GENERATION AND CHARACTERISATION OF ADIPOSE TISSUE-AND UMBILICAL-CORD-DERIVED MESENCHYMAL STEM CELLS EXPRESSING TRAIL APOPTOTIC LIGAND

By

AISHAH AMIRAH BINTI SHAMSUL KAMAL

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Chairman : Associate Professor Syahrilnizam Abdullah, D.Phil
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Recent studies have shown that mesenchymal stem cells (MSCs) expressing TNF-Related Apoptosis Inducing Ligand (MSC-TRAIL) can eliminate both lung cancer cells and lung cancer stem cells efficiently. Although there have been several studies on the efficacy of various sources of MSC-TRAIL on several cancers, there have been very limited studies on which sources of MSCs are the most efficient in expressing the TRAIL protein and biological properties of the engineered MSCs post-modification. The engineered MSCs utilised in previous projects have varying genetic constructs of the TRAIL-encoding lentiviruses transduced. Moreover, the period of time required for ADMSCs to achieve a similar level of protein expression to UCMSCs, was longer compared to UCMSCs in previous studies, suggesting UCMSCs having higher expression efficiency compared to ADMSCs. This raises the possibility of utilising other sources of MSCs, such as umbilical cord (UC) as a vector for TRAIL. This project aims to generate adipose tissue (AD) and umbilical cord (UC)-derived MSCs expressing TRAIL and characterise these cells post-modification. The hypothesis of this study is TRAIL-expressing MSCs can be generated from AD

and UC, and these engineered cells retain the MSC characteristics. The methods utilised are explained as follows. TRAIL encoding and empty vector lentivirus (LV) were produced through the transfection of LV component plasmids into 293FT cells. The virions were concentrated to $>10^6$ transducing units (TU)/mL. The MSCs were then transduced based on a range of MOIs. The TRAIL expression from the putative ADMSC-TRAIL and putative UCMSC-TRAIL was validated using ELISA. The engineered MSCs were then characterised through morphology observation, flow cytometry analysis, and differentiation assay. Analysis of transduction efficiency exhibited higher intensity of reporter gene-positive cells detected in putative UCMSC-TRAIL compared to putative ADMSC-TRAIL. Significantly higher TRAIL protein was also detected in the protein lysate and conditioned medium of putative UCMSC-TRAIL than putative ADMSC-TRAIL, empty vector (MSC-EV), and wild-type MSCs (MSC-WT). Moreover, all engineered MSCs retained their original characteristics post-transduction. The putative MSC-TRAIL were able to differentiate into adipocytes, osteocytes, and chondrocytes and maintained their MSC surface marker expression (CD44, CD90, CD105, and CD73). In conclusion, the TRAIL expression and characterisation analyses verified that the ADMSC-TRAIL and UCMSC-TRAIL have been generated. UCMSCs are discovered to be potentially more effective as a vector for TRAIL compared to ADMSCs. To further compare the potency of TRAIL-transduced MSCs in cancer therapy, functional assays incorporating MSC-TRAIL and NSCLC cell lines can be performed in the future.

Keywords: genetic engineering, mesenchymal stem cells, molecular biology

SDG: GOAL 3: Good Health and Well-Being

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

PENGHASILAN DAN PENCIRIAN SEL STEM MESENKIMA PEROLEHAN TISU ADIPOSA DAN TALI PUSAT BEREKSPRESI LIGAN PRO-APOPTOSIS TRAIL

Oleh

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Kajian terkini menunjukkan bahawa sel stem mesenkima (MSCs) mengekspresikan TRAIL (MSC-TRAIL) yang mempunyai keupayaan untuk menyingkirkan sel kanser paru-paru dan sel stem kanser paru-paru secara efisien. Meskipun terdapat beberapa kajian tentang efikasi sumber MSC-TRAIL yang berlainan terhadap beberapa jenis kanser, namun masih terdapat limitasi kajian tentang sumber MSCs yang paling berkesan dalam mengekspresi protein TRAIL dan cir-ciri biologi MSC yang telah dijuruterakan setelah proses modifikasi. MSC yang telah dijuruterakan dalam kajian-kajian yang terdahulu mempunyai konstruk genetik yang berlainan. Selain itu, tempoh yang lebih lama diperlukan oleh ADMSCs untuk mengekspresi protein pada tahap ekspresi protein yang sama seperti UCMSCs, menandakan UCMSCs memiliki keberkesanan ekspresi berbanding ADMSCs. Hal yang demikian meningkatkan kebarangkalian untuk memanfaatkan sumber-sumber MSC yang lain, seperti dari MSC tali pusat (UC), sebagai satu vektor bagi TRAIL. Objektif kajian ini adalah untuk menghasilkan MSC yang berasal daripada tisu adipos (AD) dan tali pusat (UC) yang

