UNIVERSITI PUTRA MALAYSIA

ISOLATION AND CHARACTERIZATION OF FULL-TERM AMNIOTIC FLUID-DERIVED STEM CELLS

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ISOLATION AND CHARACTERIZATION OF FULL-TERM AMNIOTIC FLUID-DERIVED STEM CELLS

By

NURFARHANA BINTI FERDAOS

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

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To my beloved parents

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

ISOLATION AND CHARACTERIZATION OF FULL-TERM AMNIOTIC FLUID-DERIVED STEM CELLS

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NURFARHANA BINTI FERDAOS

October 2010

Chairman: Norshariza Nordin, PhD

Faculty: Medicine and Health Sciences

Stem cell research has gained many attentions for their hope to cure myriad of diseases ranging from genetically linked disorders to neurodegenerative diseases as well as injuries. Pluripotent stem cells signify the perpetual source of versatile cells, making them an excellent source for cell therapy. Current discoveries have reported amniotic fluid as an alternative source for pluripotent stem cells, termed amniotic fluid-derived stem (AFS) cells. AFS cells have been shown to express embryonic and adult stem cell markers while hindering the ethical controversies, which has been the biggest issue

involving the well-known pluripotent stem cells; the embryonic stem (ES) cells. In addition, AFS cells also do not form tumors upon transplantation. To date, only amniotic fluid of mid-term pregnancies has been reported to yield AFS cells. However, the collection of mid-term AF samples involves invasive procedure (amniocentesis) that may result in several complications including abortion. Therefore, in this study, we aim to isolate and characterize AFS cells from human and rat full-term pregnancies. The heterogeneity of amniotic fluid (AF) cells was observed upon culturing the cells under the optimized conditions, prior to isolation of stem cell by immunoselection against c-Kit, a marker for stem cell factor. The isolation of cells positive for c-Kit using Magnetic-Activated Cell Sorting (MACS) technology has resulted in enrichment of the c-Kit positive cells. Characterization of the c-Kit positive cells has demonstrated the expression of Oct-4, a marker for pluripotent cells and the formation of embryoid bodies (EBs), suggesting the possibility of AFS cells being present during full-term pregnancy in mammals including human. Hence giving hopes that full-term AFS cells would be the future alternative source for stem cell therapy.

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

PEMENCILAN DAN PENCIRIAN SEL STEM CECAIR AMNIOTIK DARIPADA TEMPOH PENUH KANDUNGAN

Oleh

NURFARHANA BINTI FERDAOS

Oktober 2010

Pengerusi: Norshariza Nordin, PhD

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Penyelidikan dalam bidang sel stem telah menarik banyak perhatian kerana sel ini mempunyai potensi untuk menyembuhkan pelbagai jenis penyakit, merangkumi penyakit berkaitan genetik, sehingga penyakit berkaitan saraf termasuk kecederaan. Sel stem yang pluripoten adalah sumber semulajadi pelbagai jenis sel yang amat sesuai digunakan dalam terapi sel. Penemuan

terkini telah melaporkan bahawa cecair amniotik atau lebih dikenali sebagai air ketuban adalah sumber alternatif untuk sel stem yang pluripoten dan dinamakan sebagai sel stem cecair amniotik (AFS). Sel AFS telah terbukti berupaya mengekspres penanda sel stem untuk sel stem embrio (*embryonic stem cells*) dan sel stem dewasa (*adult stem cells*). Sel ini juga tidak mempunyai masalah etika yang kerap dikaitkan dengan sumber sel stem pluripoten yang diketahui umum iaitu sel stem embrio (*embryonic stem cells*). Tambahan pula, sel ini tidak membentuk ketumbuhan selepas proses transplantasi. Terkini, hanya air ketuban daripada kandungan tempoh pertengahan yang dilaporkan mengandungi sel AFS. Walaubagaimanapun, teknik untuk mengumpul air ketuban daripada kandungan tempoh pertengahan ini (amniosintesis) adalah berbahaya dan boleh menyebabkan beberapa komplikasi, termasuk keguguran. Oleh itu, projek ini bermatlamat untuk memencilkan dan mencirikan sel AFS daripada air ketuban dalam kandungan tempoh penuh manusia dan tikus. Kepelbagaian sel air ketuban telah didapati dalam tempoh pembiakan sel di bawah keadaan kultur sel yang optima. Proses pemencilan sel stem dijalankan dengan menggunakan proses pemilihan berdasarkan ekspresi 'c-Kit', iaitu penanda untuk faktor sel stem. Proses pemilihan ini yang menggunakan teknologi '*Magnetic-Activated Cell Sorting* (MACS)' telah menghasilkan penambahan populasi sel yang positif untuk c-Kit. Pencirian sel yang positif untuk c-Kit menunjukkan ekspresi aktiviti Oct-4, iaitu penanda untuk sel pluripoten dan pembentukan '*embryoid bodies* (EBs)'. Penemuan ini mencadangkan terdapatnya sel AFS di

dalam air ketuban daripada kandungan tempoh penuh mamalia, termasuklah manusia. Oleh itu, sel AFS daripada kandungan tempoh penuh mempunyai harapan sebagai sel alternatif untuk terapi sel stem.

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Thank you.

I certify that an Examination Committee has met on **16 November 2010** to conduct the final examination of **Nurfarhana binti Ferdaos** on her Master thesis entitled **"Isolation and Characterization of Full-Term Amniotic Fluid-derived Stem Cells"** in accordance with Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science.

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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 instituition.

NURFARHANA BINTI FERDAOS

Date: 11 October 2010

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