4 in the vicinity of L cells within <1 min from the secretion of the gut peptide. This rapid metabolism of GLP-1 raises questions about how its effects are mediated on target organs such as pancreatic β-cells. In this presentation, we will discuss possible alternative pathways for the incretin effect on pancreatic islet. These involve L-cell-derived GLP-1 via neuronal activation and β-cell-derived GLP-1 via auto/paracrine effects. The role of inflammatory pathways in this context will also be discussed. The data will highlight the importance of β-cell-derived GLP-1 for glucose homeostasis during metabolic stress, and this may influence the clinical use of systemic GLP-1 agonists versus stabilizing local β-cell-derived GLP-1 by DPP-4 inhibitors in type 2 diabetes.

**S-1-1**

**The role of genetics in beta-cell function of diabetes**

C. Hu
Shanghai Jiao Tong University Affiliated Sixth People’s Hospital

Diabetes mellitus is a complex disease characterized by hyperglycemia resulting from defective insulin secretion and action. Familial aggregation, ethnic heterogeneity and twin studies have indicated that genetic factors are of pivotal importance in the onset of diabetes. With the evolution of genetic techniques, family-based linkage analysis, candidate-gene approaches and genome-wide association studies (GWAS) have been widely used to elucidate the genetic characteristics of diabetes in Chinese population.

As a form of polygenic diabetes, over 100 genes were identified to be susceptible genes of type 2 diabetes. Most of them were linked to beta-cell function. Moreover, we constructed the genetic risk score (GRS) with multiple loci to test the effect of genetics in the 9-year follow-up perspective cohort. The weighted GRS can predict blood glucose deterioration through its effect on beta-cell function in the Chinese population. Individuals in the intermediate- or high-weighted GRS group exhibited progressive deterioration of beta-cell function. Therefore, this GRS model can be used to predict the high-risk population of diabetes in the 9-year follow-up perspective cohort.

For monogenic diabetes, we have explored more than 80 novel causative mutations responsible for special types of diabetes like maturity onset diabetes of the young (MODY), Wolfram syndromes and neonatal diabetes mellitus through whole-exome sequencing. We identified a causative mutation in INS p.Ala2Thr responsible for MODY10 in a Chinese population. We demonstrated that this mutation can activate the PERK-eIF2α-ATF4, IRE1α-XBP1, and ATF6 pathways as well as upregulate ER chaperones. Therefore, this novel mutation INS p.Ala2Thr affected β-cell function by inducing ER stress.

In summary, genetic data in Chinese population demonstrated that the main cause of diabetes in China is impaired beta-cell function.

**S-1-2**

**The genetic and clinical determinants of insulin secretagogue efficacy and the potential mechanisms**

Q. Ren
Department of Endocrinology and Metabolism, Peking University People’s Hospital

Sulfonylureas are among the most widely used oral hypoglycaemic agents in the treatment of type 2 diabetes. However, there is also considerable inter-individual variation in the response to sulfonylureas, and the mechanisms underlying these different responses to sulfonylureas are still not fully understood. In this presentation, we reviewed the clinical biomarkers and genetic background influencing the antidiabetic efficacy of sulfonylurea in type 2 diabetic patients. And we investigated the clinical and genetic biomarkers in patients with type 2 diabetes. All the participants in our study were enrolled from a clinical trial that had a prospective design and strict drug dose adjustment and data collection. We also assessed the potential utility of combining information from multiple genetic determinants and clinical biomarkers to predict sulfonylurea response. Our study may give evidence for personalized medicine in type 2 diabetes in the future.

**S-1-3**

**Insulin secretion function index as an independent predictor of major adverse cardiovascular events in a long-term population-based Chinese cohort**

Y. C. Woo
Queen Mary Hospital & Hon. Clinical Associate Professor in Medicine, Hong Kong

Insulin resistance has an important pathogenic role not only in diabetes, but also in cardiovascular diseases. Several large population-based studies have shown that hyperinsulinemia, a surrogate marker for insulin resistance, predicts incident coronary artery disease. It has been demonstrated that about 25% of an apparently healthy population is insulin resistant enough to have significantly increased risk of coronary heart disease. The homeostasis model assessment (HOMA), based on plasma levels of fasting glucose and insulin, has been widely validated and applied for quantifying insulin resistance (HOMA-IR) as well as β-cell function (HOMA-beta). While both models have been shown to associate with
Abstracts

3-D human islet histology in health and disease
S.-C. Tang
National Tsing Hua University, Taiwan

Scattered in the pancreatic parenchyma, the endocrine islets rely on neurovascular networks for signal relay to regulate and synchronize hormone secretion. However, due to the dispersed nature of the network architecture, standard microscopy-based histology cannot provide a global view of the islet tissue networks in health and disease. In this talk, I will discuss the approach of using 3-D histology with tissue clearing to examine the human islet microstructure, vasculature, and innervation in an integrated fashion. Examples will be given on how to visualize the remodeling of pancreatic and islet microenvironment in fatty infiltration, early duct lesion formation, and neuroendocrine tumor. The long-term goal of our work was to establish “3-D histology” as a preferred method for the pancreatic and islet tissue analyses.

Simplified pancreatic tissue clearing technology and three-dimensional (3D) confocal microscopy imaging to understand the role of Exchange Protein Activated by cAMP in pancreatic beta cells and islets
S. K. Chung
The University of Hong Kong, Hong Kong

BACKGROUND: cAMP is a second messenger that is involved in many biological processes. In 1998, a new target of cAMP called Exchange Protein Activated by cAMP (Epac) was discovered and thought to be responsible for cellular signals independent of the cAMP-protein kinase A (PKA) and cyclic nucleotide-gated ion channel signaling pathway. Previously, Epac1 homozygous knockout (Epac1−/−) mice were generated and found to develop metabolic syndromes with impaired glucose-stimulated insulin secretion (GSIS) and defect in pancreatic beta-cell proliferation and differentiation from ES cells with Epac1 deletion. Here, we hypothesize that Epac1 plays an important role in beta cells of pancreatic islet and justify the detailed in vivo three-dimensional (3D) imaging analysis and quantification of entire pancreatic islets and beta cells from wild-type (Epac1 +/+ ) and Epac1 knockout (Epac1−/−) mice to determine the effect of Epac1 deficiency and metabolic syndrome on the cytoarchitecture of pancreatic islets.

MATERIAL AND METHOD: The simplified faster tissue clearing technique (CLARITY) protocol was developed to minimize the pre-existing optical barriers, light scattering and light absorption in order to achieve a high-resolution 3D imaging of the entire pancreas by confocal microscopy. To determine pancreatic tissue and islet integrity, we used 6-week-old transgenic mice with overexpression of green fluorescent protein (GFP) under the mouse insulin 1 promoter (MIP). These MIP-GFP transgenic mice allowed a visualization of GFP-labeled pancreatic insulin-secreting beta cells and islets in three-dimensional (3D) imaging using the confocal microscope. To further determine the role of Epac1 in pancreatic islets and beta cells in entire pancreas, wild-type (Epac1 +/+ ) and Epac1−/− mice were crossed with MIP-GFP mice.

RESULTS: The simplified CLARITY protocol for the smaller pancreas from 6-week-old mice was able to render the pancreas transparent, preserve endogenous fluorescence and allowed high-resolution 3D confocal microscopy imaging. Analysis revealed that 6-week-old Epac1−/− mice have significantly higher beta-cell number or mass than age-matched Epac1 +/+ mice.

CONCLUSION: The new, simple and direct CLARITY method and 3D imaging of pancreas from MIP-GFP mice with or without Epac1 are useful tool to study the effect of metabolic syndrome on the cytoarchitecture of entire pancreatic islets and beta cells. The present study complements our previously reported study on the quantitation of pancreatic islets of Epac1 +/+ and Epac1−/− mice using a conventional histological methodology.

3-D histology

S-1-4

S-1-5

S-2-2

The mechanism of incretin responsiveness in pancreatic beta cells
N. Yokoi
Division of Molecular and Metabolic Medicine, Kobe University Graduate School of Medicine, Kobe, Japan

The incretins, GLP-1 and GIP, which are secreted from enteroendocrine cells in response to meal ingestion, play critical roles in preventing postprandial hyperglycemia by potentiating insulin secretion from pancreatic β cells. By a metabolomics-based approach using incretin-responsive and -unresponsive MIN6 β-cell lines, designated MIN6-K8 and -K20, respectively, we previously found that glucose produced by glucose stimulation acts as a key signaling molecule in incretin-induced insulin secretion (IIS) (Gheni et al., Cell Rep, 2014). Cytosolic glutamate is produced through the malate-aspartate (MA) shuttle linked to glycolysis and is transported into insulin granules through vesicular glutamate transporters (VGLUTs) via incretin/cAMP signaling, resulting in potentiation of insulin secretion. To confirm this finding, we recently established MIN6-K8 β-cell lines deficient for aspartate aminotransferase 1 (AST1), an enzyme in the MA shuttle, and for VGLUT1, 2, and 3 by CRISPR/Cas9 system (Murao et al., PLoS One, 2017). AST1 knockout (KO) cells or VGLUT1, 2, and 3 triple KO cells showed impaired IIS, providing direct evidence that β-cell glutamate signaling is essential in IIS.

In the course of our study of the MIN6-K20 β-cell line which is incretin unresponsive under monolayer culture (called K20-MC), we found that formation of spheroïd clusters (called K20-SC) drastically induced incretin responsiveness (Iwasaki et al., J Diabetes Investig, 2010). We recently tried to elucidate the mechanism by which incretin responsiveness is acquired using K20-SC as a model (Hashim et al., Diabetes, in press). Expression of the amino acid transporter SNAT5 was downregulated in K20-SC and pancreatic islets of normal mice, whereas it was upregulated in K20-MC and islets of mouse models of obesity and diabetes. Inhibition of SNAT5 significantly increased cellular glutamate content and improved IIS both in K20-MC and in islets of these models. These findings suggest that suppression of SNAT5 activity conveys incretin responsiveness on incretin-unresponsive β cells. Elucidation of the mechanism of SNAT5 regulation may lead to better treatment of diabetes as well as further understanding of incretin unresponsiveness in diabetes and obesity.

S-2-3

GLP-1 secretion and vagal afferent mediate anti-diabetes/obesity effects of rare sugar D-allulose
T. Yada, C. Goiswani and Y. Iwasaki
Kansai Electric Power Medical Research Institute, Center for Integrative Physiology, Chuo-ku, Kobe, Japan

Overeating and arrhythmic feeding promote obesity and diabetes. Glucagon-like peptide-1 receptor (GLP-1R) agonists are effective drugs to treat obesity, as well as anti-diabetes, but their use is limited by the side effects such as nausea, vomiting and rise of mean heart rate. Oral administration of a rare sugar D-allulose (formally called D-psicose), the non-calorie sweetener, induces GLP-1 release and thereby activates vagal afferent signaling. D-allulose reduces food intake and promotes glucose tolerance without causing adverse effects in healthy and obese-diabetic animal models. Subchronic D-allulose, when administered at the light period (LP) onset, ameliorates LP-specific hyperphagia, visceral obesity,
Abstracts

**S-2-4**

Mechanisms of gastric inhibitory polypeptide (GIP) secretion from K cells in response to nutrients

N. Harada, S. Yamane and N. Inagaki
Department of Diabetes, Endocrinology and Nutrition, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Gastric inhibitory polypeptide (GIP) is an incretin secreted from enteroendocrine K cells in response to glucose and fat ingestion. GIP potentiates insulin secretion through the GIP receptor (GIPR) expressed in pancreatic β cells. GIP plays an important role in maintaining blood glucose levels by inducing hypersecretion of insulin in high-fat diet (HFD)-induced obesity. GIPR expressed in adipose tissue is involved in HFD-induced insulin resistance. Thus, GIP is a key hormone for fat accumulation. Gene expression in K cells and mechanisms of GIP secretion remain unclear, because it is difficult to isolate K cells from intestinal mucosal epithelial cells and there is no ideal cell line to evaluate GIP secretion. We generated GIP reporter mice which enable us to visualize and isolate K cells by enhanced green fluorescent protein (EGFP).

