UNIVERSITI PUTRA MALAYSIA

NON-VIRAL TRANSFECTION OF BONE MARROW-DERIVED MESENCHYMAL STEM CELL WITH HUMAN INTERFERON-GAMMA GENE AND IN VITRO EFFICIENCY AGAINST CHRONIC MYELOID LEUKAEMIA CELLS

LIEW LEE CHUEN

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By

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

NON-VIRAL TRANSFECTION OF BONE MARROW-DERIVED MESENCHYMAL STEM CELL WITH HUMAN INTERFERON-GAMMA GENE AND *IN VITRO* EFFICIENCY AGAINST CHRONIC MYELOID LEUKAEMIA CELLS

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June 2013

Chairman : Maha Abdullah, PhD

Faculty : Medicine and Health Sciences

Chronic myeloid leukemia (CML) is a hematopoietic stem cell disorder caused by the BCR/ABL gene rearrangement, known as the Philadelphia (Ph) chromosome. To date, the only curative therapy for CML is allogeneic stem cell transplantation. However, significant morbidity and mortality are associated with the procedure and the need for a matched donor makes this option not available to the majority of the patients. Currently, various studies have been carried out to develop an alternative approach for CML treatment, for example targeted gene delivery of therapeutic cytokines. In this study, the feasibility of using bone marrow-derived mesenchymal stem cell (BM-MSC) in delivering human interferon-gamma (hIFN-γ) gene for targeted CML therapy was explored. Mesenchymal stem cells (MSC) were successfully isolated from human bone marrow aspirates and their biological properties were similar to those of MSC reported. Expanded BM-MSC were transfected with plasmid containing hIFN-γ gene (pORF-hIFN-γ) via nucleofection.

Gene transfer efficiency was determined based on intracellular hIFN-γ expression via flow cytometry and was found to be at 54.28±11.34%. The *in vitro* expression of hIFN-γ mRNA and protein in BM-MSC were also analysed at intervals of 24 h, up to 5 days post nucleofection, via real-time PCR and ELISA, respectively. Real-Time PCR data analysis showed significant up-regulation of hIFN-γ mRNA in nucleofected BM-MSC when compared to non-transfected BM-MSC (P=0.043). BM-MSC harbouring pORF-hIFN-γ could express hIFN-γ protein *in vitro*. This cytokine production was achieved as high as 3.47±1.03 ng/ml after 72 hours of nucleofection. The effect of hIFN-γ produced in nucleofected BM-MSC on the proliferation of CML cell line (K562) *in vitro* was also investigated. K562 growth was inhibited at 61.12±16.38% after seven days of co-culture with BM-MSC expressing hIFN-γ (P=0.006). In conclusion, findings in the current study indicated that hIFN-γ produced by genetically engineered BM-MSC successfully inhibited the proliferation of K562 cells *in vitro*. Thus, MSC as cellular vehicle in hIFN-γ gene delivery could be further explored as a promising treatment option for CML patients.

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

TRANSFEKSI GEN INTERFERON-GAMMA KE DALAM SEL STEM MESENKIMIA DARIPADA SUMSUM TULANG SECARA BUKAN VIRUS DAN KEBERKESANAN MENENTANG SEL LEUKEMIA MIELOGENUS KRONIK *IN VITRO*

Oleh

LIEW LEE CHUEN

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Pengerusi : Maha Abdullah, PhD

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Leukemia mielogenus kronik (CML) merupakan sejenis leukemia kronik yang dicirikan oleh peningkatan penghasilan klon sel-sel mieloid secara tidak terkawal akibat daripada translokasi gen ABL dan gen BCR, umumnya dikenali sebagai kromosom Philadelphia. Sehingga kini, transplantasi sumsum tulang merupakan satu-satunya terapi kuratif yang berpotensi untuk CML. Walau bagaimanapun, morbiditi and mortaliti yang dikaitkan dengan prosedur transplantasi and keperluan penderma yang sesuai telah menyebabkan terapi tersebut tidak dapat disediakan kepada kebanyakan pesakit. Oleh yang demikian, pelbagai penyelidikan telah dijalankan pada masa kini dengan tujuan untuk memperkembangkan satu rawatan novel untuk CML, contohnya penghantaran sitokin terapeutik ke sasaran. Dalam kajian ini, penggunaan sel stem mesenkima yang diperoleh daripada sumsum tulang dalam penghantaran gen interferon-gamma manusia (hIFN-γ) sebagai terapi sasaran

bagi CML telah dikaji. Sel stem mesenkima (MSC) telah berjaya dipencilkan daripada aspirasi sumsum tulang manusia dan sifat-sifat biologi sel ini adalah serupa dengan MSC yang dilaporkan. MSC sumsum tulang manusia (BM-MSC) ini kemudian ditransfeksi dengan plasmid mengekod gen hIFN-γ dengan menggunakan teknologi nukleofeksi. Keberkesanan transfeksi telah ditentu berdasarkan ekspresi hIFN-γ intraselular dengan menggunakan sitometri aliran. Keberkesan transfeksi sebanyak 54.28±11.34% telah tercapai selepas 24 jam nukleofeksi. Ekspresi mRNA dan protein hIFN-γ di dalam BM-MSC secara i*n vitro* juga dikaji setiap 24 jam selepas nukleofeksi, selama 5 hari dengan menggunakan tindak balas rantai polimerase masa nyata (real-time PCR) dan ELISA masing-masing. Keputusan tindak balas rantai polimerase masa nyata menunjukan peningkatan ekspresi mRNA hIFN-γ yang ketara dalam sampel nukleofeksi BM-MSC berbanding dengan BM-MSC yang tidak dinukleofeks (P=0.043). Di samping itu, BM-MSC yang telah dinukleofeks dengan plasmid pORF-hIFN-γ juga berupaya menghasilkan protein hIFN-γ secara *in vitro*. Produksi protein hIFN-γ yang paling tinggi ialah 72 jam selepas nukleofeksi, dimana sebanyak 3.47±1.03 ng/ml protein hIFN-γ telah dikesan. Selain itu, kesan hIFN-γ yang dihasilkan oleh nukleofeksi BM-MSC terhadap proliferasi sel CML (K562) juga turut dikaji. Proliferasi sel K562 telah ditindas sebanyak 61.12±16.38% apabila kultur bersama nukleofeksi BM-MSC selepas 7 hari (P=0.006). Secara kesimpulannya, penemuan dalam kajian semasa menunjukkan bahawa hIFN-γ yang dihasilkan secara modifikasi genetik BM-MSC telah berjaya menindas proliferasi sel K562 *in vitro*. Oleh itu, MSC sebagai pengangkutan selular dalam penghantaran gen hIFN-γ boleh terus diterokai sebagai rawatan alternatif yang berkesan untuk pesakit CML.

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I certify that a Thesis Examination Committee has met on 7 June 2013 to conduct the final examination of Liew Lee Chuen on her thesis entitled "Non-viral Transfection of Bone Marrow-Derived Mesenchymal Stem Cell woth Human Interferon-gamma Gene and *In Vitro* Efficiency Against Chronic Myeloid Leukaemia Cells" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the University Putra Malaysia [P.U.(A) 106] 15 March 1998. 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 the 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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LIEW LEE CHUEN

Date: 7th June 2013

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