Functional and Molecular Characterisation of Mesenchymal Stem Cells Derived From Bone Marrow and Dental Tissues

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DECLARATION

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ABSTRACT

Mesenchymal stromal/stem cells (MSCs) are multipotent, progenitor cells with the ability to differentiate into cells of mesenchymal and non-mesenchymal tissues *in vitro* and *in vivo*. Characterisation of MSC-like cells residing in dental pulp (DPSC) and periodontal ligament (PDLSC) tissues has shown that these cells exhibit similar features to bone marrow stromal cells (BMSCs). The present project proposed that BMSCs, DPSCs and PDLSCs contain heterogeneous populations of progenitor cells including a minor subset of multipotential stem cells. Identification of the precise developmental stages and generation of gene expression profiles of these different cell populations will greatly improve our understanding of the fundamental cellular processes involved in MSC differentiation and proliferation pathways. Therefore, the aim of this PhD project was to assess the unique phenotype of BMSCs, DPSCs and PDLSCs and their differentiated progeny by studying their proliferative and differentiation potentials, and by assessing their gene expression profiles.

Initial findings obtained from functional analyses of clonal populations present within BMSCs, DPSCs and PDLSCs support the hypothesis that within each of the populations, co-exist cells of varying levels of proliferation and differentiation potentials. Following on from these results, whole genome microarray analyses were used to determine gene expression patterns of clonal cell fractions exhibiting significant functional differences within each MSC population. The microarray data identified cohorts of differentially expressed genes in comparisons of cell clones of low proliferation potential and high/multi-differentiation potential within BMSC, DPSC and PDLSC populations. Further interrogation of the generated data identified 24 differentially up-regulated genes in long lived/multi-potential MSC clones, common to BMSC, DPSC and PDLSC populations. Specifically, E2F-2, LDB-2, PTTG-1 and TWIST-1, were identified as transcriptional regulators or co-regulators that may be involved in the proliferation and differentiation of these MSC populations. In light of the previously reported involvement of TWIST-1 in growth and development of BMSC, the effect of enforced TWIST-1 expression was investigated in DPSC and PDLSC populations. These findings demonstrated that TWIST-1 holds a stimulatory role in cell proliferation and is involved in the regulation of differentiation/commitment processes in MSCs.

Overall, the identification of genes associated with MSCs of high growth and developmental potential lays the foundation for further definition of molecular mechanisms involved in MSC maintenance and survival. Elucidation of these fundamental processes is highly significant as it holds a critical role in the development of MSC-based tissue regeneration therapies.

LIST OF PUBLICATIONS

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LIST OF ABBREVIATIONS

α-MEM	Minimum essential meduim, α modification
ALCAM	Activated leukocyte cell adhesion molecule
AP	Alkaline phosphatase
BALP	Bone-specific alkaline phosphatase
bHLH	Basic Helix-Loop-Helix
BM	Bone marrow
BMMNC	Bone marrow mono-nuclear cells
BMP	Bone morphogenic protein
BMSC	Bone marrow stem cells
BrdU	Bromodeoxyuridine
BSA	Bovine serum albumin
BSP	Bone sialoprotein
cDNA	Complementary deoxyribonucleic acid
CFU-F	Colony Forming Unit-Fibroblast
CMTMR	(5-(((4-chloromethyl)benzoyl)amino)-tetramethylrhodamine)
CO_2	Carbon dioxide
DEPC	Diethylpyrocarbonate
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl-sulfoxid
DNA	Deoxyribonucleic acid
dNC-PCs	Neural crest-derived progenitor cells
DP	Dental pulp
DPSC	Dental pulp stem cells
DTSC	Deciduous tooth stem cell
DTT	Dithiothreitol
ECM	Extracellular matrix
EDTA	Ethylenediaminetetra-acetic acid
EMT	Epithelial to mesenchymal transition
ESC	Embryonic stem cells
FACS	Fluorescence activated cell sorting
FCS	Foetal calf serum

FGF	Fibroblast growth factor
GAG	Glycosaminoglycan
G-CSF	Granulocyte colony-stimulating factor
GFP	Green fluorescence protein
HA/TCP	Hydroxyapatite/tricalcium phosphate
HBSS	Hanks balanced salt solution
HCl	Hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HFF	Human foreskin fibroblast
HME	Haematopoietic environment
HSC	Haematopoietic stem cell
HSP90β	Heat shock protein 90 β
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
IBMX	3-Isobutyl-1-methyl-xanthine
ICAM	Intercellular adhesion molecule
Ig	Immunoglobulin
IGF	Insulin-like growth factor
iPSC	Induced pluripotent stem cells
KCl	Potassium chloride
kDa	Kilo Dalton
LAB	Living autologous bone
LB	Luria-Bertani
LPL	Lipoprotein lipase
LTR	Long term repopulating
M	Molar
mA	Milli amps
MACS	Magnetic activated cell sorting
M-CSF	Macrophage – colony stimulating factor
ml/mm/mM	Millilitre/millimetre/millimolar
MMP	Matrix metalloproteinase
mRNA	Messenger ribonucleic acid
MSC	Mesenchymal stem cell
NaCl	Sodium chloride

NaOH	Sodium hydroxide
NF	Nuclease free
OA	Osteoarthritis
OPN	Osteopontim
P	Passage
PAGE	Poly-acrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PD	Population doublings
PDGF	Platelet-derived growth factor
PDL	Periodontal ligament
PDLP	Periodontal ligament progenitors
PE	Phycoerythrin
PG	Proteoglycans
PGP	Pre-glycoprotein
PMSF	Phenylmethanesulphonyl fluoride
PPAR	Perixosome proliferator-activated receptor
RO	Reverse osmosis
RPMI	Roswell Park Memorial Institute
RT	Room temperature
SC	Saethre-Chotzen
SCAP	Stem cells from root apical papilla
SDS	Sodium dodecyl sulphate
SIC	Stroma-initiating cells
STRO-1^{+}	STRO-1 positive
TBS	Tris buffered saline
TEMED	N, N, N, N – tetramethyl - ethylenediamine
TGF	Transforming growth factor
TMB	Tetramethyl-benzide
TNSALP	Tissue non specific alkaline phosphatise
µg/µl/µM	Microgram/microlitre/micromolar
VCAM	Vascular cell adhesion molecule
VLA	Very late antigen
v/v	Volume per volume

WST-1	4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulphonate
w/v	Weight per volume
x g	Times gravity