27th NSW Stem Cell Network Workshop

Stem Cells and Diabetes Therapies

HostCo

University of Sydney, Camperdown, NSW

Monday 18th June, 2018

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WELCOME

Welcome to the 27th Workshop of the NSW Stem Cell Network

The holy grail of a cell therapy for insulin-dependent diabetes is to be able to administer this to the large number of people with this disorder. In Australia, there are 119,000 people with type 1 diabetes, and around the world ~21 million. The first human to receive a successful cell transplant was in 1989 by David Scharp and colleagues in St Louis, USA, but as with almost all recipients since then anti-rejection drugs need to be administered.

Human islets obtained from donors after their death have been the mainstay of cell therapies, but those in the Californian company ViaCyte began a new era of hope in 2014 by using pancreatic progenitors derived from human embryonic stem cells. This Workshop is about understanding where the various researchers and companies are as regards using ß cells differentiated form stem cells.

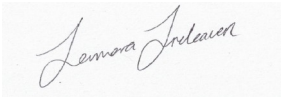
It is also about where the field is at present regarding being able to deliver ß cells to people with type 1 diabetes in bioengineered devices and without the need for anti-rejection drugs.

We are very grateful to our international guests for going out of their way to attend and present at this Workshop. From Harvard Medical School Professor Doug Melton, who has established the company Semma Therapeutics; from the pharmaceutical company Novo Nordisk in Denmark, Dr Allen Karslen; Dr Kfir Molakandov from Kadimastem in Israel; and Dr Itai Pelled from BetaO2, also from Israel.

We have divided the Workshop into three sections, starting with the differentiation of pluripotent stem cells to ß cells. Thereafter, we move to exploring the outcome of transplanting ß cells into diabetic recipients, both mice and human, all without immunosuppressive agents. After lunch we will deal with those who have bioengineered devices to deliver ß cells, and to finish off the day, we have a Panel Discussion of all participants and the audience.

This Workshop would not have been possible without the generous help from our sponsors, speakers and all of you present today. We are truly grateful for your support and contribution. We would specifically like to thank the International Society for Stem Cell Research for bringing Professor Melton to Australia for its Meeting in Melbourne. Once Professor Melton had decided to detour to Sydney, we were able to build the Program into what it has become.

We hope you enjoy the Workshop and continue to support the NSW Stem Cell Network at future events!



Tamara Treleaven
NSW Stem Cell Network
Manager

Prof. Bernie Tuch
NSW Stem Cell Network
Director

PROGRAM

9:00am	Registration opens/ Light refreshment
9:30am	**Prof. Bernie Tuch (NSW Stem Cell Network)** *Welcome*
Session 1	**Developmental Biology** **Chair: Prof. Patrick Tam (Childrens Medical Research Institute)**
9:35am	**Dr. Jacqueline Schiesser (Murdoch Childrens Research Institute)** *Exploring signaling pathways controlling endocrine cell number using human pluripotent stem cells*
10:00am	**Dr. Kfir Molakandov (Kadimastem Ltd, Israel)** *Large scale production of Insulin producing cells from hESCs: progress and future challenges*
10:30am	Morning Tea
Session 2	**Function of Beta Cells** **Chair: Prof. Tom Kay (St Vincents Institute of Medical Research)**
11:00am	**Prof. Doug Melton (Harvard Stem Cell Institute, United States)** *Making islets from human stem cells*
11:30am	**Prof. Allan Karlsen (Novo Nordisk, Denmark)** *Function, challenges and safety of using stem cell derived beta cells*
12:00pm	**Dr Itai Pelled (Beta-02, Israel)** *The quest for the cure of insulin dependence diabetes mellitus*
12:30pm	Lunch and Poster Session
Session 3	**Immunoisolation Device for Delivery** **Chair: A/Prof. Tim Dargaville (Queensland University of Technology)**
1:30pm	**Prof. Bernie Tuch (Australian Foundation for Diabetes Research)** *Microencapsulation of beta cells inside 3D scaffolds*
1:55pm	**Prof. Patrick (Toby) Coates (University of Adelaide)** *Biodegradable temporising matrix as an alternative site for islet transplantation*
2:20pm	**Dr Tom Loudovaris (St Vincents Institute of Medical Research)** *Macroencapsulation of beta cells*
2:45pm	Afternoon Tea

