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**Effects of Human Umbilical Cord Blood Mesenchymal Stem
Cells on Expression of Leukaemic Inhibitory Factor and
Interleukin-10 in Acute Myeloid Leukaemia**

A Thesis

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Fulfillment for Requirements of the Master of Science**

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List of Abbreviations

ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
ATLS	Acute tumour lysis syndrome
BM	Bone marrow
C-FU	Colony-forming unit
CLL	Chronic lymphocytic leukaemia
CML	Chronic myeloid leukaemia
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
COAP	Cyclophosphamide, Oncovin, Arabinofuranosylcytidine, Prednisone
DEPC	Diethyl pyrocarbonate
DIA	Differentiation inhibitory factor
DIF	Differentiation-inducing factor
DMEM	Dulbecco's Modified Eagle Medium
dNTP	Deoxyribosenucleoside triphosphate
DRF	Differentiation-retarding factor
EC	Embryonic carcinoma
ECCs	Embryonic carcinoma cells
EDTA	Ethylenediaminetetraacetic acid
EG	Embryonic germ
EGCs	Embryonic germ cells
ELISA	Enzyme-linked immunosorbent assay
ESCs	Embryonic stem cells
FBS	Foetal bovine serum
FITC	Fluorescein isothiocyanate
HESCs	Human embryonic stem cells
HILDA	Human interleukin for DA cells
HRP	Horseradish peroxidase
HSCs	Haematopoietic stem cells
HSCT	Haematopoietic stem cell transplantation
HSF3	Hepato-stimulating factor 3
ICM	Inner cell mass
IgG	Immunoglobulin G
IL-10	Interleukin-10

IL-6	Interleukin-6
IPSCs	Induced pluripotent stem cells
<i>LIF</i>	Leukaemia inhibitory factor gene
LIFR	Leukaemia inhibitory factor receptor
LSC	Leukaemia stem cell
MDS	Myelodysplastic syndrome
mGS	Multipotent germ stem
MLPLI	Melanoma-derived lipoprotein lipase inhibitor
M-MLV RT	Moloney murine leukaemia virus reverse transcriptase
MNCs	Mononuclear cells
MPD	Myeloproliferative disease
MRD	Minimal residual disease
MS-5	Murine stroma-5
MSCs	Mesenchymal stem cells
non-ESCs	Non-embryonic stem cells
<i>NPM</i>	Nucleophosmin gene
O. D.	Optical density
OAF	Osteoclast-activating factor
OSM	Oncostatin-M
PBS	Phosphate buffer saline
PE	Phycoerythrin
PGE ₂	prostaglandin E₂
qPCR	Quantitative polymerase chain reaction
SCNT	Somatic cell nuclear transfer
SCT	Stem cells transplantation
SD	Standard deviation
SSCs	Spermatogonia stem cells
t-AML	Therapy-related acute myeloid leukaemia
TdT	Terminal deoxynucleotidyl transferase
TMB	Tetramethylbezidine
UCB	Umbilical cord blood
VCAM-1	Vascular cell adhesion molecule-1
WHO	World Health Organization

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1. Introduction and Aim of the Work

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Acute myeloid leukaemia (AML) is the most frequent haematological malignancy in adults, with an estimated worldwide annual incidence of three to four cases per 100,000 people. Despite intensive research for new therapies and prognostic markers, it is still a disease with a highly variable prognosis among patients and a high mortality rate. Indeed, less than 50% of adult AML patients have a 5-year overall survival rate, and in the elderly, only 20% of AML patients survive for 2 years **(Gregory T. K. *et al.*, 2009)**. Haematological malignancies represent approximately 7% of all malignant diseases **(Victor Hoffbrand A. and Paul Moss A. H., 2011)**.