Fat ingestion strongly stimulates GIP secretion. From analysis of K cells isolated from GIP reporter mice, free fatty acid receptor GPR40 and GPR120 were highly expressed in K cells. GPR40/GPR120 double-knockout mice showed that GPR40 and GPR120 are essential for GIP secretion after fat ingestion.

GIPR-knockout mice are reported to be reduced fat mass and improved insulin sensitivity associated with aging. Therefore, GIP could be involved in fat accumulation and insulin resistance with aging. However, age-related changes of GIP secretion remain unclear. We found that aged mice showed hypersecretion of GIP under normal diet-fed condition. From analysis of K cells using GIP reporter mice, K cell number was increased in small intestine of aged mice. In aged mice, the mRNA expression levels of GIP and transcriptional factor pancreatic duodenal homeobox-1 (Pdx1) were increased in sorted K cells. GIP mRNA expression and content were decreased in Pdx1 knockdown small intestine treated with intestine-specific gene transfer (IGT). Thus, Pdx1 positively regulates GIP mRNA and K cell number in small intestine. GIP hypersecretion with aging is possibly due to the increase in Pdx1 expression.

In conclusion, several molecules which are involved in GIP secretion can be identified from analysis of K cells isolated from GIP reporter mice.

**S-2-5**

Macro nutrients and long-term glucose-lowering effects of DPP-4 inhibitors in individuals with type 2 diabetes

D. Yabe, H. Kawaata, S. Okamoto and Y. Seino

The incretins, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are secreted from the gut in response to ingestion of various nutrients and enhance insulin secretion glucose dependently to exert their glucose-lowering effects. GLP-1 also ameliorates postprandial glucose excursions by inhibiting glucagon secretion and delaying gastric emptying, while GIP has a role in fat accumulation and subsequently enhances insulin resistance. Therefore, enhancement of GIP function on adipose tissue by chronic fat intake could contribute to deterioration of HbA1c-lowering effects of DPP-4 inhibitors.

Furthermore, we recently found that long-term HbA1c-lowering effects of DPP-4 inhibitors, but not metformin, were also affected by dietary habits. Among those receiving DPP-4 inhibitors for 1 year without any prescription change, some of patients showed deteriorated HbA1c-lowering effects of DPP-4 inhibitors along with subtle but significant increase of body weight. The patients with deteriorated HbA1c-lowering effects of DPP-4 inhibitors tend to have significantly higher total energy intake and fat intake, while carbohydrate and protein intake were similar to those of other patients. The patients also had significantly higher intake of saturated and monounsaturated fats but not polyunsaturated fats. A stepwise multiple regression analysis showed that ΔHbA1c (1-0.5 year) that reflects deterioration of HbA1c-lowering effects of DPP-4 inhibitors was independently correlated with saturated fat intake (B = 0.032, SE=0.010, P < 0.01). These findings were specific to DPP-4 inhibitors and were not confirmed in patients receiving metformin for 1 year without any prescription change. It was known that saturated fats enhance robust secretion of GIP, which facilitates fat accumulation and subsequently enhances insulin resistance. Thus, enhancement of GIP function on adipose tissue by chronic fat intake could contribute to deterioration of HbA1c-lowering effects of DPP-4 inhibitors.

**S-2-6**

The genetic markers for the effect of GLP-1 analogue in patients with poorly controlled type 2 diabetes

C.-H. Lin
Division of Endocrinology and Metabolism, Chang Gung Memorial Hospital, Taoyuan, Taiwan

Type 2 diabetes patients have been proved to have decreased glucagon-like peptide-1 (GLP-1) levels. GLP-1 analogue therapy is associated with improved glycemic control by boosting GLP-1 levels. Nevertheless, the clinical effects are in great diversity for poorly controlled type 2 diabetes patients. Because of the heterogeneous blood glucose status, we have to optimize the insulin therapy in the first priority. Continuous subcutaneous insulin infusion (CSI) or insulin pump is a viable choice for patients with DM who want close-to-physiologic insulin treatment. By means of the insulin pump therapy, standardized sugar control profile in type 2 DM patients could be achieved in a short time. We can further evaluate the clinical response under GLP-1 analogue or not. We firstly set up the model of intensive insulin therapy with CSI for patients with T2D and assessment of GLP-1 analogue response. Secondly, we designed a randomized, two-regimen, comparative study to evaluate the blood glucose regulation and safety of GLP-1 analogue adding on the CSI therapy in poorly controlled T2D patients in Taiwan. In the GLP-1 analogue group, there was a significant higher standard deviation of plasma glucose (SDPG). The adiponectin level was significantly increased with exenatide added on. In analyzing the polymorphisms of GLP1R gene and response to GLP-1 analogue by whole exon sequencing, we found that the single nucleotide polymorphism (SNP) of rs761386 in GLP1R gene and response to GLP-1 analogue by whole exon sequencing, we found that the single nucleotide polymorphism (SNP) of rs761386 in GLP1R gene was significantly associated with changes in the standard deviation of plasma glucose. Performing whole genome methylation assay in poorly controlled T2D patients receiving GLP-1 analogue.

**S-3-2**

FABP4 mediates autoimmune destruction of beta cells by enhancing the crosstalk between innate and adaptive immunity

L. Shu, X. Wu, L. Cheung, Z.-g. Zhou, R. L. Hoo, K. S. Lam and A. Xu
State Key Laboratory of Pharmaceutical Biotechnology, Department of Medicine, The University of Hong Kong, Department of Pharmacology & Pharmacy, The University of Hong Kong, Second Xiangya Hospital, Key Laboratory of Diabetes Immunology, Central South University, National Clinical Research Center for Metabolic Diseases, Changsha, Hunan, China

**INTRODUCTION:** Type 1 diabetes (T1D) is an autoimmune disease resulted from self-destruction of insulin-producing pancreatic beta cells. However, the pathological pathways that trigger the autoimmune destruction remain poorly understood. Our previous clinical studies demonstrated that increased circulating fatty acid binding protein 4 (FABP4), a pro-inflammatory adipokine that links obesity with its related metabolic diseases, is closely associated with beta-cell autoimmunity in patients with T1D. Here, we investigate the role and underlying mechanism whereby FABP4 participates in the pathogenesis of autoimmune destruction of pancreatic beta cells in T1D.
METHODS: FABP4 +/−/NOD and FABP4+/−/NOD mice were generated by crossing FABP4+/− mice with NOD mice (a well-established model with spontaneous development of insulin and autoimmune diabetes) for at least two generations. Biochemical, immunological and in vivo imaging analysis were conducted to determine the dynamic change in the infiltration and activation of immune cells including macrophages and tissue resident memory T (Trm) cells in pancreas of FABP4+/−/NOD and FABP4+/−/NOD mice at different ages. Gain- and loss-of-function studies were employed to evaluate the effects of FABP4 in macrophages and Trm cells on insulitis and diabetes incidence.

RESULTS: A dynamic change in the expression of FABP4 was observed in macrophages and Trm cells in pancreatic islets of NOD mice at different stages. Depletion of macrophages or Trm cells in 8-week FABP4+/−/NOD mice partially alleviated insulitis and reduced the development of diabetes in NOD mice, whereas simultaneous depletion of macrophages and Trm cells prevented the onset of T1D. Flow cytomtery analysis demonstrated that FABP4 deficiency significantly attenuated the polarization and infiltration of pro-inflammatory macrophages (M1) and Trm cells into pancreas, reduced the production of inflammatory cytokines, alleviated islet inflammation and beta-cell destruction.

CONCLUSION: FABP4 activates both innate and adaptive immunity through enhancing the polarization of macrophages to pro-inflammatory M1 subtype and promoting the survival of Trm cells, respectively, thus creating an inflammatory microenvironment leading to the autoimmune attack to beta cells. Pharmacological inhibitors of FABP4 are a promising drug candidate for prevention of autoimmune diabetes.

S-3-3
CD36-dependent redoxosome regulates ceramide-induced pancreatic beta-cell apoptosis

J. S. Moon
Department of Internal Medicine, Yeungnam University College of Medicine

Altered metabolism has been implicated in the pathogenesis of β-cell failure in type 2 diabetes. Several clinical studies have confirmed the positive role of plasma and tissue levels of several ceramide species in the inflammatory and stress responses that mediate type 2 diabetes. However, the inflammatory targets of ceramide remain to be identified. Recently, we reported that CD36, a class B scavenger receptor, initiates glucolipotoxicity-induced beta-cell dysfunction and is involved in the pathogenesis of type 2 diabetes. Here, we investigated the role of CD36-dependent Vav2 signaling and its role in ceramide-induced β-cell inflammation using INS-1 cells treated with C2-ceramide (N-acetyl-sphingosine). Exposure of INS-1 cells to C2 ceramide (50 μM) induced a time-dependent increase in Src-mediated Vav2 tyrosine phosphorylation, which activated GEF activity and induced active Rac1-GTP expression. In turn, Rac1-GTP activation enhanced NADPH oxidase activity and resulted in 4.5-fold increase in ROS production. In addition, NADPH oxidase activity potentiated C2 ceramide-induced nuclear factor NF-κB transcription, which was found to be associated with TXNIP upregulation and suppression of thioredoxin activity. Interestingly, pharmacological inhibition of CD36 by sulfo-N-succinimidyl oleate (SSO) blocked C2 ceramide-induced Sreac activation and reduced Vav2 GEF activity by inhibiting Vav2 tyrosine phosphorylation. Furthermore, downregulation of active Rac1-GTP resulted in decreased NADPH oxidase activity. Under the same conditions, nuclear factor NF-κB transcription was strongly inhibited. Moreover, CD36 inhibition downregulated TXNIP expression and promoted thioredoxin activity. Finally, inhibition of CD36 by SSO or Sreac activation by SU6656 reduced INS-1 cell apoptosis. Taken together, our results revealed a novel role for CD36 during the early molecular events leading to Vav2-mediated Rac1-GTP/NADPH oxidase complex activation, which in turn contributes to ceramide-induced pathogenesis of pancreatic beta-cell dysfunction and failure.

S-3-4
Regulation of compensatory β-cell proliferation by vagal nerve signals

J. Imai
Tohoku University Graduate School of Medicine

In insulin-resistant states such as obesity, pancreatic β cells undergo compensatory proliferation and secrete more insulin to prevent blood glucose elevation. Previously, we discovered that pancreatic vagal nerve signals, elicited by activation of the hepatic extracellular signal-regulated kinase (ERK) pathway, play critical roles in triggering compensatory β-cell proliferation during obesity development. We recently recognized that, as part of this inter-organ network system, vagal nerve signals induce compensatory β-cell proliferation through a Forkhead box protein M1 (FoxM1)-dependent mechanism. Anatomically, pre-ganglionic vagal nerves terminate at the pancreatic parasympathetic ganglion (PPG), and signals are likely transmitted to postganglionic neurons. Therefore, we attempted to anatomically analyze PPG using the recently developed tissue clearing method CUBIC. These analyses showed that PPG is frequently located adjacent to pancreatic islets, suggesting significant roles of the vagal nerve system in β-cell physiology. Thus, based on these findings, we explored the molecular mechanisms by which vagal nerve signals enhance β-cell proliferation. FoxM1 and its target genes were upregulated in pancreatic islets of hepatic ERK-activated mice or obese mice, and β-cell proliferation induced by either hepatic ERK activation or obesity was completely blocked in β-cell-specific FoxM1 knockout mice (FoxM1KO mice). Either suppression of the hepatic ERK pathway or pancreatic vagotomy completely blocked activation of β-cell FoxM1 and thereby suppressed β-cell mass increases in mice during obesity development. Furthermore, combined treatment of pancreatic islets with pancreatic vagal nerve-producing factors, such as acetylcholine and PACAP/VIP, significantly upregulated the expression of FoxM1-related genes and enhanced β-cell proliferation, and these effects were completely abolished in islets from FoxM1KO mice. Our results demonstrate how vagal nerve signals induce compensatory β-cell proliferation in obesity settings. Manipulating this mechanism may lead to the development of novel therapeutic strategies aimed at increasing β-cell mass.