PROGRAM

Session 4	Panel Discussion Chair: Prof. Patrick (Toby) Coates (University of Adelaide)
3:20pm	**All previous speakers** *Cell therapy for the masses without immunosuppression*
4:00pm	Networking/ Close

Dr Jacqueline Schiesser—Murdoch Childrens Research Institute

Dr Schiesser completed her PhD in 2012 at Monash University studying human embryonic stem cell differentiation and pancreatic development under the supervision of Andrew Elefanty and Ed Stanley. Following her PhD, she undertook postdoctoral studies in the laboratory of James Wells at Cincinnati Childrens Hospital Medical Center where she studied regional identity in intestinal stem cells using an organoid model system.

In 2017, she returned to Australia and joined the Stem Cell Technology Laboratory at the Murdoch Childrens Research Institute where she is currently utilizing patient derived-induced pluripotent stem cells to create autologous beta cells in order to identify epitopes recognized by human islet-infiltrating CD8+ T-cells.

Exploring signaling pathways controlling endocrine cell number using human pluripotent stem cells

Type 1 diabetes (T1D) is an autoimmune disease that is driven by T-cell mediated destruction of the pancreatic beta cells, resulting in blood glucose dysregulation. Although islet transplantation is currently used in clinical practice, there is a shortage of donor tissue. Pluripotent stem cells (PSCs) may potentially fill this shortfall by providing an unlimited supply of endocrine cells for transplantation.

A number of protocols that promote the differentiation of PSCs towards a beta cell fate have been published, all of which aim to recapitulate the signaling processes that occur during human embryogenesis. Further improvements in these protocols have the potential to decrease the cost of production and increase the functional quality of the final product.

In order to study signalling pathways involved in controlling endocrine cell number, we generated a human PSC line that contains both GFP and mCherry fluorescent proteins that are under the control of the INSULIN and GLUCAGON loci respectively. This cell line enables endocrine cell specification to be monitored in real-time, as well as facilitating isolation of alpha and beta cells.

We utilised this INSULINGFP/w GLUCAGONmCh/w PSC line to investigate pathways involved in endocrine cell specification. RNAseq was performed on cells sorted based on their GFP and/or mCherry expression and compared to pancreatic progenitor cells. From this, a number of signalling pathways that were dynamically regulated during endocrine cell specification were identified, including Wnt signalling.

Murdoch Childrens Research Institute, The Royal Children's Hospital, Parkville, Victoria 3052, Australia
Department of Paediatrics, University of Melbourne, The Royal Children's Hospital, Parkville, Victoria

Dr Kfir Molakandov— Kadimastem Ltd, Israel

Dr Molakandov started his academic career in developmental biology with Prof. Sarah Ferber's ground breaking research on transdifferentiation of liver to pancreas using adenoviral vectors carrying pancreatic transcription factors. He was trained at the Gene Therapy Center, Alabama, USA, and specialized in adenoviral vectors construction, modifications and purification. Together with Prof. Ferber, this knowledge was exploited to construct novel adenoviral vectors to improve the reprogramming of liver to pancreas. “We have found that there is some predisposition in this reprogramming process and my research focused on designing a reprogramming vector that will exploit the characteristics of pre-disposed cells. Following completion of my thesis, I have joined to Kadimastem, an Israeli biotech company specializing in regenerative medicine solutions for neurodegenerative diseases and diabetes. In my current position, I lead the team in Kadimastem that is developing cell-therapy for diabetes using Pluripotent Stem Cells as the cell source. Our goal is to industrialize the production process for functional insulin producing cells. We have dedicated the last few years in developing a large scale robust protocol for generating islet cells in high quantities and high quality. Currently, we have finalized the production process that includes a full characterization of the different stages of differentiation using molecular tools and physiological parameters. Accordingly, we are now moving towards the clinical application of this technology, aiming to bring these therapeutic cells to clinical trials”.