mengekspresikan TRAIL, dan mencirikan modifikasi pasca sel tersebut. Hipotesis kajian ini ialah MSC daripada AD dan UC yang mampu mengekspresikan TRAIL boleh dihasilkan dan sel yang dijuruterakan ini mengekalkan ciri-ciri MSC. Metodologi yang telah dijalankan adalah seperti berikut. TRAIL yang mengekodkan- dan vektor kosong - lentivirus (LV) telah dihasilkan melalui transfeksi komponen plasmid LV kepada sel-sel 293FT. Virion ini dipekatkan kepada $>10^6$ unit transduksi (TU)/mL. MSC kemudiannya ditransduksi berpandukan MOI. Ekspresi TRAIL daripada ADMSC-TRAIL putatif dan UCMSC-TRAIL putatif telah divalidasi menggunakan ELISA. MSC yang dijuruterakan kemudian dicirikan melalui pemerhatian morfologi, analisa sitometri aliran, dan ujian diferensiasi. Analisis keberkesanan transduksi menunjukkan kadar gen pelapor-sel positif yang lebih tinggi ditemui dalam UCMSC-TRAIL putatif berbanding ADMSC-TRAIL putatif. Protein TRAIL yang lebih tinggi ditemui secara lebih ketara di dalam *protein lysate* dan media bersyarat UCMSC-TRAIL putatif berbanding ADMSC-TRAIL putatif, vektor kosong (MSC-EV) dan MSCs asal (MSC-WT). Selain itu, semua MSC yang dijuruterakan mengekalkan ciri-ciri asli pasca-transduksi. MSC-TRAIL putatif berkeupayaan untuk dibedakan menjadi adiposit, osteosit, dan kondrosit, dan mengekalkan ekspresi penanda permukaan MSC (CD44, CD90, CD105 dan CD73). Kesimpulannya, ekspresi TRAIL dan analisis pencirian mengesahkan bahawa ADMSC-TRAIL dan UCMSC- TRAIL telah dihasilkan. UCMSCs berpotensi untuk berfungsi sebagai vektor dengan lebih berkesan berbanding ADMSCs. Untuk membandingkan keberkesanan MSC yang telah ditransduksi dengan TRAIL dalam terapi kanser, ujian berfungsi yang menggabungkan MSC-TRAIL dan garisan sel NSCLC dapat dihasilkan pada masa hadapan.

Kata Kunci: kejuruteraan genetik, sel stem mesenkima, biologi molekul

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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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LIST OF ABBREVIATIONS

AD	Adipose tissue
ADMSCs	Adipose tissue-derived MSCs
AFP	Alpha-fetoprotein
ALK	Anaplastic lymphoma kinase
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
BID	BH3 interacting-domain death agonist
BM	Bone marrow
BMMSCs	Bone marrow-derived MSCs
CCL	C–C Motif Chemokine Ligand
CCR	C–C Motif Chemokine Receptor
CD	Cluster of Differentiation
CDA/UPRT	Cytosine deaminase-uracil phosphoribosyltransferase protein
CL	Cell lysate
CM	Conditioned medium
CSCs	Cancer stem cells
CT	Chemotherapy
CXCL	C-X-C Motif Chemokine Ligand
CXCR	C-X-C Motif Chemokine Receptor
DCs	Dendritic cells
DcR	Decoy receptors
DD	Death domain
DISC	Death-inducing signalling complex
DMEM	Dulbecco's Modified Eagle Medium

DNA	Deoxyribonucleic acid
DR	Death receptors
EF	Elongation factor
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ELISA	Enzyme Linked Immunosorbent Assay
EV	Empty vector
EWS	Ewing sarcoma
FACS	Fluorescence-activated cell sorting
FADD	Fas-associated Protein with Death Domain
FBS	Foetal bovine serum
FLuc	Firefly luciferase
GMP	Good manufacturing practice
hBMMSCs	Human bone marrow-derived MSCs
HGF	Hepatocyte growth factor
HIV	Human immunodeficiency virus
HLA-DR	Major histocompatibility complex (MHC) II cell surface receptor
HLA-G	Nonclassical HLA class I molecules
hUCMSCs	Human umbilical cord-derived MSCs
ICAM	Intercellular adhesion molecules
IDO	Indoleamine 2,3-dioxygenase
IFN	Interferon
IGF	Insulin-like growth factor
IL	Interleukins
ISCT	International Society for Cellular Therapy
LB	Luria-Bertani