S-3-6
Adaptive β-cell proliferation through the FoxM1/PLK1/CENP-A pathway

J. Shirakawa
Department of Endocrinology and Metabolism, Yokohama City University, Japan

Investigation of cell cycle kinetics in mammalian pancreatic β cells has mostly focused on transition from the quiescent (G0) to G1 phase. However, the mechanisms that regulate transition in subsequent phases of the cell cycle in β cells are obscure. Our studies on gene expression microarray analysis of β cells derived from β-cell-specific insulin receptor knockout (βIRKO) mice revealed several novel targets of insulin receptor signaling. In the present study, we focused on a gene, centromere protein A (CENP-A), a histone H3 variant critically involved in centromere, was also attenuated in IRKO group. Furthermore, the expression of FoxM1, a glucokinase activator (GKA) and reduced paKO mice demonstrated significant decrease in cell proliferation in response to acute insulin resistance induced by S961 (insulin receptor antagonist). We validated the significant reduction in expression of CENP-A in βIRKO mouse islets by comparing with wild-type mouse islets. Treatment of wild-type mouse islets or human islets with endogenous insulin increased CENP-A and PLK1 expression. Receptor-mediated insulin signaling promotes DNA-binding activity of FoxM1 to regulate expression of CENP-A and PLK1 by modulating cyclin-dependent kinase-1/2. CENP-A depletion at the centromere is augmented by PLK1 to promote mitosis, while knocking down CENP-A limits β-cell proliferation and survival. Inducible β-cell-specific CENP-A knockout mice (βCenpAKO) developed glucose intolerance, and decreased β-cell proliferation and mass in response to aging, pregnancy, or high-fat diet-induced chronic insulin resistance. CENP-A-deficient β cells from βCenpaKO mice demonstrated significant decrease in cell proliferation in response to a glucokinase activator (GKA) and reduced β-cell mass expansion and proliferation in response to acute insulin resistance induced by S961 (insulin receptor antagonist). Insulin-stimulated CENP-A/PLK1 protein expression is blunted in islets from patients with type 2 diabetes. These data implicate the FoxM1/PLK1/CENP-A pathway as a critical component of β-cell proliferation during pancreatic β-cell adaptation to delay and/or prevent progression to diabetes.
Type 2 diabetes is characterized by relentless decline of beta-cell function, which is clinically manifested by gradual increase in HbA1c despite standard of care according to current treatment guidelines. The pathogenesis of beta-cell dysfunction in type 2 diabetes is very complex and multifactorial. Genetic predisposition to beta-cell dysfunction is very important. However, there is no definitive target agent to address this problem except some types of genetic diabetes such as MODY and neonatal diabetes. In general, glucose toxicity is regarded as a well-established mechanism of progressive decline of beta-cell function in type 2 diabetes. Early intensive insulin therapy to normalize blood glucose levels may lead to remission of type 2 diabetes. Oral anti-diabetes agents also induce the remission of type 2 diabetes, when they are introduced early at the time of the diagnosis of type 2 diabetes. GLP-1 can restore first-phase insulin secretion or normal beta-cell glucose sensitivity in patients with type 2 diabetes. Recently, we showed that the beta-cell function can be improved in patients with type 2 diabetes by alleviating hyperglycemia with SGLT2 inhibitor. Therefore, various methods that can ameliorate hyperglycemia may lead to improvement of beta-cell function in patients with type 2 diabetes, which indicates that a glucocentric approach is important to protect beta cells from further functional decline.

S-4-1
The role of FoxO1 in the differentiation of human embryonic stem cells into islet beta cells
W. Rui
Department of Endocrinology and Metabolism, Peking University Third Hospital

BACKGROUND AND AIMS: Differentiation of human embryonic stem cells (hESCs) into islet beta cells may be one of the most promising strategies to reconstruct pancreatic beta-cell function. However, there are a variety of problems in the current differentiation schemes, such as immaturity of derived beta cells, the unclear mechanisms of differentiation and maturation. Therefore, it is necessary to clarify the molecular mechanism of the differentiation process, which can be much helpful to gain more mature islet beta cells. In this study, we aimed to explore the roles of FoxO1 in this differentiation process and the underlying mechanism.

MATERIALS AND METHODS:
1. The hESCs were differentiated into the insulin-producing cells (IPC) based on the definitive endoderm (DE) stepwise differentiation protocol. The expression of differentiation stage-specific markers and the insulin secretion capability of IPCs were analyzed by using real-time PCR, immunofluorescence and ELISA, respectively. The dynamic change of expression and localization of FoxO1 was detected by PCR and western blot.
2. The stage 3 (S3) cells (pancreatic progenitor), derived from hESCs during the IPC differentiation, were infected with the FoxO1-knockdown or FoxO1 and pancreatic important transcriptional factor Pdx1 by using lentiviral empty vectors or solvent dimethyl sulphoxide were served as the negative controls. The expression of differentiation markers was detected by real-time PCR. The glucose-stimulated insulin release response was evaluated in the IPCs by ELISA.
3. To investigate the possible mechanism of FoxO1 during the hESCs differentiation into the IPCs, we detect the nuclear/cytoplasmic localization of FoxO1 and pancreatic important transcriptional factor Pdx1 by using immunofluorescence and western blot analyses. Furthermore, the S3 cells were collected, and the targets of FoxO1 were analyzed by chromatin immunoprecipitation and sequencing assay.

RESULTS:
1. hESCs could be induced to differentiate into IPCs by using DE stepwise protocol. The expression of pluripotency marker Oct4 decreased, while the levels of pancreatic beta-cell differentiation markers such as Pdx1 and insulin increased during the differentiation stages. We noted that the mRNA and protein levels of FoxO1 were sustainable and stable, while cytoplasmic phosphorylated FoxO1 (p-FoxO1) protein level increased and nuclear FoxO1 protein level decreased as the differentiation processed.
2. FoxO1 knockdown or inhibition of FoxO1 activity by AS1842856 in the S3 cells significantly upregulated the expression of specific markers of pancreatic progenitors and beta cells such as Ngn3, Nkx6.1, Isl1, glucokinase and insulin, and enhanced insulin secretion in response to both low glucose and high glucose in the IPCs. Meanwhile, FoxO1 overexpression exerted the opposite effects.
3. Treatment with AS1842856 increased cytosolic p-FoxO1 protein level and promoted the translocation of Pdx1 into nucleus in the S3 cells.
4. Among the target genes of FoxO1 in the S3 cells, the genes related to cell morphogenesis, neuron differentiation, development and function were significantly enriched.

CONCLUSIONS:
1. The islet beta cells can be induced from hESCs through the DE protocol.
2. FoxO1 knockdown and activity inhibition both promote the differentiation of hESCs into functional islet beta cells, while FoxO1 overexpression inhibits this process.
3. Inhibition of FoxO1 promotes the differentiation of islet beta cells, which may be mediated via the nucleocytoplasmic translocation of FoxO1 and Pdx1.
4. FoxO1, an important transcription factor in human pancreatic precursors, is closely related to neural development and cell differentiation.

S-4-5
The role of pancreatic-derived factor in glucose and lipid metabolism
X. Cao
The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

Pancreatic-derived factor (PANDER, also named as FAM3B) is a cytokine identified in 2002, which is secreted from z and beta cells of pancreatic islets. Increasing evidence suggests that it may serve a hormonal function related to glycemic and lipid metabolism. The initial evaluation indicated PANDER induced apoptosis of beta cells of pancreatic islets. Recent researches showed that PANDER specifically bound to the liver cell membranes. [18F]FB-PANDER dose dependently binded to HepG2.

GLUTag membranes. And liver, pancreas and intestine had the higher original accumulations of radioactivity than brain, heart, spleen, muscle and testis using mice PET-CT scan. Studies have shown that PANDER promoted hepatic glucose production, lipogenesis and resulted in hepatic insulin resistance. In cultured GLUTag cell line, we found that PANDER exposure led to decreased glu mRNA expression and total GLP-1 secretion in a dose- and time-dependent manner. Meanwhile, PANDER reduced p-IRS-1, PI3K, p-Akt, p-GSK3beta protein level in cytoplasm and beta-catenin level in nucleus of GLUTag cells. In mice transplanted with Ad-PANDER, we observed a significant decrease of triglyceride (TG) content in both liver and adipose tissue, as well as a significant decrease of free fatty acid (FFA) content of adipose tissue. Also, the mRNA of glu gene of mice intestine, the serum total GLP-1 and active GLP-1 level significantly decreased. Furthermore, p-IRS-1, PI3K, p-Akt, p-GSK3beta protein level in cytoplasm and beta-catenin level in nucleus reduced in intestine of Ad-PANDER-transfected mice. In a cohort of 212 individuals, the plasma PANDER level was increased with the number of Metabolic Syndrome components and correlated with metabolic score. Multivariable logistic regression analysis has indicated that circulating PANDER was associated with an increased risk ratio of impaired glucose tolerance or diabetes mellitus (odds ratio 2.22, 95% confidence interval 1.15–4.42, \( P = 0.018 \)) after adjustment of the other possible confounders. All together, those data have suggested that PANDER has played a role in the glucose and lipid metabolism and is associated with an increased risk of metabolic disorder, although the mechanisms by which this occurs remain to be elucidated.
POI-3
APPL2 deficiency suppresses glucose-stimulated insulin secretion by disrupting F-actin remodeling in pancreatic β cells
B. Wang1,2, H. Lin3, X. Li4, K. S. Lam1, A. Xu1,2,5 and K. K. Cheng1
1State Key Laboratory of Pharmaceutical Biotechnology, The University of Hong Kong, Hong Kong, China, 2Department of Medicine, The University of Hong Kong, Hong Kong, China, 3Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong, China, 4Department of Endocrinology and Metabolism, Zhongshan Hospital, Fudan University, Shanghai, China, 5Department of Pharmacology & Pharmacy, The University of Hong Kong, Hong Kong, China

Defective glucose-stimulated insulin secretion (GSIS) is a key characteristic of type 2 diabetes at late stage. Previous studies have shown that the adaptor protein APPL1 potentiates first-phase GSIS by modulating the exocytotic machinery SNARE proteins in an Akt-dependent manner, whereas the role of its close homolog APPL2 in pancreatic β cells remains obscure. Here, we show that APPL2 regulates GSIS in a distinct mechanism from APPL1. Mice lacking APPL2 in pancreatic β cells displayed a dramatic reduction of first-phase and second-phase GSIS, resulting in glucose intolerance. APPL2 deficiency had no effect on glucose metabolism, calcium signaling and SNARE protein expression but impaired glucose-stimulated F-actin (Filamentous actin) remodeling in pancreatic islets. Defective GSIS in the islets lacking APPL2 was reserved by the F-actin depolymerizing drug Latrunculin A. Further analysis revealed that RNAi-mediated knockdown of APPL2 largely abolishes glucose-stimulated activation of Rac1, a small GTPase is known to regulate F-actin remodeling in pancreatic β cells. Therefore, deciphering the role of APPL2 in F-actin remodeling might provide a potential therapeutic target for type 2 diabetes by continuously evoking first- and second-phase GSIS.

POI-4
Downregulation of HuD by lower zinc in diabetic pancreatic β cells
C. Kim, W. Kim and E. K. Lee
Department of Biochemistry, College of Medicine, The Catholic University of Korea, Seoul, South Korea

Pancreatic β-cell failure is the major pathophysiologic abnormalities of type 2 diabetes (T2DM).