Large scale production of Insulin producing cells from hESCs: progress and future challenges

Despite the gradual improvement in islet transplantation procedures achieved in the last decades, the limiting factor for the wide acceptance of this procedure remains the low numbers of available islets for transplantation. Therefore, alternative abundant sources for islet transplantation are needed. This has shed light on the usage of human Pluripotent Stem Cells (hPSCs) as a potential renewable source for insulin producing cells. Based on their almost unlimited proliferation capabilities and their potential to differentiate into all cells of the three primary germ layers, hPSCs are attractive candidates for cell replacement therapy for diabetes. Utilizing embryonic developmental pathways, hPSCs were differentiated into the pancreatic and specifically into insulin producing cells. The newly formed Islet-Like-Clusters, (ILCs), grown in dynamic suspension conditions were tested both in vitro and in vivo. In vitro, ILCs exhibited molecular characteristics similar to donor human islets manifested by gene expression profile, de novo insulin synthesis and glucose dependent insulin secretion. In vivo, ILCs were transplanted under mice's skin and exhibited secretion of human insulin in physiological levels already 7 -14 days post transplantation. Here, we will describe the industrialization process for generating hESC-derived insulin producing cells in a large scale manner, using controlled bioreactors and under GMP conditions. In addition, we will describe strategies for the enrichment of functional population, necessary for both safety and encapsulation purposes.

Professor Douglas Melton— Harvard Stem Cell Institute, United States

Dr. Melton is the Xander University Professor at Harvard and an Investigator of the Howard Hughes Medical Institute. He is also a Co-Director of Harvard's Stem Cell Institute and Co-Chair of the Department of Stem Cell and Regenerative Biology in the Harvard Medical School and the Faculty of Arts and Sciences. Melton and his wife, Gail O'Keefe, are Co-Masters of Eliot House in Harvard College.

Dr. Melton earned a bachelor's degree in biology from the University of Illinois and then went to Cambridge University, U.K., as a Marshall Scholar. He earned a B.A. in history and philosophy of science at Cambridge University and remained there for a Ph.D. in molecular biology at Trinity College, Cambridge and the MRC Laboratory of Molecular Biology. Dr. Melton serves on the Scientific Advisory Boards of several pharmaceutical and biotech companies and is a scientific Co-Founder of Gilead Sciences, Curis, and Semma Therapeutics. Dr. Melton has received numerous awards for his scientific work and has twice been named to TIME magazines list of the year's 100 Most Influential People.

Research in the Melton laboratory focuses on finding a significant new treatment for Type 1 diabetes. His laboratory analyzes the normal development of pancreas, and the autoimmune attack that leads to Type1 diabetes, in order to understand how pancreatic beta cells are made and how they become the target of autoimmunity. The main focus of the lab is to use human stem cells to make insulin -producing beta cells for transplantation into diabetics.

Making islets from human stem cells

Abstract not available at time of print

Professor Allan Karlsen—Novo Nordisk, Denmark

Prof. Karlsen holds a position as Vice President and Head of Stem Cell Research at Novo Nordisk and Interim head of Novo Nordisk Research Center Oxford. After graduating from the University of Copenhagen in 1988, Allan worked in the academic milieu at the Hagedorn Research Institute and Steno Diabetes Center (both supported by NN), at the University of Washington, Seattle, USA and at the University of Lund and Karolinska Institute, Sweden, before receiving his PhD in 1995, also from University of Copenhagen. Between the academic and corporate career at Novo Nordisk starting in 2004, Allan worked for 3 years in a biotech start-up company, Inoxell. He was appointed adjunct professor in the Department of Clinical Sciences at Lund University in 2005.

