UNIVERSITI PUTRA MALAYSIA

THE ROLES OF WNT1 AND DKK1 DURING IN VITRO NEURAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELL LINES

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By

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THE ROLES OF WNT1 AND DKK1 DURING *IN VITRO* NEURAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELL LINES

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Chairman: Norshariza Nordin, PhD

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Embryonic stem cells (ESCs) possess two unique properties: self-renewal and pluripotency. These unique properties make ESCs useful tools to discover the mechanisms behind differentiation and development, to unravel the mechanism of diseases, to test the effect of drugs as well as for use in clinical trials for degenerative disease and developmental defects. Large quantities of neurons or neural precursors are required for treating neurodegenerative diseases, hence understanding the mechanisms behind neural differentiation of ESCs and establishing the appropriate protocols in generating pure population of specific neuronal precursors are indeed essential. Lots of growth factors and cell signalling molecules have been found to play important roles during neural differentiation of ESCs.

The Wnt/β-catenin pathway is one of the essential signalling pathways involved in neurogenesis *in vivo* and neural differentiation of ESCs *in vitro*. However, the activity of Wnt signalling during neural differentiation of ESCs is not constitutive but rather it is stage-dependent. The Wnt signalling pathway has been found to be downregulated upon the formation of neural precursor cells, and therefore is believed to inhibit neural differentiation of mESCs *in vitro*. Moreover, recently, the Wnt signalling pathway has also been found to regulate the self-renewal of mESCs *in vitro*.

In this project, two inducible transgenic mESC lines carrying two important components of Wnt/β-catenin signalling pathways: *Wnt1-HA* and *Dkk1* gene have been established and characterized. The system used was a binary system that combines two techniques; *Cre/loxP*-based genetic recombination and ligand-dependent activation of Cre. Expression of the transgene was induced upon exposure to a synthetic estrogen receptor, 4-hydroxytamoxifen (4'-OHT). Time course induction was carried out in both cell lines, each with

two different clones, to obtain the optimal dosage of 4'-OHT in inducing the expression of the transgene. Cytotoxicity effects of non-detrimental dosage of 4'-OHT were also determined during neural differentiation of mock-transfected inducible ES cell system (CAG-floxed-neopA-empty vector).

Using the system, I then aimed to unravel the effects of stimulating (through overexpression of Wnt1) and inhibiting (through overexpression of Dkk1) Wnt signalling pathway at specific time points during neural differentiation process on the formation of neural precursor cells and post-mitotic neurons. In addition, the system also allows the access to the role of Wnt1 in maintaining the self-renewability of undifferentiated mESCs in the absence of LIF through overexpression of Wnt1-HA in mESCs.

It was found that 1) non-detrimental dosages of 4'-OHT and different induction time were needed to induce expression of Dkk1/Wnt1-HA transgenes (in mESCs - Dkk1: 200nM/48h; Wnt1-HA: 600nM/48h; and in embryoid bodies (EBs) – Dkk1:400nM/72h; Wnt1-HA: 1000nM/48); 2) overexpression of Wnt1 maintained self-renewal and neural commitment of mESCs; 3) Wnt1 promoted the formation of NPCs and post-mitotic neurons; 4) constitutive overexpression of Dkk1 inhibited the formation of NPCs; and interestingly, 5) overexpression of Dkk1 at early and later stages significantly increased the formation of NPCs and post-mitotic neurons (T-test, $P<0.05$).

Results from this project confirm the multiple roles and stage-dependent of Wnt signal during neural differentiation process of mESCs *in vitro* as well as in maintaining the self-renewability of undifferentiated mESCs. It is hoped that the findings will add up to current knowledge regarding the understanding behind the differentiation process of ESCs, hence would be essential for future neurodegenerative cell-based therapy.

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

PERANAN WNT1 AND DKK1 SEMASA PEMBEZAAN SEL SARAF DARI SEL STEM EMBRIONIK *IN VITRO*

Oleh

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November 2013

Pengerusi: Norshariza Nordin, PhD

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Sel stem embrionik (ESCs) mempunyai dua ciri unik: pembaharuan kendiri dan pluripotensi. Ciri unik ini menjadikan ESCs sebagai bahan yang berguna untuk merungkai mechanism di sealed pembezaan dan perkembangan, untuk membongkar mekanisma penyakit, untuk menguji kesan dadah serta menggunakannya dalam ujian klinikal untuk menguji penyakit degeneratif dan kecacatan perkembangan. Sel saraf atau prekursor sel saraf yang berkuantiti tinggi diperlukan untuk merawat penyakit neurodegeneratif, maka dengan itu pemahaman mekanisma di sealed perbezaan sel saraf ESCs dan usaha mewujudkan protokol yang sesuai bagi menjana populasi tulen prekursor sel saraf spesifik adalah penting. Banyak faktor pertumbuhan dan molekul pengisyaratan sel didapati memainkan peranan yang penting dalam proses pembezaan sel saraf daripada ESCs.