HuD is a RNA-binding protein expressed in neuron and pancreatic β cells and plays diverse roles in regulations of insulin synthesis, autophagosome formation, and triglyceride (TG) synthesis.

However, the regulatory mechanism governing HuD expression has not been yet elucidated. Here, we present evidence that zinc ion is a novel upstream regulator for HuD expression in pancreatic β cells. Depletion of intracellular zinc ion using N,N,N′-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine (TPEN) resulted in a reduction of HuD mRNA, while zinc sulfate (ZnSO4) treatment raised HuD expression in pancreatic β cells. In silico analysis revealed that the promoter region of HuD has an element responsible for the binding of zinc-dependent transcription factors, and KLF6 was identified as a binding partner by chromatin-immunoprecipitation (ChIP) assay. KLF6 increased HuD expression in zinc-dependent manner by facilitating its transcription. Taken together, our results suggested that the zinc/KLF6 axis is a crucial role for regulating HuD expression in pancreatic β cells.

PO3-3
Correlation between IGF-II and Foxo1 in pancreatic adult stem cell differentiation
C. Sun
The First Hospital of Jilin University

To investigate the mechanism of rapamycin inhibiting the differentiation and proliferation of newborn porcine pancreatic adult stem cells and to explore the therapeutic methods that can effectively reduce the side effects of rapamycin.

Porcine NPCCs were treated with rapamycin alone or in combination with IGF-II, and the caspase-3 and H3-thymidine uptake assays were performed to detect apoptosis and proliferation. The expression of Insulin, PDX-1, NeuroD/Beta2 and Foxo1, a downstream transcription factor of IGFII, was analyzed by RT-PCR and Western blot to evaluate the differentiation ability of pancreatic adult stem cells.

The NPCCs treated with rapamycin inhibited the proliferation of β cells, increased the apoptosis, reduced the insulin secretion, inhibited the expression of PDX-1, NeuroD/Beta2 and Foxo1, a downstream transcription factor of IGFII, and induction of Foxo1 from the cytoplasm to the nucleus of the ectopic. The combined treatment of rapamycin and IGF-II can reduce the side effects of rapamycin, inhibit the decrease of β-cell number and insulin content, repair the expression of insulin, PDX-1, NeuroD/Beta2, inhibit Foxo1 expression and intra-cellular ectopic.

Ablerrant expression of IGF-II and Foxo1 genes is the key inducing factor of rapamycin inhibiting the proliferation and differentiation of NPCCs, and IGF-II treatment can effectively reduce the side effects of rapamycin on NPCC differentiation.

PO4-1
Bone quality in Chinese postmenopausal women with type 2 diabetes – impact of dipeptidyl peptidase-4 inhibitor usage
D. T. W. Lui1, Y. C. Woo1, C. H. Y. Fong2, V. W. K. Chau2, A. W. H. Tsui2, K. M. Y. Yeung2 and K. S. L. Lam2
1Department of Medicine, Queen Mary Hospital, Hong Kong, 2Department of Medicine, the University of Hong Kong

OBJECTIVES: To compare the bone quality in type 2 diabetes (T2D) subjects to those without T2D and evaluate the impact of dipeptidyl peptidase-4 inhibitor (DPP4-i) usage on bone quality in T2D subjects, in view of the potential effects of DPP4 and the incretins on bone biology.

METHODS: We conducted a cross-sectional study of post-menopausal women with T2D subjects recruited from the Hong Kong West Diabetes Registry and non-diabetic subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study, from November 2016 to June 2018. Subjects with fasting glucose 5.6–6.9 mmol/l, 2 hours post-load glucose 7.8–11.0 mmol/l in oral glucose tolerance test, or HbA1c 5.7–6.4% were classified as pre-diabetes and those with normal glucose tolerance as euglycaemia. Bone mineral density (BMD), vertebral fracture assessment (VFA), and trabecular bone score (TBS) were measured by dual X-ray absorptiometry. BMD and TBS in DPP4-i users and non-users with T2D were compared.

RESULTS: Three hundred and sixty subjects were studied: 98 with euglycaemia, 154 with pre-diabetes, and 108 with T2D. Using euglycaemia subjects as reference, pre-diabetes and T2D subjects were significantly older (euglycaemia 60.0 ± 4.4, pre-diabetes 61.8 ± 5.3, and T2D 63.1 ± 5.5 years, p < 0.001) and heavier (euglycaemia 56.1 ± 11.0 mmol/l, in oral glucose tolerance test, or HbA1c 5.7–6.4% were classified as pre-diabetes and those with normal glucose tolerance as euglycaemia. Bone mineral density (BMD), vertebral fracture assessment (VFA), and trabecular bone score (TBS) were measured by dual X-ray absorptiometry. BMD and TBS in DPP4-i users and non-users with T2D were compared.

Frequencies of usage of other oral antidiabetic agents were not significantly different between the two groups. More insulin usage was found among DPP4-i non-users. After adjustment for insulin usage, there was no difference in LS BMD or TBS between the two groups. More insulin usage was found among DPP4-i non-users. However, the regulatory mechanism governing HuD expression has not been yet elucidated. Here, we present evidence that zinc ion is a novel upstream regulator for HuD expression in pancreatic β cells. Depletion of intracellular zinc ion using N,N,N′-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine (TPEN) resulted in a reduction of HuD mRNA, while zinc sulfate (ZnSO4) treatment raised HuD expression in pancreatic β cells. In silico analysis revealed that the promoter region of HuD has an element responsible for the binding of zinc-dependent transcription factors, and KLF6 was identified as a binding partner by chromatin-immunoprecipitation (ChIP) assay. KLF6 increased HuD expression in zinc-dependent manner by facilitating its transcription. Taken together, our results suggested that the zinc/KLF6 axis is a crucial role for regulating HuD expression in pancreatic β cells.

Conclusions: Hong Kong Chinese subjects with T2D had lower TBS, an indirect measure of bone quality, than those without T2D, and the bone quality was not significantly different between those with or without T2D. The impact of DPP4-i usage on bone quality in T2D subjects, in view of the potential effects of DPP4 and the incretins on bone biology, needs further investigation.

CONCLUSIONS: Hong Kong Chinese subjects with T2D had lower TBS, an indirect measure of bone quality, than those without T2D, and the bone quality was not significantly different between those with or without T2D. The impact of DPP4-i usage on bone quality in T2D subjects, in view of the potential effects of DPP4 and the incretins on bone biology, needs further investigation.
P01-6
Blockade of peripheral cannabinoid 1 receptor improves GLP-1-mediated beta-cell function
J. H. Han, E.-Y. Lee, and W. Kim
Department of Molecular Science and Technology, Ajou University
Cannabinoid 1 receptors (CB1Rs) are expressed in peripheral tissues, including islets of Langerhans, where their function(s) is under scrutiny. Using mouse β-cell lines, human islets, and CB1R-null (CB1R−/−) mice, we have now investigated the role of CB1Rs in modulating β-cell function and glucose responsiveness. Synthetic CB1R agonists diminished GLP-1-mediated CAMP accumulation and insulin secretion as well as glucose-stimulated insulin secretion in mouse β-cell lines and human islets, and silencing CB1R resulted in an increase of insulin gene expression in mouse β-cell line. Furthermore, CB1R−/− mice had increased proinsulin, glucokinase and glucose transporter 2 expression in β cells. Our results suggest that CB1R signaling in pancreatic islets may be harnessed to improve β-cell glucose responsiveness and preserve β-cell function. Thus, our findings further support that blocking peripheral CB1Rs would be beneficial to β-cell function in type 2 diabetes.

P05-6
Development of non-invasive monitoring method using direct linking of Ferumoxytol to surface of PEGylated Islet
H. S. Lee1, B. J. Oh1, E. Lee1, Y. Kwon1, H. Kim1, S. Ha1, M. R. Haque2, G. Kim1, S. M. Jin1, M-K. Lee1, Y. Byun3 and J. H. Kim1
1Division of Endocrinology and Metabolism, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Research Institute of Pharmaceutical Science, College of Pharmacy, Seoul National University, Seoul, Republic of Korea, 2Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Republic of Korea

PURPOSE: Islet transplantation is promising therapy for type 1 DM patients. However, this procedure is still not ideal because a large proportion of transplanted islets are rapidly eliminated from the recipients due to immune reactions and non-specific inflammation. Therefore, there is a strong clinical need for real-time assessment of functioning islet mass in the recipients of islet grafts, because the majority of islet injury in clinical islet transplantation takes place before changes in recipient glycemic levels become apparent. The magnetic resonance imaging (MRI) is one of noninvasive monitoring methods. Contrast agent is very important to visualization of hypointense spots located at the transplantation site at days 6 and 20. These results indicate that subcutaneous transplantation of MIN6 cells embedded in temperature-sensitive mPEG-Ala hydrogels is feasible. Moreover, the IVIS and MR imaging are useful tools for detecting and monitoring cells at the subcutaneous site.

PP1-3
Function of skeletal muscle mitochondria during insulin resistance
H. Wang
The First Hospital of Jilin University

BACKGROUND: This study aims to investigate the functional changes in skeletal muscle mitochondria during insulin resistance (IR).

METHODS: Insulin resistance, type 1 diabetes mellitus (T1DM), and type 2 diabetes mellitus (T2DM) models were established in rat. Insulin resistance status was evaluated using hyperinsulinemia-glucose clamp experiments. Skeletal muscle mitochondria were extracted, and the respiratory function and fatty acid β-oxidation response to glucose stimulation. The focus of the current study is to use mPEG-poly(alanine) hydrogels to encapsulate MIN6 islet cells in order to validate the use of these hydrogels for cell therapy in diabetes treatment. Proper mixing of growth factors or cells with the copolymer solution allows them to be uniformly distributed within the structure after in situ gelation. It is also possible to mold the scaffold in situ to attain the desired location to fit the β-cell survival.

According to the in vitro study, after culturing for 14 days, the viability of MIN6 cells in mPEG-Ala hydrogel was comparable with those in medium, either assayed by MITT or LIVE/DEAD. Furthermore, static incubation showed that both had comparable insulin secretion in response to glucose stimulation. For in vivo experiments, positive IVIS images and insulin staining of tissue histology were observed up to 41 days after transplantation. Meanwhile, the graft of CSPIO-labeled MIN6 cells was visualized on Magnetic Resonance (MR) scans as distinct hypointense spots located at the transplantation site at days 6 and 20. These results indicate that subcutaneous transplantation of MIN6 cells embedded in temperature-sensitive mPEG-Ala hydrogels is feasible. Moreover, the IVIS and MR imaging are useful tools for detecting and monitoring cells at the subcutaneous site.

P06-5
Oligo (Alanine)-modified methoxy-poly (ethylene glycol) hydrogels: an in situ gelling system for MIN6 cell cultivation and transplantation
L-M. Chu1, H-C. Lin2, C-Y. Chen1, C-W. Kao2 and J-H. Juang2
1Department of Chemical Engineering, National Taiwan University, Hsinchu, Taiwan, 2Division of Endocrinology and Metabolism and Center for Tissue Engineering, Chang Gung Memorial Hospital and Chang Gung University, Taoyuan, Taiwan

A cell scaffold is an important issue in cell therapy where transplantation of functional tissues is required. Ideal scaffold materials should be able to deliver to any location with minimal invasion and maintain graft function for a long period. Thermosensitive hydrogels are capable of gelling at body temperature by physical interactions from solution state. In previous study, a 3D cell culture matrix was constructed by warming a cell-containing mPEG-poly(alanine) aqueous solution at room temperature to 37°C. Due to their unique characteristics, the hydrogels have been demonstrated to support good cell viability, proliferation, and as a source for delivery of protein growth factors.