Allan has more than 15 years of teaching experience at both undergraduate and postgraduate levels and is the author/co-author of more than 100 scientific publications, reviews and patents with focus on diabetes pathogenesis. Presently Allan is VP of Stem Cell Research at NN and has been involved in driving our stem cell program in T1D over the last 10 years and the recent expansion of our stem cell platform to other serious chronic diseases .

Function, challenges and safety of using stem cell derived beta cells

Novo Nordisk aims to treat poorly controlled T1D patients by transplanting encapsulated beta cells derived from human embryonic stem cells (hESCs). We have successfully designed a state-of-the-art cell culture protocol for suspension cultures that repeatedly generates in-vitro mature glucose-responsive beta cells that are functional both in-vitro and in-vivo, responding to glucose challenges by increasing their human insulin release and controlling glucose levels in diabetic mice.

The hESC-beta cells can be cryopreserved, encapsulated in a prototype device and transplanted to diabetic mice leading to normalization of blood glucose. The developing of an optimal device for human use is progressing. In parallel, high quality GMP compliant processes are being developed and a first-in-class global approvable clinical grade hES cell line is generated. This is all parts of the complex path of transferring the protocol and processes from research into the development phase of a cell based product to be taken into the clinic. Based on the knowledge we have gained over the last 10 years in our beta-cell project,, we are now also expanding our stem cell platform into other serious chronic diseases.

Dr Itai Pelled—Beta-02, Israel

Dr Pelled joined in 2016 to Beta-O2 Technologies as Executive Vice President of Research and Development. Dr. Pelled previously served for 16 years in Edwards LifeSciences (Israeli branch, Caesarea and HQ, Irvine, California), a global leader in the science of heart valves and hemodynamic monitoring, as distinguished developing engineer, bio polymers specialist and R&D and production manager. During that period, he was developing the first and second generations of the transcatheter heart valve (TAVI), leading the polymeric material section (Advance Material & Technology Center at Edwards LifeSciences HQ), and managing the R&D production team that supported the development of the Trans-catheter Heart Valve' 3rd generation (Caesarea, IL). Most recently, Dr. Pelled was involved in developing several other medical devices as well as bio-compostable flexible films for the food packaging industry.

Dr. Pelled background includes undergraduate degree in Chemistry, a master degree in Biomedical Engineering and a Ph.D. in biodegradable polymers from the Tel-Aviv University, Israel and the Hebrew University in Jerusalem, Israel. He is also a co-inventor of eleven patents.

The quest for the cure of insulin dependence diabetes mellitus

Since the 1960s, researchers have been interested in the possibility of treating type 1 diabetes by transplanting islet cells—the pancreatic cells that are responsible for producing insulin and the most adequate way is by encapsulating them to prevent the need for immunomodulation.

Implementing this approach has proven challenging, however.

One obstacle is that once the islets are encapsulated and transplanted, they will not perform and die if they don't receive an adequate supply of oxygen. To help overcome that challenge, we, at Beta-O2 Technologies, have implant our proprietary capsule that furnishes islet cells with their own supply of oxygen, we have gained positive results in different models (small animals, large animals and humans) and with different cells type (Rat islets, pig islets human islets and stem cells derived islets).

In this talk, I will discuss the essences of beta cells macroencapsulation and share some of the data that we gathered in the human trials in Dresden, Germany and Uppsala, Sweden.

Professor Bernie Tuch—Australian Foundation for Diabetes Research

Professor Tuch is a practising endocrinologist with a strong interest in translational research. During three decades of research he has been involved in 3 clinical trials for type 1 diabetes utilizing a cell therapy. Initially this was human fetal pancreas, then encapsulated human islets, and by association with Living Cell Technologies encapsulated pig islets. At all times this has been without immunosuppression. Recently, he has joined forces with colleagues at Queensland Institute of Technology to incorporate 3D printed scaffolds into the bioengineered device for implanting ß cells. Professor Tuch is a Director of the Australian Foundation for Diabetes Research, which provided seed funding for the current project, an Honorary Professor at the University of Sydney and Monash University, and directs the NSW Stem Cell Network.

