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MESENCHYMAL STEM CELL-MEDIATED IMMUNOMODULATION OF MICROGLIA

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By

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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

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Dedicated to my parents and my beloved wife

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

MESENCHYMAL STEM CELL-MEDIATED IMMUNOMODULATION OF MICROGLIA

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September 2013

Chairperson: Sharmili Vidyadaran, PhD

Faculty : Medicine and Health Sciences

Neuroinflammation is a key pathogenic event of neurological diseases. The continuous inflammatory responses of microglia exacerbate disease by increasing neuronal damage, an effect that may warrant control. An approach for this is the utilisation of mesenchymal stem cells (MSC). This study explores the mechanisms through which MSC modulate inflammatory responses of microglia. For this, mouse bone marrow-derived MSC and BV2 (a microglial cell line) were cocultured at different ratios. The anti-proliferative effect of MSC on microglia was deciphered by examining cell cycle, apoptosis and the role of nitric oxide (NO).

Migration of both cell types was also examined along with differential expression of soluble mediators. Direct contact of BV2 with MSC at the 1:0.2 (BV2:MSC) seeding ratio inhibited proliferation of LPS-stimulated BV2 microglia to 71.2 ± 9.7% ($p<0.05$), an effect also conferred by MSC soluble factors. At the same 1:0.2 ratio, MSC also increased NO expression in cocultures, inducing a 25% surge from 56.94 ± 2.65μM to 76.59 ± 3.08μM at 48hrs ($p<0.05$). However, NO was not implicated in the anti-proliferative effect as inhibiting NO did not restore BV2 proliferation. Role of apoptosis in the reduction of BV2 proliferation was also ruled out as the number of Annexin-V^-/PI^- cells remained high. A slowdown of cell cycle was identified as the mechanism through which MSC exert their anti-proliferative effect on microglia. Coculture with MSC reduced the population of BV2 microglia in S-phase by 6.25 ± 1.5% ($p<0.001$) and restored the percentage of BV2 cells at the G2/M-phase to levels similar in unstimulated BV2 microglia. The immunomodulatory effects reported here were also accompanied by an MSC cell cycle arrest at G0/G1-phase (percentage of MSC in G0/G1-phase increased from 55.34 ± 2.6% to 86.32 ± 2.0% ($p<0.001$)). Using protein array, galectin-1 was identified as a possible immunomodulator as its levels increased significantly to 2.16-fold in coculture ($p<0.05$). Presence of MSC also increased IL-6 (by 30-fold, $p<0.05$) whereas TNF-α was reduced by 4-fold ($p<0.05$). The study then explored the role of IL-6

and TNF-α in MSC-mediated modulation of NO production and proliferation of microglia. By using neutralising antibodies, it was shown that IL-6 did not play a role in the NO production of cocultures or inhibition of microglial proliferation, whilst neutralisation of TNF-α abolished the NO surge although leaving proliferation unaffected in coculture. As blocking TNF-α reduced LPS-stimulated BV2 proliferation, proliferation of microglia was proposed to be mediated by TNF-α and that MSC inhibit microglial proliferation by downregulating TNF-α levels in coculture. This study then pursued the migratory properties of microglia and MSC. The results demonstrated that BV2 cells showed remarkable migration to MSC, paralleled by an increase in MMP-9 activity (12.35 ± 2.89-fold). It was also shown that MSC intrinsically migrated towards microglia (208.5 ± 10.6), and more so in inflammatory conditions (295.3 ± 43.8). MSC also migrated towards LPS-stimulated astrocytes. However, LPS alone did not influence MSC migration, indicating the importance of a cellular impetus that MSC requires for their migration and the reciprocity in migration of BV2 microglia and MSC. The findings from this study shed light on mechanisms of immunomodulatory functions of MSC on microglia which improve our understanding for MSC-mediated therapy of neuroinflammatory conditions.

