UNIVERSITI PUTRA MALAYSIA

CHARACTERISATION OF CAPRINE ISLET-RELATED GENES AND ENHANCEMENT OF ISLET VIABILITY AND FUNCTION BY HEME OXYGENASE 1 GENE

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By

FAEZEH VAKHSHITEH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

February 2013

DEDICATION

With the name of ALLAH,

To my beloved parents and my lovely sisters for providing me with their enormous kindness, love and compassionate

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairperson: **Associate Professor Zeenathul Nazariah Allaudin, PhD**

Institute: **Bioscience**

Type 1 diabetes is an important health issue since diabetes is related with serious mortality and morbidity worldwide. One promising approach to treat the disease would be transplantation of the islets which are isolated from the donor pancreas into the patient liver via portal vein. However, a high loss of islets after isolation and transplantation is commonly noted which negatively affects the transplant outcomes. Heme oxygenase 1 (HO-1) is a stress protein induced as a protective mechanism in response to a variety of stimuli. Hence, targeted induction of this protein may be considered as a valuable therapeutic strategy for the protection against inflammatory processes and oxidative tissue damages. The first aim of the current study was to sequence goat HO-1 mRNA. Once the HO-1 mRNA was sequenced, the vector encoding goat HO-1 was constructed and later transfected into the islets. Insulin is the most important hormone of the pancreas, hence, the second objective of the study

was aimed to clone and sequence a cDNA encoding an insulin protein in the caprine species. The study was also designed to investigate the transient response of the caprine islets for insulin secretion as well as insulin gene expression after glucose exposure in short period (1 h). The current study also aimed to determine and differentiate the insulin secretion and cell death in association with the size of caprine islets. In this regard, caprine islets were isolated by collagenase digestion and purified by discontinuous Ficoll density gradient centrifugation. Isolated caprine islets were transfected with expression vector containing goat HO-1 gene and cultured for 5 days. The efficacy of GFP transfer to islets was quantified by flow cytometry. Western blots were applied to verify the expression of HO-1 protein. Glucose stimulated insulin release was measured using insulin ELISA assays. Meanwhile, the islet viability was evaluated by the Cell-Titer blue viability assay. Goat HO-1 cDNA was encoding a 288-amino acid protein with a predicted molecular mass of 32.75 kD. The cDNA of goat insulin was successfully amplified using a pair of specifically designed primers. Pairwise comparison showed that goat insulin protein (partially sequenced) was highly similar to insulin protein deposited in public databases, especially cattle preproinsulin with 93% homology. Goat insulin was also similar to human preproinsulin with 72% identity. It was shown that stimulation of insulin-producing cells with high glucose concentrations for only an hour resulted in transient elevation of insulin gene transcription, as verified by measurements of insulin mRNA levels. It was found that isolated caprine islets of large diameter could not be maintained efficiently in culture compared to small islets. After 48 h of culture, small islets showed 2.33% necrosis while a large proportion of necrotic cells was detected in cultured large islets (29.5%). The small and large islets showed an apoptotic death

