The Role of *TWIST-1* in the Regulation of Mesenchymal Stem Cell Growth, Fracture Repair and Bone Loss

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Abstract

Mesenchymal stem cells (MSC) have the capacity to differentiate into osteoblasts, chondrocytes and adipocytes *in vivo*. It is well established that the basic Helix-Loop-Helix transcription factor *TWIST-1* plays an important regulatory role within the context of bone remodelling and formation in both developmental and adult settings by regulating the proliferation, differentiation and commitment of MSC. Recent studies have demonstrated that elevated levels of *TWIST-1* in MSC results in the maintenance of an immature population of cells with enhanced proliferative and adipogenic potential while simultaneously reducing the cells capacity to undergo osteogenesis and chondrogenesis.

The aim of this thesis was to define the role of *TWIST-1* in MSC commitment and differentiation within the context of bone fracture healing and bone loss during osteoporosis. Microarray results highlighted differential expression of Wnt pathway genes *WNT2* and *WNT2B* in MSC with enforced *TWIST-1* expression. Purified MSC isolated from bone marrow aspirates obtained from normal human donors were used to generate over-expressing *WNT2* and *WNT2B* MSC lines using established retroviral based systems. Genetically modified MSC were used to examine the effect of *WNT2* and *WNT2B* on osteogenesis, chondrogenesis, adipogenesis and proliferation of MSC *in vitro*. Results showed regulation of *WNT2* and *WNT2B* by *TWIST-1* through direct interactions between *TWIST-1* protein and the proximal promoter regions of *WNT2* and *WNT2B*. Furthermore, functional analysis demonstrated enhanced adipogenesis in MSC with enforced expression of *WNT2B*.

To assess the role of *TWIST-1* deficiency in fracture healing and the disease state of osteoporosis *in vivo,* a heterozygous *TWIST-1*$^{+/-}$ mutant mouse model was used. The role of *TWIST-1*

deficiency in fracture healing was assessed by induction of a femoral fracture followed by analysis at one, two four and eight weeks post fracture to examine the different stages of fracture healing. Analysis showed enhanced osteoblastic differentiation and accelerated fracture repair in heterozygous *TWIST-1*$^{+/-}$ mutant mice when compared to relevant wild type littermate control mice. The role of *TWIST-1* deficiency in osteoporosis was examined by induction of osteoporosis by ovariectomy. Results from this study showed heterozygous *TWIST-1*$^{+/-}$ mice maintained bone volume following ovariectomy induced osteoporosis.

Declaration

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Signed

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Date:

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Abbreviations

3D	Three dimentional
AGE	Agarose gel electrophoresis
ALK PHOS	Alkaline phosphatase
ATCC	American Type Culture Collection
ATP	adenosine triphosphate
bHLH	beta helix loop helix
BLAST	Basic Local Alignment Search Tool
BMD	Bone mineral density
BMMC	Bone marrow mononuclear cell
BMP	Bone morphogenetic protein
BMSC	Bone marrow stromal cell
BMSSC	Bone marrow stromal stem cell
Brdu	5-bromo-2-deoxyuridine
BSA	Bovine serum albumin
BSP	Bone sialoprotien
C/EBPα	CCAAT/Enhancer Binding Protein alpha
CBFA1	Core binding factor-1
cDNA	Complementary deoxyribonucleic acid
CDS	Coding DNA sequence
CFU	Colony forming unit
CFU-F	Colony forming unit-fibroblast
ChIP	chromatin immunoprecipitation
CoA	Coenzyme A
COL2a1	Collagen type II alpha 1
CSA	chondroitin sulphate A
DAVID	Database for Annotation, Visualization and Integrated Discovery
DEPC	Diethylpyrocarbonate
DERMO	Dermis-expressed protein 1
DLX5	Distal-Less homeobox 5
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide

DNA	Deoxyribonucleic acid
DNAse	Deoxyribonuclease
dNTP	Deoxyribonucleotide triphosphate
DTT	dithiothreitol
E47	Immunoglobulin Enhancer Binding Factors
EB	Elution buffer
ECF	Enhanced chemifluorescence
EDTA	Ethylenediaminetetraacetic acid
EZH2	Enhancer of zeste homolog 2
FACS	Fluorescence activated cell sorting
FCS	Foetal calf serum
FGFR2	Fibroblast growth factor receptor 2
GAG	glycosaminoglycan
GFP	Green fluorescent protein
GM-CSF	Granulocyte/macrophage-CSF
HBSS	HANKS balanced salt solution
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
Het	Heterozygous
hMSC	Human mesenchymal stem cell
HSC	Haematopoietic stem cells
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IP	Intraperitoneal
iPSC	Induced pluripotent stem cell
LB	Luria Broth
LDB2	Lim domain binding 2
MACS	Magnetic cell sorting
MAF	Musculoaponeurotic fibrosarcoma
M-CSF	Macrophage colony-stimulating factor
MEM	Minimum essential medium
MPP	Multipotent progenitor cells
mRNA	Messenger ribonucleic acid

MSC	Mesenchymal stem cell
MSX2	Muscle segment homeobox 2
NBF	Neutral buffered formalin
NK	Natural killer
NOD	Normal osteoblast donor
OCN	Osteocalcin
OPN	Osteopontin
OVX	Ovariectomised
PAGE	Polyacrylamide gel electrophoresis
PBND	PCR buffer with non-ionic detergents
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PD	Population doublings
PGP	Pre-glycoprotein
PGP-2	Prostaglandin E2
PTH	Parathyroid hormone
PTHrP	Parathyroid hormone-related protein
PTTG1	Pituitary tumor-transforming 1
Q-PCR	Real-time polymerase chain reaction
RANK	Receptor activator of nuclear factor kappa-B
RANKL	Receptor activator of nuclear factor kappa-B ligand
RBC	Red blood cell
RNA	Ribonucleic acid
RO	Reverse osmosis
ROI	Region of interest
rpm	revolutions per minute
RT	Room temperature
RT-PCR	Real-time polymerase chain reaction
RUNX2	Run related transcription factor
SDF-1/CXCL12	Stromal cell dirived factor 1
SDS	Sodium dodecyl sulfate
SMAD	SMAD protein

SOC	Super optimal broth with cotabolite repression
STRO-1	Stromal precursor antigen - 1
TAE	Buffer containing Tris base, Acetic acid and EDTA
TAZ	Tafazzin protein
TBS	Tris buffered saline
TE	Buffer containing Tris and EDTA
TGF-β	Transforming growth factor beta
TRAP	tartrate-resistant acid phosphatase 5
Tris HCL	Tris Hydrochloride
Tween 20	Polyethylene glycol sorbitan monolaurate
TWIST-1	Transcription factor Twist-1
TWIST-2	Transcription factor Twist-2 (*DERMO-1*)
uCT	micro-computed tomography
UV	Ultra violet
VSVG	Vesicular stomatitis virus G-protein
Wnt	Wnt family of signalling molecules
WNT2	Wingless-type MMTV integration site family, member 2
WNT2B	Wingless-type MMTV integration site family, member 2B
WST-1	4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3- benzene disulphonate
WT	Wild Type
α-MEM	α-modified Eagle's medium

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