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

IMMUNOMODULASI MIKROGLIA OLEH PERANTARA STEM SEL MESENKIMA

Oleh

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Keradangan neuron adalah peristiwa penting patogenik bagi penyakit saraf. Respons keradangan berterusan yang dihasilkan oleh mikroglia akan memburukkan penyakit dengan meningkatkan kerosakan saraf. Imbangan respons keradangan adalah penting disamping mengurangkan kerosakantisu. Salah satu pendekatan adalah dengan menggunakan sel stem mesenkima (MSC). Kajian ini menjelaskan

mekanisme MSC dalam meminda respons keradangan oleh mikroglia. Dengan ini, MSC yang dipencilkan daripada sumsum mencit dan BV2 (titisan sel mikroglia) telah dikultur bersama pada nisbah yang berbeza. Kesan anti-proliferatif mikroglia oleh MSC ditentukan oleh pemerbadanan tritium timidina, dengan memeriksa kitaran sel, apoptosis dan peranan nitric oksida (NO). Penghijrahan kedua-dua sel juga ditentukan bersama penghasilan perantara-perantara kimia. Hubungan langsung antara BV2 dan MSC pada nisbah 1:0.2 (BV2:MSC) bilangan sel telah merencatkan proliferasi BV2 mikroglia yang telah diransang oleh LPS kepada 71.2 ± 9.7% ($p<0.05$), yang mana disumbangkan oleh perantara-perantara kimia MSC. Pada nisbah sama 1:0.2, MSC juga meningkatkan penghasilan NO dalam kultur bersama dengan merangsang peningkatan sehingga 25% daripada 56.94 ± 2.65μM kepada 76.59 ± 3.08μM pada jam ke-48 ($p<0.05$). Walau bagaimanapun, NO tidak diimplikasikan sebagai kesan anti-proliferatif kerana perencatan NO tidak mengembalikan proliferasi sel BV2. Peranan apoptosis dalam merencat proliferasi sel BV2 juga tidak diambil kira disebabkan jumlah bilangan sel Annexin-V^-/PI^-adalah kekal tinggi. Penurunan kadar kitaran sel dikesan sebagai mekanisme yang mana MSC menunjukkan kesan anti-proliferatif pada mikroglia. Kultur bersama MSC mengurangkan populasi BV2 mikroglia pada fasa-S daripada 54.77 ± 1.5% (kawalan) kepada 48.54 ± 1.0% ($p<0.001$) dan

peratusan sel BV2 telah kembali pada fasa-G2/M kepada 7.71 ± 1.01% yang mana didapati turun pada 3.31 ± 2.38% selepas diaruh LPS. Berpandukan keputusan penting daripada cerakin proliferasi, modulasi kitaran sel BV2 juga berkaitan dengan perantara kimia. kesan immunomodulasi adalah diiringi dengan perencatan kitaran sel pada fasa-G0/G1 (peratus MSC dalam fasa G0/G1 meningkat daripada 55.34 ± 2.6% kepada 86.32 ± 2.0% ($p<0.001$)). Berpandukan tatasusun protein, galektin-1 muncul sebagai salah satu perantara immunomodulasi yang mana tahap peningkatan signifikan dikesan sehingga 2.16 kali ganda di dalam kultur bersama ($p<0.05$). Kehadiran MSC juga meningkatkan penghasilan IL-6 (30 kali ganda, $p<0.05$) manakala TNF-α turun sehingga 4 kali ganda ($p<0.05$). Kajian ini kemudian menghuraikan peranan IL-6 dan TNF-α oleh MSC dalam memodulasikan penghasilan NO dan proliferasi mikroglia. Dengan menggunakan kaedah peneutralan antibodi, IL-6 didedahkan bahawa tidak memainkan peranan dalam penghasilan NO di dalam kultur bersama atau perencatan dalam proliferasi mikroglia, manakala peneutralan TNF-α menghapuskan kesan peningkatan NO manakala sebaliknya tiada kesan pada proliferasi sel. Dengan kesan perencatan proliferasi BV2 akibat daripada penghalangan TNF-α, ajukan dibuat bahawa TNF-α berperanan dalam proliferasi mikroglia teraktif dan MSC merencatkan proliferasi mikroglia dengan merendahkan tahap

