

## **Pancreatic transcription factors: implications for diabetes therapy**

Tali Avnit-Sagi, Keren Bahar, Revital Sharivkin, Lia Kantorovitch, Sara Weiss, and Michael D. Walker

*Department of Biological Chemistry, Weizmann Institute of Science, Rehovot*

Beta cell replacement is one of the most promising approaches towards improved therapies for diabetes. As a result, a variety of experimental approaches are being investigated in an effort to generate novel sources of functional beta cells. A key aspect of these efforts involves manipulation of transcription factors, since these proteins lie at the heart of developmental decisions and the maintenance of mature beta cell function. To better understand the network of transcription factors controlling beta cell development and function, we study the regulation of genes expressed selectively in beta cells. GPR40 and GPR41 are fatty acid-binding receptors encoded by adjacent genes that are expressed selectively in pancreatic beta cells. Unexpectedly, analysis using reporter gene transfections, Northern blot and 5'-RACE showed that the GPR41 gene appears to lack a dedicated promoter: GPR40 and GPR41 mRNA share the same transcription start site, located at the GPR40 promoter. Thus transcription from the GPR40 promoter generates a bicistronic mRNA containing two separate protein coding regions. This unusual gene structure suggests a possible mechanism for coordinate expression of the proteins, perhaps related to the response of the beta cell to changes in blood levels of fatty acids. microRNAs (miRNAs) play an essential role in controlling a broad range of biological processes including animal development. miR-375, which is selectively expressed in pancreatic islets, has been implicated both in the development of islets, and the function of mature pancreatic beta cells. Using a reporter gene approach in transgenic mice and cultured cells, we have characterized the promoter of the miR-375 gene and shown that islet-specific expression is controlled at the transcriptional level. Identification and characterization of beta cell transcription factors is essential for a detailed understanding of beta cell development and function. Importantly, this knowledge will improve our ability to “reprogram” cells towards a beta cell phenotype, and advance the goal of generating an unlimited supply of beta cells for replacement therapy.

## **Ex-vivo expansion of adult human pancreatic beta cells**

Shimon Efrat

*Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv*

Expansion of insulin-producing beta cells from adult human cadaver islets could alleviate donor shortage for cell-replacement therapy of diabetes. Culture of adult human islet cells results in a rapid loss of expression of insulin and other beta-cell markers. In the absence of a stable marker, it is difficult to determine whether this loss reflects beta-cell death or dedifferentiation. We developed a genetic cell-lineage tracing approach based on viral vectors for following the fate of cultured human beta cells. This labeling system demonstrated that cultured human beta cells survived, dedifferentiated and were induced to proliferate for a maximum of 16 population doublings. Labeled cells could be isolated and expanded in the absence of other pancreas cell types, if provided with medium conditioned by non-beta pancreatic cells. These findings provide for the first time direct evidence for ex-vivo replication of cells derived from adult human beta cells, unlike mouse beta cells. These cells may maintain open chromatin structure at loci important for beta-cell phenotype, and thus represent good candidates for redifferentiation. Redifferentiation may be facilitated by elucidation of the signaling pathways altered during the adaptation of these cells to growth in culture. Our studies show that human beta-cell dedifferentiation and entrance into the cell cycle *in vitro* correlates with activation of the NOTCH pathway and downregulation of the cell cycle inhibitor p57. Inhibition of upregulation of the NOTCH target HES1 using small hairpin RNA (shRNA) resulted in higher levels of p57 in beta cells and reduced beta-cell dedifferentiation. Thus, components of the NOTCH pathway represent attractive molecular targets for induction of redifferentiation in the expanded cells.

## **Mechanisms of pancreatic beta cell proliferation and regeneration**

Yuval Dor

*Hebrew University, Jerusalem*

Recent evidence suggests that pancreatic beta cell mass is more plastic than assumed, but the mechanisms involved and the regenerative potential of beta cells are poorly understood. We have developed a transgenic mouse model, based on doxycycline-regulated expression of diphtheria toxin in insulin-producing cells, to study the dynamics of beta cell regeneration. Following the administration of doxycycline to transgenic mice we observe beta cell apoptosis, destruction of islet architecture, a reduction in beta cell mass and pancreatic insulin content, and the development of diabetes. Surprisingly, doxycycline withdrawal results in spontaneous normalization of blood glucose levels and islet architecture, and a significant regeneration of beta cell mass. Genetic lineage tracing shows that beta cell regeneration in this model relies mostly on the enhanced proliferation of surviving beta cells, rather than the differentiation of stem cells. Treatment with Sirolimus and Tacrolimus, the non-steroidal immunosuppressants used in the Edmonton protocol for islet transplantation, completely abolishes recovery from diabetes and beta cell regeneration. The results suggest that regenerative therapy for type 1 diabetes is possible given that autoimmunity is blocked before beta cells are depleted and using regeneration-compatible drugs. Ongoing experiments utilize this system as a screening platform for drugs that affect beta cell regeneration, and for testing hypotheses regarding the mechanisms that control beta cell proliferation. Specifically, we focus on understanding the role of insulin-glucose metabolism in beta cell regeneration.

## **Closing the Loop – Towards an Artificial Pancreas – The Schneider Medical Center Experience**

Revital Nimri, Eran Atlas, Shahar Miller, Eli Grunberg, Moshe Phillip

*Institute of Endocrinology and Diabetes, Schneider Children Medical Center, Petah Tikva*

The ultimate goal in diabetes treatment is the development of an autonomous system that mimics the activity of the pancreatic beta cells and is capable of maintaining normal physiologic blood glucose levels. The Institute for Endocrinology and Diabetes in Schneider Children's Medical Center of Israel has established recently a new Diabetes Technology Center aiming at developing a control algorithm that will communicate between a continuous glucose sensor and delivery pumps. We developed the MD-Logic system which allows real-time control of the blood glucose levels based on readings from continuous glucose sensor. The MD-Logic system is based on a model which imitates the logic of diabetes care givers. Our system is subject-specific and changes the control parameters according to the subject's glucose response. Our system can operate in two different delivery and sensing routes: 1.subcutaneous glucose sensing and insulin delivery and 2.intravascular glucose sensing and insulin delivery. The S.C. MD-Logic system uses readings from subcutaneous continuous glucose sensor to maintain euglycemia between and during meals. Four adult type 1 patients participated in six closed loop sessions. Each patient's blood glucose levels were controlled for 8 hours using the S.C MD-Logic system during a fasting and meals stages. The S.C. MD-Logic succeeded in adjusting and maintaining the blood glucose levels at the range of 92-150 mg/dL in three sessions. The I.V. MD-Logic system was tested on diabetic swine. Eighteen closed loop experiments were conducted on six streptozotocin-induced diabetic swine. The I.V. MD-Logic system lowered high blood glucose levels to the physiological range of 80-130 mg/dl with no hypoglycemic episodes for at least one hour. We conclude that although further clinical studies are needed, our preliminary results suggest that the MD-Logic system has clinical potential for both iv and sc closed loop systems.