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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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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**
LIF	**Leukaemia inhibitory factor gene**
LIFR	**Leukaemia inhibitory factor receptor**
LSC	**Leukaemia stem cell**
MDS	**Myeloydysplastic 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**
NPM	**Nucleophosmin gene**
O. D.	**Optical density**
OAF	**Osteoclast-activating factor**
OSM	**Oncostatin-M**
PBS	**Phosphate buffer saline**
PE	**Phycoerythrin**
PGE_2	**prostaglandin E_2**
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

1. Introduction and Aim of the Work

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)**.

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

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)**.