Laluan Wnt/β-catenin adalah salah satu laluan pengisyaratan penting yang terlibat dalam neurogenesis *in vivo* dan pembezaan sel saraf ESCs *in vitro*. Walau bagaimanapun, aktiviti pengisyaratan Wnt semasa pembezaan sel saraf daripada ESCs tidak konstitutif tetapi bergantung pada sesuatu peringkat pembezaan. Laluan pengisyaratan Wnt didapati menurun semasa pembentukan prekursor sel saraf, dan oleh itu dipercayai merencat pembezaan sel saraf daripada mESCs *in vitro*. Tambahan pula, baru-baru ini, laluan pengisyaratan Wnt juga didapati mengawal atur pembaharuan kendiri mESCs *in vitro*.

Dalam projek ini, dua titisan mESC transgenik yang membawa dua komponen penting dalam laluan pengisyaratan Wnt / β-catenin: Gen *Wnt1-HA* dan *Dkk1*, dihasilkan dan dicirikan. Sistem ini merupakan sistem binari yang menggabungkan dua teknik: penggabungan semula genetik berasaskan Cre*/loxP* dan pengaktifan Cre yang bergantung kepada ligan. Ekspresi

transgen akan terangsang apabila didedahkan kepada reseptor estrogen sintetik, 4-hydroxytamoxifen (4'-OHT). Induksi berpandukan masa dijalankan di dalam kedua-dua titisan sel, masing-masing dengan dua klon yang berbeza, untuk mendapatkan dos optimum 4'-OHT bagi merangsang ekspresi transgen itu. Kesan sitotoksik dos tidak memudaratkan 4'-OHT juga ditentukan semasa pembezaan sel saraf dari sistem sel ES terinduksi yang ditransfeksi dengan vektor kosong (vektor CAG-floks–*neopA*-kosong).

Dengan menggunakan sistem ini, kesan merangsang (melalui ekspresi berlebihan Wnt1) dan merencat (melalui ekspresi berlebihan Dkk1) laluan pengisyaratan Wnt pada detik masa spesifik semasa proses pembezaan prekursor sel saraf dan sel saraf pasca mitosis ditentukan. Di samping itu, sistem ini juga membolehkan pengaksesan peranan Wnt1 dalam mengekalkan pembaharuan kendiri sel asal mESCs tanpa kehadiran LIF melalui ekspresi berlebihan Wnt1-HA di dalam mESCs.

Keputusan kajian mendapati bahawa 1) dos tidak memudaratkan 4'-OHT dan masa induksi yang berbeza diperlukan untuk merangsang ekspresi transgen Dkk1/Wnt1-HA (dalam mESCs - Dkk1: 200nM/48h; Wnt1-HA: 600nM/48h dan dalam badan embrioid (EBs) - Dkk1: 400nM/72h; Wnt1-HA: 1000nM/48); 2) ekspresi berlebihan Wnt1 mengekalkan ciri pembaharuan kendiri dan pembezaan mESCs kepada sel saraf; 3) Wnt1 menggalakkan pembentukan NPC dan sel saraf pasca mitosis; 4) ekspresi berlebihan Dkk1 yang konstitutif merencat pembentukan NPC dan yang menariknya, 5) ekspresi berlebihan Dkk1 pada peringkat awal dan berikutnya meningkatkan tahap pembentukan NPC dan sel saraf pasca mitosis dengan signifikan (ujian-T, $P<0.05$).

Keputusan hasil daripada projek ini mengesahkan pelbagai peranan dan pengisyaratan Wnt yang bergantung kepada peringkat semasa proses pembezaan sel saraf mESCs *in vitro* serta dalam pengekalan ciri pembaharuan kendiri sel mESCs asal. Penemuan ini diharapkan akan menambah kepada pengetahuan semasa mengenai pemahaman di sebalik proses pembezaan ESCs, yang menjadi penting untuk terapi berasaskan sel neurodegeneratif pada masa hadapan.

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To my families, thank you for your support. I'm sorry Ranka, I can't stay with you and keep you waiting for too long.

APPROVAL

I certify that a Thesis Examination Committee has met on (20th November 2013) to conduct the final examination of (Liyang Gao) on her thesis entitled "THE ROLE OF WNT1 AND DKK1 DURING IN VITRO NEURAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELL LINES" 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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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.

GAO LIYANG

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