Microencapsulation of beta cells inside 3D scaffolds

The goal of diabetes cell therapies for the future is to deliver them in a manner that does not require toxic anti-rejection drugs. In this way, the treatment should be available to all those that currently require exogenous insulin administration to control their blood glucose levels and stay alive. We have engineered a device which when seeded with allogeneic insulin-producing (ß) cells normalizes blood glucose levels without immunosuppression.

The device consists of the ß cells encapsulated within immune-isolating alginate microcapsules, which are seeded into a 3D scaffold made by the relatively novel highly sophisticated process of melt electrospin writing. Part of the art requires the insertion of the device subcutaneously, where the host reaction to materials is less than at the more traditional site of the peritoneal cavity. The scaffold material is poly-e-caprolactone. The microcapsules are the same we have used in a clinical trial with human islets previously (Diabetes Care 2009; 32: 1887).

We have demonstrated that blood glucose levels (BGL) of encapsulated MIN6 cells are lowered to normal (from 30 ± 3 to 4 ± 2mmol/L) in diabetic NOD/SCID and BALB/c mice, within 25-41 days. Long -term normalization of BGL (up to 82 days) was achieved in BALB/c mice receiving encapsulated QS islets in a device pre-vascularised for 3 weeks. There was minimal insulin content in the pancreas of all diabetic mice, but plasma insulin was significantly higher in treated (2.5 ± 1.6µg/L) vs untreated (0.2 ± 0.1) mice. Insulin was released from cultured ex-vivo grafts. Intra-device vascularization was evident (CD31+), and increased linearly with time (R=0.65), as assessed by 3D ultrasound. Inflammatory infiltration of neutrophils (myeloperoxidase+), macrophages (CD68+) and B-lymphocytes (CD19+) was present on MEW scaffolds but not on microcapsules, which had small amounts of fibrosis (α-SMA+).

Our next goal is to test the robustness of the device with human ß cells, and if confirmed, to move to a phase 1b/2a clinical trial.

Australian Foundation for Diabetes Research, The University of Sydney and Queensland University of Technology

Professor Patrick (Toby) Coates—University of Adelaide

Prof Coates is a full time clinician-scientist at the Royal Adelaide Hospital and Renal Transplant Nephrologist and Clinical Professor in Medicine at the University of Adelaide. He undertook his PhD in Transplant Immunology at the University of Adelaide. He was the recipient of South Australian Tall Poppy Science Award for his research from the Government of South Australia in 2004. In June 2016 he received JDRF USA funding for development of alternative sites for islet transplantation and was appointed to the International JDRF Islet Encapsulation Consortium.

He is currently the Director of South Australia's first (and only) Nationally Funded Centre for Islet Transplantation and the Head of Kidney and Pancreas Islet Transplantation at the Royal Adelaide Hospital. He was awarded the Ian McKenzie Prize for Outstanding Contribution to Transplantation in 2013, from the Transplantation Society of Australia and New Zealand. He is a consultant to the National Transplant Service and a member of the Renal Transplant Advisory Committee of the Transplantation Society of Australia and New Zealand.

Biodegradable temporising matrix as an alternative site for islet transplantation

