UNIVERSITI PUTRA MALAYSIA

GENERATION AND CHARACTERISATION OF HUMAN MESENCHYMAL STEM CELLS FROM HUMAN UMBILICAL CORD

TONG CHIH KONG

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GENERATION AND CHARACTERISATION OF HUMAN MESENCHYMAL STEM CELLS FROM HUMAN UMBILICAL CORD

By

TONG CHIH KONG

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

January 2010

Specially dedicated to my beloved parents, family and friends

For their unconditional love, understanding, patience, support and constant faith.

My mother always uses to tell me:

“People may look down on you, but never look down on yourself.”

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

GENERATION AND CHARACTERISATION OF HUMAN MESENCHYMAL STEM CELLS FROM HUMAN UMBILICAL CORD

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January 2010

Chairman: Dr Rajesh Ramasamy, PhD

Faculty: Medicine and Health Sciences

Mesenchymal stem cells (MSC) are multipotent stem cells that possess the ability to self-renew, capable of differentiating into mesodermal lineage and exert an immunomodulatory activity. These qualities grant MSC great potential in regenerative medicine, immunotherapy and gene therapy. The increasing demand of tissue regeneration and allogenic transplantation necessitate the need for a readily available source of MSC as an 'off-the-shelf' product for quick and effective treatment. To date, MSC can be generated from human umbilical cord which was once considered clinical waste. The aim of this study is to establish an optimal method to generate and characterise MSC from human umbilical cord samples. In this study, a novel generation method by combining mild enzymatic digestion and mechanical dissociation is established. Briefly, the outer layer of umbilical cord is separated from the blood vessels and remnant cord blood prior to processing. The umbilical cord samples are subjected to explants culture, enzymatic degradation or a combination of enzymatic and mechanical dissociation to obtain a single cell suspension for the generation of umbilical cord MSC (UC-MSC). Once UC-MSC expand, they are characterised by immunophenotyping and differentiation assays.

The data show that UC-MSC express common MSC surface markers (CD105+, CD73+, CD29+, CD90+, CD34-, CD45-, MHC class I+, MHC class II-, CD80-, CD86-) and are capable of differentiating into osteoblasts and adipocytes. Simultaneously, the properties of UC-MSC are evaluated in the presence and absence of basic fibroblast growth factor (bFGF). The results show that bFGF supplementation significantly affects the morphology, growth kinetics, cell cycle and cellular functions of UC-MSC. Furthermore, bFGF enhances the growth rate of UC-MSC by reducing the doubling time (about 3-4 fold) and skews the cytokine secretion profile (decreases MMP3 and VEGF production). Similar to bone marrow MSC, UC-MSC also exert an immunomodulatory effect on T cells. In the presence of UC-MSC, T cell activation is preserved. However their proliferation is profoundly inhibited in a dose-dependent manner. T cells are arrested anergy in G_0 phase of the cell cycle and this inhibitory activity requires cell-to-cell contact. This study reveals that UC-MSC share similar characteristics with BM-MSC and potentially serve as a future source of MSC for clinical use.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

GENERASI DAN PENCIRIAN SEL INDUK MESENKIMA MANUSIA DARIPADA TALI PUSAT MANUSIA

Oleh

TONG CHIH KONG

Januari 2010

Pengerusi: Dr Rajesh Ramasamy, Ph.D.

Fakulti: Perubatan dan Sains Kesihatan

Sel induk Mesenkima (MSC) merupakan sel induk multipotensi yang memiliki keupayaan pembaruan diri yang tinggi, mampu membezakan dirinya kepada sel yang bercirikan mesoderma dan menunjukkan kegiatan immunotindasan. Sifat-sifat ini menjadikan MSC sebagai calon yang berpotensi dalam perubatan generasi semula, imunoterapi dan terapi gen. Untuk memenuhi permintaan yang kian bertambah dalam kes regenerasi tisu dan pemindahan sel induk alogen, penemuan sumber baru MSC adalah penting untuk rawatan yang cepat dan berkesan. Sehingga kini, MSC telah dihasilkan dari tali pusat yang pernah dianggap sebagai sisa klinikal. Kajian ini adalah bertujuan untuk menubuhkan satu kaedah optima dalam penjanaan dan pengelasan MSC daripada sampel tali pusat manusia. Satu protokol generasi yang novel dengan menggabungkan pencernaan berenzim sederhana dan teknik penceraian mekanikal telah dihasilkan. Lapisan luar tali pusat dipisahkan daripada urat darah dan darah tali pusat sebelum pemprosesan seterusnya. Tali pusat sama ada diproses dengan kultur 'explant', pendegradan enzim, atau gabungan pendegradan enzim dan mekanikal penceraian untuk memperolehi satu ampaian sel tunggal bagi penjanaan MSC tali pusat (UC-MSC). Setelah UC-MSC dijanakan dan

diperkembangkan, mereka dikelaskan melalui profil immunophenotyping dan keupayaan pembezaan kepada sel-sel yang bercirikan mesoderma. UC-MSC menunjukkan penanda-penanda permukaan biasa MSC (CD105+, CD73+, CD29+, CD90+, CD34-, CD45-, MHC class I+, MHC class II-, CD80-, CD86-) dan mampu membezakan diri kepada osteoblasts dan adipocytes. Pada masa yang sama, ciri-ciri UC-MSC juga dikaji dalam kehadiran faktor pertumbuhan fibroblast asas (bFGF). Penambahan bFGF ke dalam media kultur jelas sekali memberi kesan kepada morfologi, kinetik pertumbuhan, kitar sel dan fungsi-fungsi selular UC-MSC. Tambahan pula, bFGF meningkatkan kadar pertumbuhan UC-MSC dengan penurunan masa bergandanya (sebanyak 3-4 kali ganda) dan memencongkan profil rembesan sitokin (mengurangkan pengeluaran MMP3 dan VEGF). Menyerupai sumsum tulang MSC, UC-MSC juga mempamerkan keupayaan immunotindasan pada sel-sel T. Dalam kehadiran UC-MSC, pengaktifan sel T terpelihara. Bagaimanapun, proliferasi mereka disekat bergantung kepada dos. T cell diperangkap anergi dalam fasa G_0 kitaran sel dan kegiatan perencatan ini memerlukan hubungan antara sel-sel. Kajian ini mendedahkan keserupaan ciri-ciri dengan BM-MSC. UC-MSC berpotensi sebagai sumber masa depan MSC untuk penggunaan klinikal.

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I certify that a Thesis Examination Committee has met on 11th Jan 2010 to conduct the final examination of **Tong Chih Kong** on his Master of Science thesis entitled '**Generation and Characterisation of Human Mesenchymal Stem Cells from Human Umbilical Cord**' 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 degree of 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 requirements for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date: 17 March 2010

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.

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TONG CHIH KONG

Date: 30th January 2010

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