UNIVERSITI PUTRA MALAYSIA

GENERATION AND CHARACTERISATION OF MESENCHYMAL STEM CELLS DERIVED FROM PLACENTA AND ADULT CARDIAC TISSUE

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By

SHALINI VELLASAMY

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfilment of the Requirements for the Degree of Doctor of Philosophy

December 2011

I dedicate this specially to my late mother for her lifetime of love, support, inspiration and motivation that had led me thus far

For you, Mom -

Regina Rajahdurai

with all my love.

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

GENERATION AND CHARACTERISATION OF MESENCHYMAL STEM CELLS DERIVED FROM PLACENTA AND ADULT CARDIAC TISSUE

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SHALINI VELLASAMY

December 2011

Chairman: Rajesh Ramasamy, PhD

Faculty: Medicine and Health Sciences

Mesenchymal Stem Cells (MSC) are adherent fibroblastic cells that are able to self-renew and can differentiate to give rise to mesodermal lineages. MSC are non-haematopoietic cells for they do not give rise to blood cell lineages but they support the haematopoiesis development in foetal and adult body. In addition, MSC were found to exhibit immunomodulatory effect on immune cells and cancer cells. In line with this, MSC have emerged as novel-cell based therapy to treat inflammations, autoimmune diseases, tumour growths and degenerative disorders which are considered as permanent and incurable diseases. This has increased the demands for MSC and has initiated the necessity to find alternative sources of MSC for an immediate and cost effective treatment. To date, there are some studies conducted on placenta, but little information is available on their immunomodulatory effects. Therefore, a comparative study was designed to investigate MSC derived from

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placenta tissues including umbilical cord, placenta (chorion and decidua basalis) and placental membrane (amnion) using enzymatic-dissociation method under one experimental condition. Besides, MSC were also generated from human adult cardiac tissues which were previously considered as terminally differentiated, post-mitotic organs lacking self renewal and regeneration potential. These MSC were characterised for their mesenchymal properties by means of cell surface markers via flowcytometric analysis, embryonic stem cell markers via reverse transcriptase PCR, differentiation potential and their immunomodulatory effect through tritiated thymidine cell proliferation, transwell, apoptosis and cell cycle assays. Placenta derived MSC were further assed for the effect of basic fibroblastic growth factor bFGF on the morphology and cell cycle kinetics of MSC. Both placenta and cardiac tissue derived MSC expressed common mesenchymal cell surface markers, embryonic stem cells markers and were able to differentiate into mesodermal lineages (adipocytes, osteocytes and chondrocytes). Interestingly, cardiac MSC had shown a more predetermined commitment into cardiac lineages as compared to bone marrow derived MSC. Supplementation of bFGF had significantly altered the morphology of MSC into more defined fibroblastic shape, enhanced growth kinetics, shortened doubling time, induced active cell cycle progression, delayed senescence, skewed the cytokine secretion profile and abundantly produced VEGF as compared to the MSC cultures without bFGF supplementation. Further functional study had demonstrated that UC-MSC and PLC-MSC significantly exerted a dose dependent inhibition on mitogenic stimulated T cell proliferation mainly via cell-to-cell contact than soluble factors ($p<0.05$). This inhibition was due to the arrest of T cells in G0 phase of cell cycle and not induced by apoptosis. However, this inhibitory effect was not specific to T lymphocytes, but also seen on other lymphocytes such as B cells

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and NK cells. Differently from T cells, MSC significantly prevented the transition from G_1 S phase of NK cells ($p<0.05$). In addition, we have also shown for the first time that cardiac derived MSC exert immunosuppressive effect on immune cells apart from their predetermined commitment into cardiac lineages. This study revealed that UC-MSC stands out to be a potential alternative source of MSC as they display mesenchymal properties and had significantly suppressed various immune cells as compared to PLC-MSC. Furthermore, we also show that cardiac MSC could be a potential therapeutic tool in regenerating and treating a diseased heart. Our findings are fundamental and provide a platform for future use of these MSC as a cell based therapy in controlling unwanted immune responses and organ regeneration.