Clinical Islet Transplantation developments over the past 2 decades have improved the success rate of achieving insulin independence. However, the efficacy of the treatment is still limited by the need for multiple infusion and lack of islet survival post transplantation. There is a clear consensus in the field to develop alternative transplantation sites to address these clinical roadblocks. A clinically proven biodegradable temporising matrix (BTM) polyurethane-based scaffold vascularises the skin and may serve as a tool to generating a hyper-vascularised site in the skin to house human islet transplants. In this study, we tested the feasibility of this scaffold to provide an environment that is conduit to the survival of islets. Using a large animal (porcine) preclinical model the kinetics of re-vascularisation of the BTM in the flanks of pigs was determined and found that BTM implants create a highly vascularised neo-dermis by day 7 post implantation. In separate mouse in vitro experiments the capacity of mouse islets to survive in the presence of the BTM was determined. The BTM did not affect islet viability or function. Human islets were transplanted into BTM neo-dermis for 7 days and were found to survive and maintained their islet architecture. Moreover, when autologous neonatal islets were transplanted into BTM neo-dermis, we found that islets remain differentiated as insulin producing cells and survived long-term over 100 days (n=3). In conclusion, the BTM polyurethane material is inert and non-toxic to islets and this study provides proof of concept in a pre-clinical large animal model that intracutaneous transplantation is able to sustain the survival of NPIs over 100 days.

Dr Tom Loudovaris—St Vincents Institute of Medical Research

Dr Loudovaris is the Islet Transplant Program Manager in the Tom Mandel Islet Transplant Program at St Vincent's Institute in Melbourne, Australia. Dr Loudovaris' involvement in encapsulation research began over 30 years ago at the Walter and Eliza Hall Institute on a Baxter Healthcare funded project. There he was studying the immunological protection of allografts and xenografts by encapsulation. He then continued his interest in cell therapies through encapsulation as a scientist and then senior manager in the Cell and Gene Therapy Unit at Baxter Healthcare, USA. In 1999, Dr. Loudovaris co-founded the cell encapsulation company, TheraCyte Inc., in California. He returned to Australia in 2005 to become Islet Program Manager where has taken the human isolation process from a laboratory research procedure to a process producing islets for human transplantation. This lead to Victoria's and South Australia's first successful islet transplants. In addition to his role in the human islet program, he has a continued interest and research involvement in cell transplantation and encapsulation and has collaborated with Professor Klearchos Papas at the University of Arizona on oxygenated device projects over the past seven years.

Macro encapsulation of beta cells

If islet transplantation is the cure for insulin dependant diabetes (IDD) then a means of protecting them from the destructive immune system must be found. Immune suppression with drugs has demonstrated that islet transplantation can cure IDD, but at the expense of continuous treatment with drugs that have their own adverse effects. Encapsulation can provide a physical immune barrier that should eliminate the need for these drugs. Encapsulation comes in several forms, microencapsulation and macroencapsulation. This presentation will discuss macroencapsulation, its advantages, disadvantages and the different forms. The advantage of macrocapsules is that of safety. Every device can be tested and inspected for leaks or defects during manufacture and every device can be quantitatively removed after implantation. Macrocapsules are also usually made of durable materials such medical grade polymers.

Macroencapsulation comes in different forms, in the form of vascular implants, islets are enclosed around a permeable membrane tube implanted as a shunt in the vascular system. Blood flowing through the tube would provide glucose and nutrients to the islets, the islets would detect the level of glucose and release insulin appropriately. However, biocompatibility can be a serious issue with vascular devices as they can suffer clotting which can be life threatening.

Diffusion chambers are typically planar or tubular in design. Because of intimate contact between macrocapsules and the hosts tissues, biocompatibility is an issue that needs addressing. Biocompatibility has been approached either by adjusting the outer chemistry of the device membranes or adjusting the outer physical structure of the implant.

Once biocompatibility has been resolved, devices can be tested for the immune protective capabilities. Highly permeable membranes have been found to be protective of allografts but not xenografts. Membrane permeability can be altered to be restrictive of immune molecules but this also restricts nutrient flow into the device, meaning less islets can be supported and making the device less practical in size. Alternatively, xenogeneic islets can be co-transplanted in devices with protective cells such as Sertoli cells or cells engineered to release immune suppressive factors in the vicinity of the device.