The MIN6 cell line derived from a mouse insulinoma is one of a few cell lines that display characteristics of pancreatic β cells, such as insulin secretion in response to glucose stimulation. The focus of the current study is to use mPEG-poly(alanine) hydrogels to encapsulate MIN6 islet cells in order to validate the use of these hydrogels for cell therapy in diabetes treatment. Proper mixing of growth factors or cells with the copolymer solution allows them to be uniformly distributed within the structure after in situ gelation. It is also possible to mold the scaffold in situ to attain the desired location to fit the β-cell survival.

According to the in vitro study, after culturing for 14 days, the viability of MIN6 cells in mPEG-Ala hydrogel was comparable with those in medium, either assayed by MITT or LIVE/DEAD. Furthermore, static incubation showed that both had comparable insulin secretion in response to glucose stimulation. For in vivo experiments, positive IVIS images and insulin staining of tissue histology were observed up to 41 days after transplantation. Meanwhile, the graft of CSPIO-labeled MIN6 cells was visualized on Magnetic Resonance (MR) scans as distinct hypointense spots located at the transplantation site at days 6 and 20. These results indicate that subcutaneous transplantation of MIN6 cells embedded in temperature-sensitive mPEG-Ala hydrogels is feasible. Moreover, the IVIS and MR imaging are useful tools for detecting and monitoring cells at the subcutaneous site.

PP1-7
Modulation of adipocyte differentiation by M5T1-mediated phosphorylation of miRNA-binding protein AUFl
J.-H. Yoon, M. Fomin and K.-W. Min
Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC, USA

mRNA degradation is an important step in post-transcriptional gene expression regulation during cellular proliferation and differentiation. One way in which
mRNA decay can be regulated is the MST1 (Mammalian Ste20-like kinase 1) pathway, where MST1 controls the activity of RNA-binding proteins whose binding ultimately regulates mature mRNA abundance. Through the action of those miRNAs, MST1 also ultimately controls mRNA-mediated mRNA decay. Previously, we have shown that the phosphorylation of Dcp2 by Ste20 in yeast and eIF4E by MST1 in mammals inhibits mRNA decay and translation, suggesting that MST1 has a certain role in posttranscriptional gene regulation. Here, we identified novel signaling pathways wherein MST1 is activated in adipocytes to modulate the abundance of mature miRNAs and their target mRNAs by differential phosphorylation of MST1's target miRNA-binding proteins. The consequences of these phosphorylation events include upregulation of specific mature mRNA, suppression of abundance of mRNAs targeted by those miRNAs, and suppression of adipocyte differentiation. Our findings indicate that MST1 signaling pathways control the stability of target mRNAs by differential phosphorylation of miRNA-binding proteins and implicate that MST1 is a crucial factor for the degradation of mature mRNAs during adipocyte differentiation.

mRNA degradation is an important step in posttranscriptional gene expression regulation during cellular proliferation and differentiation. One way in which mRNA decay can be regulated is the MST1 (Mammalian Ste20-like kinase 1) pathway, where MST1 controls the activity of RNA-binding proteins whose binding ultimately regulates mature mRNA abundance. Through the action of those miRNAs, MST1 also ultimately controls mRNA-mediated mRNA decay. Previously, we have shown that the phosphorylation of Dcp2 by Ste20 in yeast and eIF4E by MST1 in mammals inhibits mRNA decay and translation, suggesting that MST1 has a certain role in posttranscriptional gene regulation. Here, we identified novel signaling pathways wherein MST1 is activated in adipocytes to modulate the abundance of mature miRNAs and their target mRNAs by differential phosphorylation of MST1's target miRNA-binding proteins. The consequences of these phosphorylation events include upregulation of specific mature mRNA, suppression of abundance of mRNAs targeted by those miRNAs, and suppression of adipocyte differentiation. Our findings indicate that MST1 signaling pathways control the stability of target mRNAs by differential phosphorylation of miRNA-binding proteins and implicate that MST1 is a crucial factor for the degradation of mature mRNAs during adipocyte differentiation.

PO5-1

Effects of imeglimin on insulin secretion, beta-cell proliferation, or apoptosis in mouse islets

J. Li, J. Shikarawa, Y. Togashi, T. Oyakuma, M. Kyehara and Y. Terasuchi

Department of Endocrinology and Metabolism, Graduate School of Medicine, Yokohama City University, Japan

Imeglimin, an anti-hyperglycemic agent, is thought to have beneficial effects on mitochondrial function of the liver, skeletal muscle, and pancreatic beta cells in type 2 diabetes. However, the molecular mechanisms of imeglimin action have been still unknown. The aim of this study was to clarify the effects of imeglimin on insulin secretion, beta-cell proliferation, or beta-cell apoptosis in mouse islets. Mouse islets were isolated from 8- to 10-week-old male C57BL/6J wild-type mice. Glucose-induced insulin secretion from isolated islets was examined at concentrations of 3.9, 11.1, or 16.7 mM glucose in the presence or absence of imeglimin (0.1 mM or 1.0 mM) or liraglutide (100 nM). Liraglutide increased glucose-induced insulin secretion from islets under 11.1 mM glucose (4.2-fold, n = 5, P < 0.05) or 16.7 mM glucose (4.1-fold, n = 5, P < 0.01). Treatment of islets with 1.0 mM imeglimin, but not 0.1 mM imeglimin significantly increased insulin release in response to glucose (2.1-fold, n = 5, P < 0.01 at 11.1 mM glucose, 1.9-fold, n = 5, P < 0.05 at 16.7 mM glucose). Both liraglutide and imeglimin had no effect on the insulin content in the islets compared to control. Treatment with a combination of liraglutide and 1.0 mM imeglimin did not show an additional increase in insulin secretion compared to the treatment with liraglutide alone (1.1-fold, n = 6, P = 0.53 at 11.1 mM glucose), beta-cell proliferation was evaluated by the proportion of EdU-incorporated insulin-positive beta cells in islet sections. TUNEL staining was performed to assess beta-cell apoptosis. Liraglutide and 1.0 mM imeglimin, but not 0.1 mM imeglimin, significantly increased EdU-incorporated proliferating beta cells in the islets under 11.1 mM glucose (1.02-fold in vehicle control, 2.21-fold in liraglutide, n = 5, P < 0.01 vs. vehicle; 1.46-fold in 1.0 mM imeglimin, n = 5, P < 0.01 vs. vehicle). The proportion of TUNEL-positive apoptotic beta cell was increased by high glucose (0.62% in 3.9 mM glucose, 1.07% in 11.1 mM glucose, n = 4, P < 0.05). Liraglutide and 1.0 mM imeglimin prevented beta-cell apoptosis induced by high glucose (57% of control in liraglutide, n = 4, P = 0.07; 32% of control in imeglimin, n = 4, P < 0.01). Collectively, imeglimin enhanced glucose-induced insulin secretion, increased beta-cell proliferation, and prevented beta-cell apoptosis in a glucose-dependent manner.

PP2-7

Thyroid hormone in normothyroid diabetes: a population-based study

K. Qin, Q. Wu, L. Gao, Y. Huaq, F. Zhang, H. Yang, J. Tan, Z. An, S. Li and S. Li

1Physical Examination Center, West China Hospital,Sichuan University, Chengdu, China
2Chinese Evidence-based Medicine Center, West China Hospital,Sichuan University, Chengdu, China
3Department of Endocrinology and Metabolism, West China Hospital,Sichuan University, Chengdu, China
4General Medical Center, West China Hospital, Sichuan University, Chengdu, China

OBJECTIVE: To investigate the thyroid hormone changes in patients with newly diagnosed and previously diagnosed diabetes.

METHODS: Participants aged 20 to 79 with the negative thyroid autoantibodies and free of a history of thyroid disease were recruited from candidates undergoing health examination in West China Hospital, Sichuan University in 2016. Participants were divided into the groups of diabetes and the non-diabetes according to the fasting serum glucose levels. The group of diabetes was further divided into the group of newly diagnosed diabetes (NDD) and the group of previously diagnosed diabetes (PDD). Independent t-test and the multivariate Logistic regression were used in comparing the thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3) and FT4/FT3 ratios between groups.

RESULTS: We recruited 32,557 participants, 7% and 36.3% of which were diabetes and NDD, respectively. FT4 and FT4/FT3 in the group of diabetes were higher than those in the group of non-diabetes (FT4: 17.09 ± 2.35 vs. 16.62 ± 2.22 pmol/L, P = 0.001; FT4/FT3: 3.47 ± 0.56 vs. 3.31 ± 0.48, P = 0.004), while FT3 was lower (5.00 ± 0.97 vs. 5.07 ± 0.65 pmol/L, P < 0.001). Compared to the NDD group, FT4 and FT4/FT3 in the PDD group were elevated (16.96 ± 2.30 vs. 17.23 ± 2.41 pmol/L, P = 0.008; FT4/FT3: 3.38 ± 0.56 vs. 3.58 ± 0.55, P < 0.001), while FT3 in the PDD group was reduced. The Logistic regression suggested that compared to the non-diabetes group, the per-one-odds ratio (OR) of TSH, FT4, FT4/FT3 and FT3 in the diabetes group was 0.88 (95%CI: 0.82–0.95), 1.11 (95% confidence interval (CI): 1.08–1.14), 2.05 (95% CI: 1.81–2.32) and 0.85 (95% CI: 0.78–0.93), respectively. Compared to the NDD group, the per-one-OR of TSH, FT4, FT4/FT3 and FT3 of the PDD group was 0.81 (95% CI: 0.71–0.92), 1.08 (95% CI: 1.04–1.12), 1.76 (95% CI: 1.49–2.08) and 1.01 (95% CI: 0.92–1.12), respectively.

CONCLUSION: Diabetes was associated with changes in the thyroid hormone levels in different stages. Long-term diabetes may be associated with suppressed TSH level and elevated FT4/FT3 ratio, indicating a reduced basal metabolic status.
PO1-1
Thiazolidinediones (TZDs) enhance insulin secretory response via GPR40
M. Hwang, H-S. Kim, J-H. Hong, S-M. Jin, K-Y. Hur, J-H. Kim and M-K. Lee
Division of Endocrinology and Metabolism, Samsung Biomedical Research Institute, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

BACKGROUND: Thiazolidinediones (TZDs) are synthetic PPARγ ligands which enhance insulin sensitivity and could increase GSIS from β cell. However, the functional role and mechanism(s) of action in pancreatic β cell have not been investigated in detail.

METHODS & RESULTS: To identify potential molecular mechanisms of PPARγ in the pancreatic β cell, we treated INS-1 cells with TZDs. TZDs significantly increased GSIS and GPR40 expression in INS-1, but not in cells transfected with PPARγ shRNA. Using RT-PCR and western blot, we detected expression of the GPR40, a G protein-coupled receptor recently identified as a receptor for free fatty acids and TZDs in INS-1 cells. GPR40 mRNA and protein levels were increased by 80 and 60%, but not in cells transfected with GPR40 or PPARγ shRNA. GPR40 overexpression without TZD led to a significantly enhanced insulin secretion in response to glucose, and GPR40 activity, measured by fluorescence spectroscopy, was also significantly increased. The increase of GPR40 by TZDs was associated with the increased glucagon-like peptide-1 (GLP-1) sensitivity in INS-1 cells. TZDs also showed protective effects against the palmitate-induced aggravation in insulin secretion.

CONCLUSION: TZDs significantly increased the incretin sensitivity and protected against the lipotoxicity through the stimulation of GPR40 expression in pancreatic β cell.