The stem cells are undeveloped cells capable of proliferation, self renewal, conversion to differentiated cells and regenerating tissues. There are two main types of stem cells: embryonic and non-embryonic. The embryonic stem cells (ESCs) are pluripotent because they can differentiate into all cell types. Non-embryonic stem cells (non-ESCs) are multipotent because their potential to differentiate into cell types is comparatively limited. The embryonic stem cells are more prevalent than the non-ESC and have a greater potential to spontaneously differentiate than the non-ESCs **(Bernard E. T., 2006)**.

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Leukaemia inhibitory factor protein (LIF) is a pluripotent cytokine with pleiotropic activities. The protein LIF is a member of a family of cytokines called "neuropoietic cytokines" that includes the ciliary neurotrophic factor (CNTF), interleukin-6 (IL-6), IL-11, oncostatin-M (OSM), and cardiotropin-1 (**Hall A. K. and Rao M. S., 1992; Sylvian B. *et al.*, 2007**). The *LIF* gene was first cloned in 1987, and is characterized by its ability to stimulate the differentiation of the murine myeloblastic leukaemia cell line "M1" (**Gearing D. P. *et al.*, 1987**).

Interleukin-10 (IL-10) is a polypeptide produced by the Th2 subset of T helper lymphocytes, B lymphocytes, macrophages, and monocytes in response to an immunological challenge (**De Waal Malefyt R. *et al.*, 1991**). The IL-10 is an efficient inhibitor of tumour metastases *in vivo* at doses that do not have a direct effect on normal cells (**Zheng L. M. *et al.*, 1996**).

The hypothesis that the mesenchymal stem cells (MSCs) have unique immunomodulatory properties attracted much interest as they may be harnessed for novel therapeutic approaches in immune-mediated diseases. So, these stem cells may be potential candidates for immunotherapeutic approaches in acute myeloid leukaemia (AML) patients.

The MSCs seem to have a relevant role in AML as they prevent spontaneous and induced apoptosis and may attenuate

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chemotherapy-induced cell death. This possibility has been confirmed by the finding that co-cultivation of a leukaemic cell line with the murine stroma cell line "MS-5" can block apoptosis (**Konopleva M. et al., 2002**).

Aim of the Work

The aim of the present work is to detect the effect of the human MSCs on the expression of *LIF* gene, and the cytokine IL-10 level in human AML. The MSCs were separated from human umbilical cord blood (HUCB), and co-cultured with samples collected from peripheral blood (PB) of AML-insulted adults prior to chemotherapy. Cells identification was done using flow cytometric analyses.

The expression of *LIF* gene and the cytokine IL-10 level were measured using the real-time polymerase chain reaction (q-PCR) and enzyme-linked immunosorbent assay (ELISA) techniques, respectively before and after the co-culture in order to evaluate the immunomodulatory and anti-inflammatory effects of the human MSCs on the human AML.

2. Previous Work

2.1 LEUKAEMIA

Previous work

Cancer is an increasingly-important cause of morbidity and mortality. The majority of cancers are epithelial malignancies. Haematological malignancies represent approximately 7% of all malignant diseases (**Figure 1**). There are major geographical variations in occurrence of such diseases; for example, chronic lymphocytic leukaemia (CLL) is the most common leukaemia in the West, but rare in the Far East (**Victor Hoffbrand A. and Paul Moss A. H., 2011**).

Biomedical research has focused on the study of cancer with notable success. A rather simplistic view of how cancer arises (cancer pathogenesis) can be based on the consideration of a normal cell's fate. All cells in the body appear to have three possible fates: they may proliferate to produce more cells, differentiate to carry out specialized functions, or die at a predetermined time (by a process termed “apoptosis” or “cell suicide”), and then be eliminated.

The body requires an appropriate balance of the cells undergoing each of these fates for normal function and survival. The normal cells usually die after 40 to 60 cycles of replication. In contrast, cancer arises when proliferation consistently and aberrantly exceeds apoptosis in a single (clonal) population of cells (**Tariq M. I. et al., 2013**).