pengawalseliaan TNF-α di dalam kultur bersama. Kajian ini dilanjutkan kepada ciri-ciri penghijrahan mikroglia dan MSC. Hasil membuktikan bahawa LPS meningkatkan penghijrahan mikroglia sebanyak 3.8 kali ganda kepada 505.3 ± 94.0 ($p<0.05$) tetapi tidak mempengaruhi penghijrahan MSC. Ulungnya, sel BV2 berhijrah secara setara ke MSC (521.7 ± 52.3), juga ditonjolkan dengan peningkatan aktiviti MMP-9 (12.35 ± 2.89 kali ganda). MSC juga dipamerkan sebagai berhijrah secara intrinsic ke mikroglia (208.5 ±10.6), dan begitu juga dalam persekitaran keradangan (295.3 ± 43.8). Dengan ini, BV2 mikroglia dan MSC ditunjukkan untuk berhijrah secara silang, mengesahkan potensi kedua-dua sel untuk mendiami tempat yang tercedera. Penemuan daripada kajian ini menyorot fungsi mekanisme immunomodulasi oleh MSC pada mikroglia yang mungkin dapat meningkatkan pemahaman bagi terapi menggunakan MSC sebagai perantara dalam keradangan neuron.

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Last but most importantly, Sona; my wife! This thesis has witnessed the transition of this beautiful lady from a childhood friend to my wife. Am not sure how to thank you for all what you have done for me. All what I want to say is “see you soon!”

I certify that a Thesis Examination Committee has met on 3rd September 2013 to conduct the final examination of Shinsmon Jose on his thesis entitled "Mesenchymal Stem Cell-Mediated Immunomodulation of Microglia" 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 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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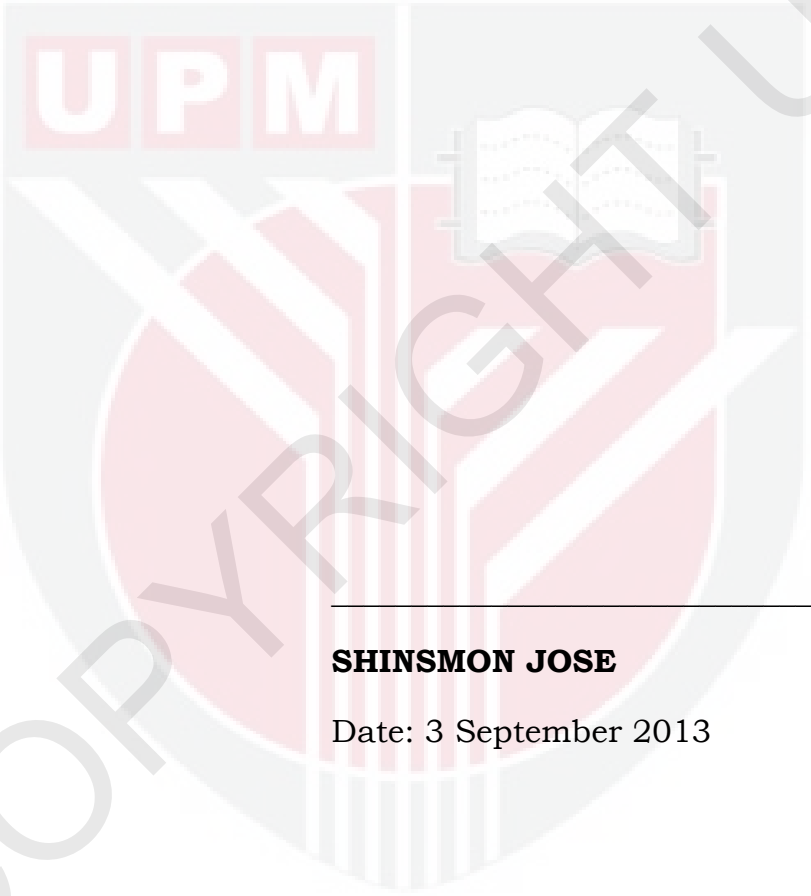
DECLARATION

I declare that the thesis is my original work except for the 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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