pattern of 5.21 and 7.34%, respectively. Small islets were 92.46% viable, while the viability of the large islets was 63.16%. At 48 h post isolation, under basal conditions, the small islets release 1.39 ± 0.2 ng/IE insulin. With a high glucose concentration, the secreted insulin increased to 2.95 ± 0.33 ng/IE. For the large islet equivalencies, the insulin release under basal conditions and with a high glucose concentration were 0.489 ± 0.2 and 1.01 ± 0.26 ng/IE, respectively. Based on the results, after 2 days of culture, the insulin release with low and high glucose stimulation in large and small islets decreased significantly which clearly indicated that the islet function decreased gradually over time in *in vitro* culture. In the present study, to ensure the lipid-mediated transfection efficiency into the islets, optimization was first done using a reporter protein (Green Fluorescent Protein, GFP). After optimization, transfection and expression of the goat native HO-1 protein in the islet were investigated to determine islet functional outcome after 5 days of cultures *in vitro*. The insulin stimulation index (SI) in control islets (non-transfected) was 2.02 ± 0.026 while the SI in GFP and HO-1 transfected islets was 1.97 ± 0.026 and 2.07 ± 0.030, respectively. In conclusion, caprine islets could be suitable alternatives for human transplantation with regard to high similarity of the insulin gene to that of pig and human. The study showed that small islets were superior large islets in viability and insulin release under low and high glucose conditions in vitro culture 48 h post-isolation. Therefore, protection from core cell death in transplanted islets may improve the success of transplantation by reducing the routine non-functionality of the grafted islets. Transfection of caprine islets with native HO-1 can improve viability and function of the cultured islets which might subsequently increase success of islet transplantation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENCIRIAN GEN TERKAIT PEPULAU SEL KAPRIN DAN PENINGKATAN DAYA MAJU DAN FUNGSI PEPULAU SEL OLEH GENHEME OKSIGENASE 1

Oleh

FAEZEH VAKHSHITEH

Februari 2013

Pengerusi: Profesor Madya Zeenathul Nazariah Allaudin, PhD

Institut: Biosains

Diabetes tip 1 adalah satu isu kesihatan yang penting kerana diabetes dikaitkan dengan kematian dan morbiditi yang serius di seluruh dunia. Salah satu pendekatan berpotensi untuk merawat penyakit ini adalah dengan pemindahan pepulau sel (*islet*) yang diasingkan daripada pankreas penderma ke dalam hati pesakit melalui vena portal. Walau bagaimanapun, kehilangan pepulau sel yang tinggi selepas pengasingan dan pemindahan biasanya berlaku dan ini memberi kesan negatif terhadap hasil pemindahan. Heme oksigenase 1 (HO-1) adalah protein tekanan yang teraruh sebagai mekanisme pelindung dalam gerak balas terhadap pelbagai rangsangan. Justeru itu, aruhan tersasar protein ini boleh dianggap sebagai satu strategi terapi yang bernilai dalam perlindungan terhadap proses keradangan dan kecederaan oksidatif pada tisu. Tujuan pertama kajian ini ialah untuk menjujuk mRNA HO-1 kambing. Apabila

mRNA HO-1 telah dijujuk, vektor yang mengekodkan HO-1 kambing dibina dan kemudiannya ditransfeksikan ke dalam pepulau sel. Insulin adalah hormon paling penting dalam pankreas, maka, objektif kedua kajian ini ialah untuk mengklon dan menjujuk cDNA pengekod protein insulin dalam spesies kaprin. Kajian ini juga bertujuan untuk menyiasat gerak balas fana pepulau sel kaprin dalam rembesan insulin dan dalam penyataan gen insulin selepas pendedahan singkat (1 jam) kepada glukosa. Kajian ini juga bertujuan untuk menentu dan membezakan rembesan insulin dan kematian sel berhubung dengan saiz pepulau sel kaprin. Pepulau kaprin telah diasingkan melalui pencernaan kolagenase dan ditulenkan melalui pengemparan cerun ketumpatan Ficoll. Pepulau sel ditransfeksikan dengan vektor ekspresi yang mengandungi gen HO-1 kambing dan dikultur selama 5 hari. Keberkesanan pemindahan GFP ke dalam pepulau sel telah disukat mengguna sitometri aliran. Sap Western telah digunakan untuk mengesahkan penyataan protein HO-1. Pembebasan insulin terrangsang glukosa disukat mengguna assai ELISA insulin. Sementara itu, daya maju pepulau sel dinilai melalui assai daya maju biru Cell-Titre. cDNA HO-1 kambing mengekodkan protein 288-asid amino dan jisim molekul ramalannya ialah 32.75 kD. cDNA insulin kambing telah berjaya diperkuatkan dengan menggunakan sepasang primer direka bentuk khusus. Perbandingan berpasang menunjukkan protein insulin kambing (separa jujuk) adalah tinggi keserupaannya dengan protein insulin yang telah didepositkan dalam pangkalan data awam, terutamanya preproinsulin lembu dengan homologi 93%. Insulin kambing juga serupa dengan preproinsulin manusia dengan kesamaan 72%. Adalah didapati bahawa pepulau sel kaprin berdiameter besar yang diasingkan itu tidak dapat dikekalkan dengan berkesan dalam kultur jika dibandingkan dengan pepulau sel kecil. Selepas dikultur selama 48 jam,