MHC	Major histocompatibility complex
MMP	Matrix metalloproteinase
MOI	Multiplicity of infection
MSCs	Mesenchymal stem cells
MSC-TRAIL	TRAIL-transduced MSCs
NK	Natural Killer
NSCLC	Non-small cell lung cancer
OD	Optical density
ORF	Open Reading Frame
OX40L	TNF ligand superfamily member 4
PDGF-AB	Platelet derived growth factor-AB
pDNA	Plasmid DNA
PEDF	Pigment epithelium-derived factor
PFS	Progression-free survival
PGE2	Prostaglandin E2
PI3K	Phosphoinositide 3-kinase
PMSF	Phenylmethylsulfonyl fluoride
qRT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction
rhEGF	Recombinant human epidermal growth factor
rhTRAIL	Recombinant human TRAIL
RIPA	Radioimmunoprecipitation assay
RP2D	Recommended Phase II dose
SCLC	Small cell lung cancer
SDF	Stromal cell-derived factor
sTRAIL	Soluble TRAIL
tBID	Truncated BID

TGF	Transforming growth factor
TKI	Tyrosine kinase inhibitors
TNF	Tumour necrosis factor
TRAIL	Tumour necrosis factor-related apoptosis inducing ligand
TU	Transducing units
UC	Umbilical cord
VCAM	Vascular cell adhesion molecules
VEGF-A	Vascular endothelial growth factor A
VSV-G	Glycoprotein of vesicular stomatitis virus
WT	Wild-type
ZsGreen	Green fluorescent protein
293FT	Human embryonal kidney cell line

CHAPTER 1

INTRODUCTION

1.1 Research Background

Lung cancer is the most frequently diagnosed cancer worldwide, accounting for more than 2 million cancer fatalities in 2020 (Siegel et al., 2022). Of all lung cancer cases, non-small cell lung cancer (NSCLC) comprises 80% of them, followed by small-cell lung cancer (Sung et al., 2021). Third-generation anticancer agents such as paclitaxel and vinorelbine paired with traditional chemotherapies and platinum drugs, like carboplatin and cisplatin, have been proven to increase the overall survival rate of NSCLC patients. However, acquired chemoresistance and microscopic tumour spread would ultimately cause these treatments to fail (Mascaux et al., 2017).

Cancer stem cells (CSCs) are a rare subpopulation of cells within tumours that could promote tumour recurrence and chemoresistance (Hong et al., 2014). Nanoparticles (Huang et al., 2017; Y. Li et al., 2018; Mi et al., 2018; Qi et al., 2016; Y. Zhang et al., 2018) or antibody-conjugated nanoparticles (Mandal et al., 2018; Ning et al., 2016) have recently been used to target these CSCs. Despite the promising outcomes of these approaches, safety problems such as cellular toxicity and lack of specificity (Shen et al., 2013; Uboldi et al., 2016) are some of the issues that may hinder their development toward clinical application. Hence, due to the limitations of current conventional therapies, there is a necessity to venture into alternative therapeutic strategies, such as targeted therapy via the apoptotic pathway.

Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) has been demonstrated to be immensely effective in targeting and destroying many types of tumours, including liver cancer (Xie et al., 2017), breast cancer (Ma et al., 2015), and glioma (Smith et al., 2015) with minimal toxicities. The findings render TRAIL a potential candidate in cancer therapy. TRAIL preferentially induces apoptosis via death receptors DR4 and DR5 in cancer cells compared to normal cells. Contrastingly, decoy receptors DcR1 and DcR2, which lack functional death domains, are significantly upregulated in normal cells. Therefore, TRAIL does not induce cell death of normal cells (LeBlanc & Ashkenazi, 2003; K. Min et al., 2019). However, despite it being a promising anti-apoptotic ligand for prospective use in cancer therapy, it requires a delivery vector to be effective due to its inclination toward being eliminated via renal filtration, besides its short half-life.