Posters

Naturally occurring diabetes in dogs as a translational model for iPSC based therapies

Maryam Moshref

Kol Laboratory, University of California, Davis

Pluripotent stem cells can give rise to all adult tissue types. Over the last decade, protocols for the differentiation of human and murine pluripotent stem cell derived β-cells (SC-β) have been developed, in an effort to cure diabetes mellitus (DM). Pre-clinical studies suggest that SC-β grafts induce sustainable normoglycemia in rodents with chemically induced diabetes-like disease model. Nevertheless, critical issues such as immune compatibility, cellular product scale-up, delivery method and complex and long lasting cell-host interactions are yet to be resolved. Dogs suffer from naturally occurring DM that mirrors some aspects of the pathologic and clinical features of human type 1 DM, primarily pancreatic β-cell loss and persistent hyperglycemia. Achieving long term glycemic control in dogs with DM is a major clinical challenge in veterinary medicine given the nature of current treatment (i.e. daily insulin injections) and the long term complications. Moreover, canine DM may better reflect the complex genetic, environmental and physiologic variation present in humans, and may prove to be a robust platform for novel regenerative medicine translational research. Finally, the fact that certain breeds (i.e. Samoyed, Keeshond etc.) are at increased risk of developing diabetes, decreases genetic variability/polymorphism and enhances our capacity to discover novel therapeutic targets. We have established canine iPSC lines using the four Yamanaka factors transduced via a lentiviral vector. We have further optimized a feeder-free culture system that sustain canine iPSC pluripotency. We now pursue different approaches do induce β-cell differentiation in these iPSC. Our goal is to develop a canine-specific SC-β differentiation protocol to enable translational research in pet dogs with spontaneous DM, to inform parallel efforts in human patients.

Role of Sphingosine Kinase in Insulin Signaling and Its Action in Hepatocytes

Mei Li NG1, 2, Pu Xia2

1 Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia (USM), Malaysia. 2 Centenary Institute, Sydney Medical School, University of Sydney, Australia

Background and aims: Sphingosine kinase (SK) is a key enzyme involves in the homeostasis of sphingolipids metabolism by catalysing phosphorylation of sphingosine to sphingosine 1-phosphate (S1P). Increased level of sphingolipids are associated with insulin resistance. In this study, we aimed to examine the potential role of SK in insulin signaling and its metabolic actions in hepatocytes.

Methodology: A human hepatocarcinoma cell line (Huh7), a nonneoplastic human hepatic cell line (PH5CH8) and freshly-isolated mouse primary hepatocytes from wild-type (WT) or SK1 knockout (SK1-/-) were used as experimental models. SK expression and activity were manipulated by gene overexpression of WT or dominant-negative SK, siRNA-mediated knockdown, and using a specific chemical inhibitor, SKI-II.

Findings: Treatment of hepatocytes with insulin resulted in two-folds increase in SK enzyme activity. Inhibition of SK activity by overexpressing a dominant-negative mutant (SKG82D) or the enzyme inhibitor SKI-II significantly attenuated insulin-induced Akt activation, while overexpression of WT-SK markedly enhanced Akt activation.

Inhibition of SK activity markedly inhibited the association of IRS-1 with p85 regulatory subunit of PI3-K, suggesting a molecular target for the action of SK on insulin signaling. Interestingly, we revealed that SK2 was predominantly insoform for insulin-induced Akt activation in hepatocytes. Insulin-induced Akt activation and phosphorylation of two important Akt downstream targets, GSK3α and 70S6K were significantly attenuated in the hepatocytes where SK2 was knockeddown by its specific siRNA. Insulin-induced suppression of phosphoenolpyruvate carboxykinase (PEPCK) gene, a rate-limiting enzyme in gluconeogenesis, was abolished by knockdown of SK2 expression. In contrast, the siRNA-mediated knockdown of SK1 expression had no significant effects on insulin-induced Akt activation and PEPCK suppression, indicating an isoform-specific effect of SK2 on insulin action in hepatocytes. **Conclusion:** Our data suggest a potential role of SK2 in regulation of insulin signaling and glucose metabolic action in hepatocytes.

Notes

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