PO2-6
Activation of GLP-1 receptor inhibits hepatocarcinogenesis through CAMP-PKA-EGFR-STAT3 axis
M. Zhou1,2,3, T. M. Hadl4,5, Anthony, W. Chan6,8, Y. Huang1,4, A. S. Cheng1,4,5,7 and G. Xu1,8
1Division of Endocrinology and Metabolism, Samsung Biomedical Research Institute, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, 2Centre for Cancer Biology, University of South Australia, Australia, 3Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong, China, 4School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China, 5Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen, China, 6Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Hong Kong, China, 7State Key Laboratory of Digestive Disease, The Chinese University of Hong Kong, Hong Kong, China, 8Diabetes Center and Department of Endocrinology, The Eighth Affiliated Hospital of Sun Yat-sen University, Shenzhen, China

Type 2 diabetes contributes to the development of hepatocellular carcinoma (HCC), shown by the accumulating evidences from both clinical and experimental studies. Exendin-4 (Ex-4), a potent anti-diabetes drug targeting glucagon-like peptide-1 receptor (GLP-1R), was reported to protect the liver from non-alcoholic fatty liver disease (NAFLD). However, it is unknown about the Ex-4 function and GLP-1R signaling in HCC. In this study, we investigated the effect of Ex-4 in diethylnitrosamine (DEN)-induced HCC mice consuming normal control or high-fat high carbohydrate diet. Ex-4 treatment significantly improved obesity-induced hyperglycemia and hyperlipidemia. Furthermore, Ex-4 administration dramatically reduced HCC multiplicity and tumor weight in DEN-treated mice, in which suppressed proliferation and induced apoptosis were confined to tumor cells. In addition, it is found that high GLP-1R expression, subsequent activation of cyclic AMP (AMP) and protein kinase A (PKA) are critical to the tumor suppressive effects of Ex-4. The tumor inhibitory effects of Ex-4 were only observed in GLP-1R-abundant human hepatoma cell lines. Inhibition of PKA activity attenuated the growth suppressive effect of Ex-4 in hepatoma cells. Importantly, Ex-4 also downregulated epidimal growth factor receptor (EGFR) and signal transducer and activator of transcription 3 (STAT3), which are the downstream effectors of CAMP/PKA signaling, resulting in suppression of multiple STAT3-targeted pro-oncogenes including c-Myc, cyclin D1, survivin, Bcl-2 and Bcl-xl. Besides the regulation of oncogene signaling, we also found Ex-4 effectively suppressed inflammatory and fibrotic phenotypes in mice fed with methionine-choline deficient (MCD) diet and choline-deficient ethionine-supplemented (CDE) diet, respectively. MCD diet-induced inflammatory symptoms were effectively improved by Ex-4 treatment, evidenced by reduced lobular inflammation, TNF-alpha and MCP1 mRNA expressions. In CDE diet induced liver fibrosis model, Ex-4 treatment remarkably alleviated hepatotoxic steatosis and fibrosis and diminished the hepatic progenitor cell amount. In summary, Ex-4 elicits protective functions against DEN induced HCC through CAMP-PKA/EGFR-STAT3 signaling, suggesting Ex-4 administration as a novel approach to reduce HCC risk in diabetic patients.
Abstracts

POI-5  
Global and pancreatic beta-cell-specific Ogt deletion revealed multiple effects of O-GlcNAcylation in glucose metabolism  
S. Ida1, O. Sekine1, N. Ohashi1, S. Kame1, K. Iwasaki2, N. Harada2, N. Inagaki2, S. Ugi3, H. Maegaawa4 and K. Morino1  
1Department of Medicine, Shiga University of Medical Science, Otsu, Shiga, Japan,  
2Department of Diabetes, Endocrinology and Nutrition, Graduate School of Medicine, Kyoto University, Kyoto, Japan

O-GlcNAcylation is one of the post-translational modifications that is characterized by the addition of N-acetylgalactosamine to various proteins by O-GlcNac transferase (Ogt) and serves as “an intracellular nutrient sensor” by modulating various cellular processes. Although it has been speculated that O-GlcNAcylation is associated with glucose metabolism, its exact roles in whole body glucose metabolism and the relationship with the pathogenesis of diabetes have not yet been fully elucidated. To examine the role of O-GlcNAcylation in glucose metabolism, tamoxifen-inducible global Ogt knockout mice were generated by cross-breeding Ogt-flox mice with R26-Cre-ERT2 mice. Various metabolic phenotypes including glucose metabolism were evaluated in both models. To evaluate the relationship between O-GlcNAcylation and the pathogenesis of diabetes, we performed immunohistochemical analysis of O-GlcNAcylation over time in pancreas of LETO (Control) and OLETF (Obese type 2 diabetes model) rats.

Tamoxifen-inducible global Ogt knockout mice exhibited a lethal phenotype with severe weight loss, hypoglycemia and hypoproteinemia, although food intake was preserved. Moreover, pancreatic beta-cell-specific Ogt knockout mice displayed transient hypoglycemia associated with enhanced insulin secretion and accelerated adiposity, followed by subsequent hyperglycemia with insulin depletion accompanied by beta-cell apoptosis. In diabetic rat models, immunohistochemistry for O-GlcNAcylation (RL2) partially decreased in pancreatic islets of 50-week-old OLETF rats. In this study, we demonstrated that O-GlcNAcylation in adult mice is essential for survival and glucose homeostasis maintenance and is important for pancreatic beta-cell function and survival. Moreover, the result from diabetic rat model suggested a relationship between O-GlcNAcylation and diabetes. Our results provide new insights into O-GlcNAc biology and beta-cell physiology in glucose metabolism.

PP1-4  
Loss of Epac2A KO enhances resistance to cold via leptin-mediated mechanism  
S.-K. Shin, S.-E. Song, S. Y. Kim, H.-W. Cho, S.-S. Im, J.-H. Bae and D.-K. Song  
Department of Physiology & Obesity-related Disease Research Center, Keimyung University School of Medicine, Daegu, Korea

OBJECTIVE: Leptin is a critical hormone that links energy intake and storage to energy consumption. Non-obese humans and animals are responsive to exogenous leptin, which increases food satiety and energy consumption. In Epac2A KO mice, plasma leptin level is found to be higher as compared with wild-type mice (C57BL/6J, WT). Thus, we investigated whether Epac2A KO mice are resistant to cold ambient temperature.

METHOD: Seven-week-old WT and Epac2A KO mice were placed in an individual incubator for a 72-hr period to 4℃. Body temperature was measured every 2 hrs throughout this period, after 72 hrs of cold exposure, mice were euthanized and tissues were harvested. Mice were individually caged during experiment and fed ad libitum with free access to tap water. All values are expressed as mean ± SEM. Statistical analysis was performed by using SPSS.

RESULTS: During cold exposure, Epac2A-KO mice better-maintained body temperature in response to cold stress, and the decrease in body temperature induced by cold exposure was smaller than that in WT mice. Serum creatine kinase concentrations in the two groups were not different, which is a parameter of compensatory shivering. The thermogenic genes uncoupling protein-1 (UCP-1) and peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) in interscapular brown adipose tissue, interscapular white adipose tissue and epididymal fat showed greater elevation in the KO mice. The KO mice also exhibited higher expression of type 2 deiodinase in the brown and white adipose tissues. Plasma levels of T3 and T4, and T3/T4 ratio were parallel to these results. The decrease in subcutaneous, epididymal, perirenal and retroperitoneal fat depots by cold stress was dramatically greater in the KO mice.

CONCLUSION: Epac2A KO mice are more resistant to cold stress with mechanisms independent of shivering effects. A mechanism may be leptin-mediated increase in sympathetic nerve activity to promote adipocytic lipolysis and thermogenesis as well as thyroid hormonal signaling.

PP2-3  
Role of adaptive and innate immunity in type 2 diabetes mellitus  
S. Yang  
Department of Endocrinology and Metabolism, The First Hospital of Jilin University, Changchun, Jilin, China

Type 2 Diabetes Mellitus (T2DM) which is characterized by abnormally elevated levels of blood glucose due to impaired insulin secretion, glucose intolerance, and hyperglycemia has become a health burden worldwide. Nowadays, the pathogenesis of T2DM is considered to be linked to both innate and adaptive immune factors that are recognized as important etiological components in the development of insulin resistance. According to the recognition of the essential role of immune system in the progression of type 2 diabetes mellitus(T2DM), increasing number of issues focused on the effects produced by the abnormal differentiation of immune system. In patients with obesity or T2DM, there were changed proliferations of T cells and macrophages, impaired function of NK cells and B cells, which represented abnormal innate and adaptive immunity. Either the abnormality of innate immunity or adaptive immunity was involved in the progression of T2DM. Although previous studies revealed the functional involvement of T cells in T2DM, and the regulation of metabolism by the innate or adaptive immune system in the pathogenesis of T2DM, respectively, literature reviewing the relevant role of adaptive and innate immunity in the pathogenesis of T2DM in recent years still remains blank. In the current study, we reviewed the relevant research accordingly, purposing to provide new thought for the development of immune therapy for T2DM. A large body of evidence supported linkage between immune system and T2DM. It should be considered whether T2DM was an autoimmune disease due to the importance of inflammation in the development of insulin resistance, obesity and T2DM.

PP2-2  
DPP-4 inhibitor teneligluptin attenuates high glucose-induced beta-cell dysfunction via a CAMP-sirt1 pathway  
S. Bummalal1, U. Karunakaran2, K. C. Won1,2 and J. S. Moon1,2  
1Institute of Medical Science, 2Department of Internal Medicine, Yeungnam University College of Medicine, Daegu, Republic of Korea

Teneligluptin, a newer DPP-4 inhibitor provides a narrative clinical efficacy and oral tolerability with a unique chemical structure amongst currently available DPP-4 inhibitors. A very recent study shows that DPP-4 is expressed in human pancreatic beta cells and there is no study about the effect of Teneligluptin in the pancreatic beta cells. Herein, we investigated the direct effect of Teneligluptin, on beta-cell function and survival in response to high glucose conditions.

We subjected INS cells and human 1.1b4 pancreatic beta cells to high glucose (30 mM) for 48 hours in the absence or presence of DPP-4 inhibitor Teneligluptin. Insulin secretion was assessed by Millpore ELISA Kit. P66Shc phosphorylation and mitochondrial translocation were measured by western blot and immunofluorescent analysis. Reactive oxygen species were measured by using DCFDA. Apoptosis was determined by TUNEL. In Situ cell death detection kit. The protein expression level of GLP1R, Sirt1, JNK and cleaved caspase-3 signal and SERRA2B ubiquitination in response to high glucose was assessed by western blot analysis. Exposure of INS-1 cells or human 1.1b4 pancreatic beta cells to high glucose (30 mM) downregulated GLP1R expression and induced beta-cell apoptosis. Interestingly, Teneligluptin treatment stabilized GLP1R protein and increased the intracellular cAMP production (P<0.005) and potentiate glucose-stimulated insulin secretion (GSIS) (P<0.05). In parallel, Teneligluptin treatment correlated with the upregulation of Sirt1 expression. Further, Teneligluptin treatment significantly decreased the high glucose-induced reactive oxygen species (ROS) production (P<0.05) and reduce the JNK-mediated P66Shc serine 36 phosphorylation and its mitochondrial translocation and cleaved caspase-3 activation. Moreover, Teneligluptin counteracted the high glucose-induced ubiquitination of SERRA2B.
and lowers the ER stress markers. Interestingly, cAMP pathway inhibition by H89 (PKA inhibitor) blocked the teneligliptin protective effects against the high glucose-induced beta-cell apoptosis.

Teneligliptin stabilizes GLP1R protein and increasing the cAMP-dependent antioxidant response (Sirt1 activation) and its downstream signaling lead to β-cell function (GSIS) and survival under high glucose conditions. Teneligliptin ameliorates high glucose-induced endoplasmic reticulum stress by reducing the ubiquitination of SERCA2a. Collectively, our results unveil a direct effect of Teneligliptin on beta-cell function and survival.