pepulau sel kecil menunjukkan 2.33% nekrosis manakala kadar necrosis (29.5%) dikesan dalam kultur pepulau sel besar adalah tinggi. Pepulau sel kecil dan besar menunjukkan pola kematian apoptosis masing-masing 5.21 dan 7.34%. Pepulau sel kecil berdaya maju 92.46%, manakala daya maju pepulau sel besar ialah 63.16%. Pada 48 jam pasca-pengasingan, dalam keadaan basal, pepulau kecil membebaskan 1.39 ± 0.2 ng/IE insulin. Dengan kepekatan glukosa tinggi, insulin yang dirembeskan meningkat kepada 2.95 ± 0.33 ng/IE. Bagi setara pepulau sel besar, dalam keadaan basal dan kepekatan glukosa tinggi, pembebasan insulin masing-masing ialah 0.489 ± 0.2 dan 1.01 ± 0.26 ng/IE. Berdasarkan keputusan ini, selepas 2 hari dalam kultur, pembebasan insulin dengan rangsangan glukosa rendah dan tinggi dalam pepulau besar dan kecil menurun dengan ketara, menunjukkan bahawa fungsi pepulau sel menurun secara beransur-ansur mengikut masa dalam kultur *in vitro*. Dalam kajian ini, untuk memastikan kecekapan transfeksi berantarakan lipid ke dalam pepulau sel, pengoptimuman dilakukan terlebih dahulu dengan menggunakan protein pelapor (protein pendarfluor hijau, GFP). Selepas pengoptimuman, transfeksi dan penyataan protein HO-1 kambing asli dalam pepulau sel disiasat untuk memastikan hasil fungsian pepulau sel selepas 5 hari dalam kultur *in vitro*. Indeks rangsangan insulin (SI) dalam pepulau sel kawalan (bukan ditranfeksi) adalah 2.02 ± 0.026 manakala SI dalam pepulau sel tertransfeksi GFP dan HO-1, masing-masing adalah 1.97 ± 0.026 dan 2.07 ± 0.030. Justeru itu, pepulau kaprin boleh dijadikan alternatif sesuai untuk pemindahan dalam manusia mengambil kira adanya persamaan tinggi di antara gen insulin babi dan manusia. Kajian menunjukkan bahawa daya maju dan pembebasan insulin pepulau sel kecil lebih unggul daripada pepulau sel besar dalam keadaan glukosa rendah dan tinggi dalam kultur *in vitro* 48 jam pasca-pengasingan. Justeru itu

perlindungan daripada kematian sel teras pepulau sel yang dipindah itu mungkin meningkatkan kejayaan pemindahan dengan mengurangkan ketidakfungsian rutin pepulau sel yang dicantumkan. Transfeksi pepulau sel kaprin dengan HO-1 asli boleh meningkatkan daya maju dan fungsi pepulau sel terkultur yang kemudiannya mungkin meningkatkan kejayaan pemindahan pepulau sel.

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I certify that a Thesis Examination Committee has met on **19th February 2013** to conduct the final examination of Faezeh Vakhshiteh on her thesis entitled "**characterization of caprine islet-related genes and enhancement of islet viability and function by Heme oxygenase 1 gene**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Master of Science.

Members of the Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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FAEZEH VAKHSHITEH

Date:

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