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

GENERASI AND PENCIRIAN SEL INDUK MESENKIMA DARIPADA TISU PLACENTA DAN TISU JANTUNG DEWASA

Oleh

SHALINI VELLASAMY

Disember 2011

Pengerusi: Rajesh Ramasamy, PhD

Fakulti: Perubatan dan Sains Kesihatan

Sel induk mesenkima adalah sel yang boleh melekat pada permukaan plastik, berbentuk fibroblastik, memiliki keupayaan pembaharuan diri yang tinggi dan boleh mengubah dirinya kepada sel yang bercirikan mesoderma. Memandangkan sel induk mesenkima adalah bukan sel haematopoietik, jadi ia tidak akan mengubah dirinya kepada keturunan sel-sel darah yang lain, tetapi sel induk mesenkima ini boleh menyokong proses haematopoiesis dalam badan janin dan orang dewasa. Selain itu, sel induk mesenkima juga boleh mengenakan kegiatan immunotindasan ke atas sel-sel imun dan kanser. Selaras dengan ini, sel induk mesenkima telah muncul sebagai novel terapi yang berasaskan sel untuk merawat kebanyakkan penyakit kekal dan yang tidak boleh diubati seperti keradangan, penyakit autoimun, ketumbuhan dan penyakit gangguan degeneratif. Keadaan ini telah meningkatkan permintaan terhadap sel induk msenkima sebagai sumber alternatif untuk rawatan segera yang berkesan dan kos efektif. Sepanjang masa ini hanya sedikit kajian dijalankan ke atas sel induk

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mesenkima yang diperoleh daripada tisu sisa kelahiran manusia dan maklumat mengenai kesan kesan immunotindasan sel induk mesenkima ini adalah terhad. Oleh itu, kami telah mengendalikan satu kajian perbandingan antara sel-sel induk mesenkima yang dijana dari tali pusat, plasenta (chorion dan decidua basali), dan selaput plasenta (amnion) menggunakan kaedah penceraian enzim di bawah keadaan eksperimen yang sama. Selain ini, kami juga telah menghasilkan sel induk mesenkima daripada tisu dewasa manusia seperti tisu jantung yang sebelum ini dianggap sebagai organ yang kekurangan keupayaan mitotik, pembaharuan diri dan potensi untuk pertumbuhan semula. Kesemua sel-sel induk mesenkima yang dijana telah menunjukkan penanda-penanda sel induk mesenkima yang lazim, petanda sel stem embrio dan dapat membezakan dirinya kepada keturunan mesoderma (adipocyte, osteocyte, chondrocyte). Suplemen bFGF dengan ketaranya telah mengubah morfologi sel menjadi lebih fibroblatik, mempertingkatkan kinetik pertumbuhan, memendekan masa pergandaan, meangaktifkan kitar sel, melewatkan proses penuaan sel, memesongkan profil rembesan sitokin, dan juga telah menghasilkan lebih banyak VEGF berbanding dengan sel induk mesenkima yang dikultur tanpa suplemen bFGF. Selain itu, tisu jantung dewasa juga telah menunjukkan penanda-penanda sel induk mesenkima yang lazim, petanda sel stem embrio, petanda awal keturunan jantung, dapat membezakan dirinya kepada keturunan mesoderma dan juga telah mengenakan kesan-kesan immunotindasan. Kajian terhadap fungsi sel induk mesenkima selanjutnya telah menunjukkan bahawa UC-MSC dan PLC-MSC dengan ketaranya telah mengenakan perencatan dos bergantung ke atas proliferasi sel T yang telah dirangsang dengan mitogen, terutamanya mealui kenalan sel-sel (cell contact) dan faktor-faktor larut (soluble factors). Proses immuotindasan oleh sel induk mesenkima ke atas sel T adalah

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disebabkan oleh pemerangkapan sel T dalam fasa kitaran sel G_0 dan bukan melalui proses apoptosis sel. Proses sekatan proliferasi ini bukan sahaja dilihat spesifik pada sel T, malah juga wujud pada sel sel limfosit yang lain seperti sel B dan NK 92-MI. Berbeza dengan sel T, sel induk mesenkima telah mencegah peralihan sel NK 92-MI dari fasa G_1 ke S. Di kalangan semua sel induk mesenkima yang dijana, kajian kami mendedahkan bahawa UC-MSC berkongsi ciri ciri sel induk mesenkima yang sama dengan sel induk mesenkima yang lain dan jugan menunjukkan kesan immunotindasan yang lebih ketara dan tinggi terhapad pelbagai sel imun. Ini menjadikan UC-MSC menonjol sebagai sumber alternatif sel induk mesenkima yang berpotensi sebagai teapi sel yang boleh dieksploitasi untuk menghindari tindak bals yang tidak diingini oleh sel sel imun.

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I certify that a Thesis Examination Committee has met on 29 December 2011 to conduct the final examination of Shalini Vellasamy on her thesis entitled Generation and Characterisation of Mesenchymal Stem Cells Derived from Placenta and Adult Cardiac Tissue in accordance with 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 Doctor of Philosophy.

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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 **Doctor of Philosophy**. 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 the questions 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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