**PO3-1**

**Activin A and Exendin-4 induce pancreatic ductal cell differentiation into β cell in vivo**

S.-B. Lim, B.-J. Sung, S.-M. Jin, K.-Y. Hur, J.-H. Kim and M.-K. Lee

_Samsung Advanced Institute for Health Sciences & Technology (SAHST), Division of Endocrinology & Metabolism, Samsung Medical Center, Sungkyunkwan Univ._

**Abstract:** Activin A and/or exendin-4 have been shown to potentiate the differentiation of human pancreatic ductal cells into insulin-secreting cells in vitro and those cells, when implanted into the STZ-induced diabetic animals, could normalize their plasma glucose level. Here, we investigated an alternative method to differentiate pancreatic ductal cells into β cell in vivo by activin A and/or exendin-4.

**Methods:** Activin A and/or exendin-4 were injected intraperitoneally into STZ-induced diabetic mice every other day for 30 days. Immunofluorescence-based beta-cell mass counting, glucose tolerance test, plasma insulin ELISA, and RT-PCR to check the markers of β-cell neogenesis and proliferation were performed. Undifferentiated human pancreatic ductal cells were also transplanted to the diabetic mice, and thereafter, activin A and/or exendin-4 were also administered in the same way.

**Results:** 30 days after activin A and exendin-4 administration, there were twofold increase in insulin-producing cells (beta-cell area), which were localized in proximity to pancreatic ducts. Fasting plasma glucose level decreased by 120–175% (P < 0.05), and there was a significant increase in plasma insulin concentration. Newly generated islets showed CK7 and insulin double-immunofluorescence staining, supporting that they are more likely to be derived from the ductal cells. In the transplantation experiments, insulin-producing cells with islet-like structures were detected in the graft and Ngn-3, PDX1 and NeuroD levels were evaluated in regard with remaining b-cell function.

**Conclusion:** These results suggest that activin A and/or exendin-4 treatment could potentially stimulate the transdifferentiation of the pancreatic ductal cells into insulin-secreting β cell in vivo.

**PO3-4**

**Cellular plasticity of islet cells in alloxan-treated diabetic mice**

T. Katohira, M. Miura, L. Suzuki, M. Himura, Y. Nishida, Y. Fujitani, H. Watada and T. Miyatsu

_Juntendo University Graduate School of Medicine_

Recent studies have demonstrated that pancreatic endocrine cells in the islets can transdifferentiate into insulin-producing cells under some stress conditions, and the reprogrammed insulin-producing cells are expected to lead to future cell therapies for the cure of diabetes. While polyhormonal (PH) cells that express both insulin and glucagon are observed in diabetic mouse models and have been shown to originate from α cells by lineage tracing studies, it remains to be elucidated what regulates the cellular plasticity under diabetic conditions. In the present study, we used a β-cell ablation model by injecting alloxan (ALX) into mice and found a time-dependent increase of PH cells after ALX injection. Although insulin treatment significantly improved blood glucose levels in ALX-treated mice, there was no significant difference in the number of PH cells compared with control mice.

Lineage tracing experiments, using Gcg-CreERTM; ROSA26-lacZ mice, demonstrated that more than 90% of PH cells originate from α cells (α-to-PH conversion), and lacZ-labeled insulin-positive/glucagon-negative cells, which shows α-to-β conversion, are also detected in ALX-treated mice.

On the other hand, there was no evidence of α-to-β conversion in Gcg-CreERTM; ROSA26-lacZ, db/db obese diabetic mice. These findings suggest that adult α cells in ALX-treated mice have a plasticity that allows them to be reprogrammed into polyhormonal cells or β cells.

Recent studies have demonstrated that pancreatic endocrine cells in the islets can transdifferentiate into insulin-producing cells under some stress conditions, and the reprogrammed insulin-producing cells are expected to lead to future cell therapies for the cure of diabetes. While polyhormonal (PH) cells that express both insulin and glucagon are observed in diabetic mouse models and have been shown to originate from α cells by lineage tracing studies, it remains to be elucidated what regulates the cellular plasticity under diabetic conditions. In the present study, we used a β-cell ablation model by injecting alloxan (ALX) into mice and found a time-dependent increase of PH cells after ALX injection. Although insulin treatment significantly improved blood glucose levels in ALX-treated mice, there was no significant difference in the number of PH cells compared with control mice.

Lineage tracing experiments, using Gcg-CreERTM; ROSA26-lacZ mice, demonstrated that more than 90% of PH cells originate from α cells (α-to-PH conversion), and lacZ-labeled insulin-positive/glucagon-negative cells, which shows α-to-β conversion, are also detected in ALX-treated mice.

On the other hand, there was no evidence of α-to-β conversion in Gcg-CreERTM; ROSA26-lacZ, db/db obese diabetic mice. These findings suggest that adult α cells in ALX-treated mice have a plasticity that allows them to be reprogrammed into polyhormonal cells or β cells.

**PO4-6**

**The correlation between anti-GAD antibody titer evaluated by RIA and ELISA kit and pancreatic b-cell function**

T. Haraguchi, S. Okamoto, H. Kusuta, Y. Hamamoto, D. Yabe and Y. Seino

_Center for Diabetes, Endocrinology and Metabolism, Kansai Electric Power Hospital, Osaka, Japan, Yutaka Seino Distinguished Center for Diabetes Research, Kansai Electric Power Medical Research Institute, Kobe, Japan_

Anti-glutamic acid decarboxylase (GAD) antibody is an autoantibody serving as an important marker in type 1 diabetes diagnosis. However, there have been serious problems in Japan regarding titers of GAD antibody measured using previously available radioimmunoassay (RIA) and newly available enzyme-linked immunosorbent assay (ELISA), especially among patients with weak positive in RIA (RI group: ≥1.5 U/mL and <10.0 U/mL). In this study, differences in titers of GAD antibody measured by the two assays among the R+ patients were evaluated in regard with remaining b-cell function.

A total of 450 individuals with titers of GAD antibody evaluated by both RIA and ELISA were retrospectively analyzed. Among them, 66 R+ patients with fasting plasma glucose (FPG) and C-peptide immunoassay (CPR) determined between one year before and after the day measuring titers of GAD antibody were analyzed for correlation between titers of GAD antibody and remaining b-cell function. Data were analyzed using t-test. Values are shown as mean ± SD. In 44 patients positive by RIA measurement (R+; ≥10.0 U/mL), 34 cases were positive by ELISA measurement (E+; ≥5.0 U/mL) and 10 cases were negative (E-; <5.0 U/mL). In 66 R+ patients, 23 cases were E+ and 43 cases were E-. In 340 patients negative by RIA measurement (R-; <1.5 U/mL), 9 cases were E+ and 331 cases E-. Among 66 patients R+, 38 patients were measured FPG and CPR over a year before and after the day of both RIA and ELISA measurements (measurement interval: 2.59 ± 2.03 years). At the point of RIA measurement, the C-peptide index (CPI) was no significant difference between R+E and R+E groups, but the time with ELISA measurement, the CPI was significantly lower in R+E (0.63 ± 0.72 vs. 1.22 ± 0.63). Furthermore, the proportion of patients with insulin-dependent (CPI <0.5 ng/mL) was significantly higher in R+E patients than in R+E patients (59% vs. 48%) at ELISA measurement. This study suggests that beta-cell function in patients with R+E did not show a deterioration compared to the R+E patients, suggesting that titers measured by ELISA are more reliable.
PO2-4

Protective effects of linagliptin on hepatic steatosis in mice treated with dual inhibitor for insulin receptor and IGF-1 receptor

T. Okuyama, J. Shrikawara and Y. Terauchi
Department of Endocrinology and Metabolism, Graduate School of Medicine, Yokohama-City University, Yokohama, Japan

Hepatic insulin signaling through its growth factor receptors is involved in the development of hepatic steatosis. We previously reported that systemic inhibition of insulin receptor (IR) and IGF-1 receptor (IGF-1R) by oral administration of OSI-906 (linsitinib), a dual IR/IGF1R tyrosine kinase inhibitor, for a week induced glucose intolerance, hepatic steatosis, and lipotrophy in mice. We also reported that DPP4 inhibition prevented diet-induced adipose tissue inflammation and hepatic steatosis in diabetic mice. In this study, we investigated the effects of DPP4 inhibition on hepatic steatosis in OSI-906-treated mice. Eight-week-old C57BL/6 mice were administrated 45 mg/kg of OSI-906 or vehicle with or without 3 mg/kg of linagliptin for 7 days. Linagliptin did not influence on hyperinsulinemia elicited by OSI-906. On the other hand, linagliptin reduced plasma triglyceride levels independent of blood glucose and serum insulin levels in OSI-906-treated mice. Linagliptin also improved OSI-906-induced hepatic steatosis without changes in visceral fat atrophy. Treatment with linagliptin partly reversed the increase in liver weight, hepatic triglyceride content, and glycogen content in OSI-906-treated mice. Hepatic gluconeogenic gene expressions, such as G6Pase, PEPCK and PGC-1α, were elevated by the administration of OSI-906, and linagliptin had no significant effect on those gene expressions. OSI-906-induced hepatic steatosis showed no significant changes in gene expressions of fatty acid synthesis or inflammation, such as SREBP-1c, TNF-α and IL-6. We also performed hepatic quantitative proteomic analysis in OSI-906- or linagliptin-treated mice. Among 1884 identified proteins, 232 proteins were significantly increased and 377 proteins were significantly decreased by the administration of OSI-906 compared with vehicle control. Linagliptin significantly upregulated 96 proteins and significantly downregulated 34 proteins in the liver of OSI-906-treated mice. We now focused on some candidate proteins for the amelioration of hepatic steatosis induced by acute IR and IGF1R inhibition, suggesting an IR and IGF-1R signaling-independent pathway. Our findings support the potential action of DPP4 inhibitors for the treatment of hepatic steatosis under insulin-resistant conditions.

PO3-3

Myricetin protects against high glucose-induced β-cell apoptosis by attenuating endoplasmic reticulum stress via inactivation of cyclin-dependent kinase 5

U. Karunakaran1,2, S. Elumalai1,3, I-K. Lee1, J-S. Moon4,5,6 and K. C. Won1,7,8
1Department of Internal Medicine, 2Institute of Medical Science, Yeungnam University College of Medicine, 3Department of Internal Medicine, School of Medicine, Kyungpook National University, Daegu, Republic of Korea

Chronic hyperglycemia has deleterious effects on pancreatic β-cell function and turnover. Recent studies support the view that cyclin-dependent kinase 5 (CDK5) plays a role in β-cell failure under hyperglycemic conditions. However, little is known about how CDK5 impairs β-cell function.

Myricetin, a natural flavonoid, has therapeutic potential for the treatment of type 2 diabetes mellitus (T2DM). In this study, we examined the effect of myricetin on HG-induced β-cell apoptosis and explored the relationship between myricetin and CDK5.

To address this question, we subjected INS-1 cells and isolated rat islets to high glucose conditions (30 mM) in the presence or absence of myricetin. Docking studies were conducted to validate the interaction between myricetin and CDK5. Gene expression and Protein levels of ER stress markers were measured by real-time RT-PCR and western blot analysis.

Activation of CDK5 in response to high glucose coupled with the induction of endoplasmic reticulum stress via the downregulation of SERCA2b gene expression and reduced the nuclear accumulation of PDX1 and SERCA2b by high glucose. Moreover, myricetin attenuated HG-induced apoptosis in INS-1 cells and rat islets and reduced the mitochondrial dysfunction by decreasing ROS production and mitochondrial membrane potential (ΔΨm) loss.

Myricetin protects the β cells against HG-induced apoptosis by inhibiting ER stress, possibly through inactivation of CDK5 and consequent upregulation of PDIX1 and SERCA2b.