MSCs have been documented as a suitable vector for TRAIL because of their immunomodulatory characteristics, which resulted from the absence of major histocompatibility complex (MHC) class II, ability to execute paracrine activity at target site, convenience of isolation, and extensive expansion capacity (Samsonraj et al., 2017). MSCs have been utilised as a factory for drug production (Nowakowski et al., 2016) and employed as a vector for several biological agents comprising antitumour cytokines (X. Liu et al., 2018), pro-drug converting enzymes (Cavarretta et al., 2010; J. Zhang et al., 2015), and oncolytic viruses (Ahmed et al., 2011; Leoni et al., 2015a). Although several cancer models, including glioblastoma (Choi et al., 2019), breast cancer (Chulpanova et al., 2023), and pancreatic adenocarcinoma (Rossignoli et al., 2019) were examined for the antitumour effect of mesenchymal stem cells (MSCs) expressing TRAIL (MSC-TRAIL), studies that specifically

compare and characterise the properties of various sources of MSCs engineered via lentiviral transduction of TRAIL are still insufficiently reported.

1.2 Problem Statement

Considering the specific antitumour characteristics of TRAIL and the fact that MSCs may migrate actively to tumour sites, the potential therapeutic effect of MSC-TRAIL in killing NSCLCs and their cancer stem cells seems promising (Fakiruddin et al., 2019). However, the MSCs obtained in previous studies were specifically derived solely from the adipose tissue (AD). This raises the possibility of utilising other sources of MSCs as the vector for TRAIL in cancer therapy and whether MSC-TRAIL from other sources could be more potent in inducing apoptosis.

Although MSCs share similar cell surface markers and immunomodulatory characteristics, previous studies suggest that MSCs isolated from different origins show varied biological effects, cytokine profiles, and transcriptome properties. For example, a prior study using murine lung carcinoma model revealed that the combination of cytosine deaminase-uracil phosphoribosyltransferase protein (CDA/UPRT)-transfected adipose tissue-derived MSCs (ADMSCs) and lysomustine chemotherapy exhibited higher efficacy compared to monotherapy-based treatment with either lysomustine only or transfected ADMSCs alone (Krassikova et al., 2016). This study was able to prove that engineered MSCs demonstrated higher potency in eliminating lung tumours, compared to wild-type MSCs. In another study, apoptotic activity was successfully induced in breast cancer cells (MCF-7) and lung tumour cells (A549, H1299, and H460) by interferon IFN-γ-modified human bone marrow-derived MSCs (hBMMSCs) expressing TRAIL. Interestingly, the development of a lung

carcinoma xenograft model was also inhibited by IFN-γ-secreting hBMMSCs (Yang et al., 2014). The discovery implies the possibility of engineered MSCs from various sources having different therapeutic effects in specific clinical indications. However, studies on the differences between these differently sourced engineered MSCs in terms of transgene expression and biological properties post-modification are still scarce. Moreover, the engineered MSCs utilised in previous projects have varying genetic constructs of the TRAIL-encoding lentiviruses transduced (Chulpanova et al., 2023; Fakiruddin et al., 2019; Quiroz-Reyes et al., 2023). The period of time required for ADMSCs to achieve a similar level of protein expression to UCMSCs, was longer compared to UCMSCs in previous studies, suggesting UCMSCs having higher transduction efficiency compared to ADMSCs (Amadeo et al., 2023).

Therefore, to explore this aspect, it is necessary to generate apoptotic-inducing MSCs transduced with TRAIL-encoding lentiviral vector of the same genetic construct, from differently sourced MSCs using genetic engineering techniques. The genetically modified MSCs would be analysed for the expression of exogenous apoptotic-inducing protein using several assays related to gene expression and protein expression. Subsequently, the modified MSCs would be characterised based on the MSC criteria proposed by the International Society for Cellular Therapy (ISCT).

1.3 Significance of Study

The generation of the *bona fide* ADMSC-TRAIL and UCMSC-TRAIL will allow the comparison of the type of MSC-TRAIL that is more efficient in killing lung cancer cells and its cancer stem cells in the following in vitro study. This may facilitate future

researchers to make an informed decision on the best MSC-TRAIL for future in vivo or preclinical experiments and, subsequently, in clinical trials for lung cancer.

1.4 Hypothesis and Objectives

It is hypothesised that TRAIL-expressing MSCs can be generated from AD and UC, UCMSCs exhibit higher TRAIL expression compared to ADMSCs, and these engineered cells retain the MSC characteristics. To explore this hypothesis, this study aims to generate AD and umbilical cord (UC)-derived MSCs expressing TRAIL, compare the TRAIL expression in both engineered UCMSCs and ADMSCs, and characterise these cells post-modification.

The specific objectives of this research are as follows:

1. To determine and compare the transduction and TRAIL expression of MSCs upon lentiviral transduction of TRAIL into MSCs
2. To determine whether the engineered MSCs still retain their MSC characteristics post-transduction

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