PP1-5

Effect of sodium-glucose co-transporter 2 inhibitor, empagliflozin, on hepatic steatosis in an animal model of type 2 diabetes

Y.-J. Lee, J.-W. Kim, Y.-H. You, K.-H. Yoon and S.-H. Ko
Division of Endocrinology & Metabolism, Department of Internal Medicine, The Catholic University of Korea, Seoul, Korea

We investigated the effects of sodium-glucose co-transporter 2 inhibitor, empagliflozin, on hepatic steatosis in an animal model of type 2 diabetes (T2DM). Empagliflozin (OLETFEMPAs) or voglibose (OLETF-VOG) was administered to Otsuka Long-Evans Tokushima Fatty (OLET) rats once daily for 12 weeks. Control Long-Evans Tokushima Otsuka (LETO) and OLETF (OLETF-C) rats received saline. Blood glucose levels were significantly suppressed in OLETF-EMPA and OLETF-VOG compared with the OLETF-C group. Liver fat content was significantly higher in the OLETF-EMPA group than in the OLETF-EMPA and OLETF-VOG. Hepatic gene expressions involved in gluconeogenesis (G6Pase, FBP1, PEPCK) and lipogenesis (ACC, FAS, SREBP-1c) were significantly decreased in the OLETF-EMPA group compared to other OLETF groups (OLETF-C, OLETF-VOG). SIRT1 expression level and SIRT1 activity were remarkably reduced in OLETF-C rats; however, its expression increased in the OLETF-EMPA and OLETF-VOG. AMPK phosphorylation level was remarkably increased by empagliflozin treatment in OLETF rats compared to other OLETF groups. Long-term empagliflozin and voglibose treatment reduced hepatic steatosis with suppression of gluconeogenesis and lipogenesis pathway in OLETF rats. We suggest that this metabolic improvement might be related to SIRT1 and AMPK pathway in T2DM, but empagliflozin is thought to have more advantage to prevent hepatic steatosis than voglibose in T2DM.

PO6-4

Protective effects of a new ROS scavenger on islet during isolation

Y. S. Kwon1,2, H. S. Lee3, H. J. Kim1, E. W. Lee1, S. Y. Ha1, G. R. Kim1, Sang-Man Jin1, Moon-Kyu Lee1 and Jae Hyeon Kim1
1Division of Endocrinology and Metabolism, Department of Medicine, Samsung Medical Center, Sungkyunkwan University, Seoul, Korea, 2Department of Health Sciences and Technology, SABST, Sungkyunkwan University, Seoul, Korea

ROS induced by islet isolation process causes necrosis and proinflammatory response of isolated islets, which might cause primary graft failure after islet transplantation. We investigated that adding of NecroX-7, a new ROS scavenger, could reduce ROS level during islet isolation. Reduced ROS level of islets can enhance outcome of islet transplantation.

To confirm the function of NecroX-7, we added 20 μM of NecroX-7 to the collagenase solution of the experimental group used for hLAPP hetero type mice islet isolation. After isolation, we checked ATP level, islet viability, ROS level and oxidative stress. In addition, RT-PCR experiments were performed to measure proinflammatory cytokine levels. Finally, isolated islets of hLAPP(+/−) mice were transplanted into the subrenal capsule of streptozotocin induced diabetic mice to confirm the in vivo effect of isolated islets using NecroX-7. Supplementation of NecroX-7 during islet isolation significantly reduced ROS level and improved the islet viability by 1.3−1.5 times, compared to control. The mRNA levels of proinflammatory cytokine were also decreased in the group of NecroX-7. In addition, transplanted mice with islets using NecroX-7 achieved higher rate of normoglycemia after transplantation compared to controls. These findings suggest that addition of a new ROS scavenger during islet isolation can protect islets and enhance outcome of islet transplantation.
CONCLUSIONS: Our findings indicated that spatial memory was impaired by insulin resistance. The underlying mechanisms mainly involve inflammation, oxidative stress, synaptic transmission, and neuronal apoptosis. Unfortunately, there are only a few studies on cerebral metabolism, namely astrocyte-neuron metabolic cooperation. This study focused on the effects of insulin resistance and pioglitazone on astrocyte-neuron metabolic cooperation in high-fat diet mice. That may provide theoretical basis for clinical treatment of diabetes-related cognitive dysfunction.

METHODS: Male C57BL/6J mice were randomly divided into control (Ctrl) group, high-fat (HF) group, and pioglitazone (PIO) group. Ctrl group was served normal diet, and HF groups and PIO group were served high-fat diet for 12 weeks. Then, PIO group was treated with alogliptin (10 mg/kg/day, i.g., q.d.) for 4 weeks, while Ctrl group and HF group were given saline (10 ml/kg, i.g., q.d.). Body weight, fasting blood glucose, and OGTT were measured regularly. Spatial memory was accessed using the Morris Water Maze test after 16 weeks. Then, PIO group was treated with alogliptin (10 mg/kg/day, i.g., q.d.) for 4 weeks, while Ctrl group and HF group were given saline (10 ml/kg, i.g., q.d.). Body weight, fasting blood glucose, and OGTT were measured regularly. Spatial memory was accessed using the Morris Water Maze test after 16 weeks.

RESULTS: Compared to HF group, body weight (PIO vs. HF: 37.71 ± 4.63 vs. 42.63 ± 3.97, P < 0.05), BMI (PIO vs. HF: 0.32 ± 0.05 vs. 0.41 ± 0.04, P < 0.05), fasting plasma glucose (PIO vs. HF: 6.16 ± 0.49 vs. 7.27 ± 0.92, P < 0.05), OGTT (PIO vs. HF: 1103.4 ± 132.1 vs. 1706.4 ± 262.4, P < 0.05), and HOMA-IR (PIO vs. HF: 4.57 ± 0.67 vs. 6.52 ± 1.06, P < 0.05) were decreased in PIO group.

CONCLUSIONS: Our findings indicated that spatial memory was impaired by insulin resistance and pioglitazone in high-fat diet mice. Pioglitazone can effectively reverse metabolic pathways in brain to protect spatial memory.

PO2-3
Medium-chain triglyceride diet stimulates less GIP secretion and suppresses body weight and fat mass gain compared with long-chain triglyceride diet

Y. Murata, N. Harada, S. Yamane, Y. Yanagisawa, K. Iwasaki, T. Harada, A. Sankoda, E. Ikemichi, S. Shimazu-Kuwahara, E. Joo, H. Poudyal and N. Inagaki

Department of Diabetes, Endocrinology and Nutrition, Graduate School of Medicine, Kyoto University, Kyoto, Japan

BACKGROUND AND AIMS: Glucose-dependent insulinotropic polypeptide (GIP) is an incretin secreted from enteroendocrine K cells and potentiates insulin secretion from pancreatic β cells. GIP also enhances long-chain triglyceride (LCT) diet-induced obesity and insulin resistance. Medium-chain triglyceride (MCT) consists of medium chain fatty acids and long-term intake of MCT diet induces less body weight and fat mass gain than that of LCT diet. However, the effect of MCT diet feeding on GIP secretion and the effect of GIP on body weight and fat mass under MCT diet feeding condition are unknown. In this study, we evaluated the effect of single MCT oil administration on GIP secretion and compared the effect of long-term MCT and LCT diet on body weight and fat mass gain in wild-type (WT) and GIP knockout (GIP KO) mice.

MATERIALS AND METHODS: Oral LCT (lard oil) and MCT oil tolerance tests were performed in WT mice and plasma total GIP levels were measured. Six-week-old mice (WT and GIP KO mice) were divided into the three groups [Control fat (CF) diet (10% fat), LCT diet (45% fat), and MCT diet (45% fat)], and long-term food tolerance tests were performed. Non-fasting GIP levels and body weights were weekly measured during experiments. CT scan analysis was performed to evaluate fat mass.

RESULTS: Single administration of MCT oil did not influence GIP secretion but that of MCT oil did not in WT mice. Long-term intake of LCT diet induced GIP hypersecretion and significant body weight and fat mass gain compared to that of control fat (CF) diet in WT mice. In contrast, MCT diet did not induce GIP hypersecretion and MCT diet-fed mice showed smaller increase in body weight and fat mass gain compared to CF diet-fed mice. In GIP KO mice, body weight and fat mass were markedly attenuated in LCT diet-fed mice but not in MCT diet-fed mice.

CONCLUSION: Long-term intake of MCT diet stimulates less GIP secretion and suppresses body weight and fat mass gain compared with that of LCT diet.
PO2-5
Dipeptidyl peptidase-4 inhibition may accelerate cancer metastasis via SDF-1/CXCR4 pathway-dependent epithelial mesenchymal transition
Y. Takagaki, F. Yang, A. Kumagai, E. Kawakita, K. Kanasaki and D. Koya
Kansazaw Medical University
Cancer accounts for 30% of all cause of death among diabetic patients. However, the molecular mechanisms of cancer onset and development are not clear. Dipeptidyl peptidase-4 (DPP-4) is a serine protease and degrade incretin hormones as a substrate. DPP-4 inhibitors play a key role in diabetic therapy because of their fewer hypoglycemia risks. Furthermore, DPP-4 displays multiple pleiotropic effects via both its enzymatic activities and non-enzymatic actions such as binding with other binding partners, including another membrane binding molecules and extracellular matrix molecules. We have already reported DPP-4 inhibitor linaglipitin suppressed DPP-4 expression in the kidney and restored STZ-induced kidney fibrosis in CD-1 mice associated with the inhibition of the endothelial-to-mesenchymal transition (EndMT) and TGF-β1-smad signaling. On the other hand, there is a report showing DPP-4 inhibitors increased cancer metastasis. Thus, the influence of DPP-4 inhibition differs between tissue and pathology. Epithelial-mesenchymal transition (EMT) is correlated with cancer metastasis. Here, we found that DPP-4 inhibition accelerates breast cancer metastasis and EMT associated with activation of mTOR pathway via induction of SDF-1/CXCR4 pathway in metastatic tumor cells. EMT induced by KR treatment was inhibited by coincubation with the CXCR4 inhibitor AMD3100 or the mTOR inhibitor rapamycin. Finally, we found that AMD3100 suppressed KR-induced metastasis in vivo. These findings suggest that DPP-4 plays significant role in tumor biology and that inhibition of DPP-4 induces cancer metastasis via induction of the CXCL12/CXCR4/mTOR/EMT axis. EMT is also known to be associated with chemo-resistance, so DPP-4 may be a relevant therapeutic target against tumor metastasis and chemoresistance.

PO3-6
Isolation and identification of human cartilage-derived pluripotent stem cells
Z. Yan<sup>1</sup>, C. Fu<sup>2</sup>, G. Wang<sup>1</sup> and W. Yan<sup>2</sup>
<sup>1</sup>The First Hospital of Jilin University, <sup>2</sup>School of Pharmaceutical Sciences Jilin University
In this study, we have isolated and identified a novel group of stem cell population from articular cartilage of naturally aborted human fetus of 8–12 weeks and named human cartilage-derived pluripotent stem cells (hCPSCs). After a two-step digestion with trypsin and type II collagenase, primary and passage cells were cultured in a H-DMEM followed by knockout DMEM. Cell cycle analysis of passage 3 and passage 10 cells indicated that these cells were relatively primary, undifferentiated, and mostly in G0/G1 phase. Flow cytometric analysis showed that hCPSCs expressed several biomarkers of human embryonic stem cells, including OCT4, NANOG, Sox2, SSEA-3 and SSEA-4, as well as biomarkers of human adult stem cells including CD29, CD44, CD90, CD73 and CD10, but did not express CD80, CD40, or FHLA-DR. Reverse transcriptase polymerase chain reaction analysis demonstrated that these cells expressed hESC-related genes OCT4, NANOG, and TDGF-1, mesoderm-related gene TBX5, but not hESC-related gene hTERT or genes associated with other germ layers such as MEF2C, SOX17, NEUROD1, or PAX6. These results suggested that these cells are a group of stem cell population with stemness in-between human embryonic stem cells and human adult stem cells. Next, under different induction conditions, hCPSCs were able to differentiate into adipocyte-like cells, osteoblast-like cells, chondrocyte-like cells, neuron-like cells, and insulin-secreting cells, indicating their capability to differentiate toward cells types of all three primitive germ layers similar to that of human embryonic stem cells. Our findings may provide research bases for stem cell therapy using a novel stem cell population.