

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

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A thesis submitted to the University of Adelaide
For the degree of Doctor of Philosophy
May 2013

Table of Content

TABLE OF CONTENTS	I
ABSTRACT	V
DECLARATION	VII
ACKNOWLEDGEMENTS	VIII
ABBREVIATIONS	X
PUBLICATIONS	XIV
1 INTRODUCTION	1
1.1 OVERVIEW	1
1.1.1 Bone Structure and Composition	2
1.2 BONE FORMATION	2
1.2.1 Bone Remodelling	4
1.3 MESENCHYMAL STEM CELLS	5
1.3.1 The transcriptional regulation of adipogenic MSC differentiation	10
1.3.2 The transcriptional regulation of chondrogenic MSC differentiation	11
1.3.3 The transcriptional regulation of osteogenic MSC differentiation	11
1.4 <i>TWIST-1</i>	12
1.4.1 The effect of <i>TWIST-1</i> mutations on humans and mice	13
1.4.2 <i>TWIST-1</i> regulation of osteogenic differentiation	14
1.5 CONCLUDING REMARKS	16
1.6 HYPOTHESES	18
1.7 AIM	18
2 MATERIALS AND METHODS	19
2.1 CELL CULTURE	19
2.1.1 Culture Media	19
2.1.2 Cell Culture Buffers	20
2.1.3 Cell Culture Conditions	20
2.1.4 Isolation of mesenchymal stem cells using Magnetic Activated Cell Sorting	21
2.1.5 Isolating mononuclear cells	22
2.1.6 Culture of human MSC	22
2.1.7 Trypsin Digestion	23
2.1.8 Human osteosarcoma cell line MG63s	23
2.1.9 Adherent retroviral HEK 293T packaging cell line	23
2.1.10 Cryopreservation of cells	23
2.1.11 Thawing of cryopreserved cells	24
2.1.12 Counting Cells	24
2.2 FUNCTIONAL ANALYSIS OF <i>WNT2</i> AND <i>WNT2B</i> OVEREXPRESSING MSC	25
2.2.1 Assessment of Population Doublings	25
2.2.2 Senescence Assay	25
2.2.3 Assessment of Osteogenic Differentiation Potential	26
2.2.4 Picogreen DNA Assay	27
2.2.5 Assessment of Adipogenic Differentiation Potential	28
2.2.6 Assessment of Chondrogenic Differentiation Potential	28

2.2.7	Histological Assessment	30
2.2.8	CFUF	31
2.2.9	Staining CFU-F Colonies	32
2.2.10	Flow-Cytometric Analysis	33
2.3	CELL IMAGING	36
2.4	MOLECULAR TECHNIQUES	36
2.4.1	Microarray Analysis	36
2.4.2	Analysis of Gene Expression - PCR	37
2.4.2.1	Preparation of total RNA	37
2.4.2.2	Quantification and purity analysis of RNA	38
2.4.2.3	Complementary DNA (cDNA) synthesis	38
2.4.2.4	Real-time PCR	39
2.4.2.5	Sequencing	43
2.4.2.6	Isolation of DNA from agarose	45
2.4.2.7	PCR Product Purification	45
2.4.2.8	Heat Shock Transformation	46
2.4.2.9	FastPlasmid™ Mini Kit (5 PRIME)	47
2.4.2.10	Maxiprep plasmid preparation	47
2.4.2.11	Agarose Gel Electrophoresis (AGE)	49
2.4.3	Cloning	49
2.4.3.1	Gateway™ Cloning	49
2.4.3.2	Isolation of genomic DNA	50
2.4.3.3	Cloning Promoter Fragments into PGL3 Basic	51
2.5	PROTEIN ANALYSIS	54
2.5.1	Preparation of protein lysates	54
2.5.2	Western blot analysis	55
2.5.3	Retroviral Techniques	57
2.5.3.1	Retroviral supernatant preparation	57
2.5.3.2	Retroviral infection	58
2.5.3.3	FACS sorting of infected cells based on GFP expression	58
2.5.3.4	Assessment of overexpression	59
2.5.4	Luciferase assays	59
2.5.5	Chromatin Immunoprecipitation	61
2.6	ANIMAL TECHNIQUES	63
2.6.1	<i>TWIST-1</i> mouse colony	63
2.6.2	Genotyping	64
2.6.3	Fracture Surgery	66
2.6.4	Ovariectomy Surgery	68
2.6.5	μCT – Skyscan 1174 Scanning	68
2.6.6	μCT – Analysis	69
2.6.6.1	Fracture	69
2.6.6.2	Ovariectomy	70
2.6.7	Histology	71
2.6.7.1	Methacrylate Processing	71
2.6.7.2	Paraffin Processing	72
2.6.7.3	Trap Staining	72
2.6.7.4	Haematoxylin and Eosin Staining	73
2.6.7.5	Von Kossa Staining	75
2.6.7.6	Safranin O Staining	75
2.6.7.7	Imaging Slides	76
2.6.7.8	Histoporphometric Analysis	76

2.6.8	X-Ray.....	77
2.6.9	Mechanical Testing.....	77
2.7	STATISTICAL ANALYSIS.....	78
3	IDENTIFICATION OF <i>TWIST-1</i> TARGET GENES INVOLVED IN MSC PROLIFERATION AND DIFFERENTIATION.	79
3.1	INTRODUCTION	79
3.2	RESULTS	83
3.2.1	Microarray analysis identified differentially expressed <i>TWIST-1</i> targets	83
3.2.2	Confirmation of differential gene expression by RT-PCR.....	84
3.2.3	Canonical Wnt signalling pathway is activated by <i>TWIST-1</i>	84
3.2.4	<i>TWIST-1</i> expression enhances <i>WNT2</i> and <i>WNT2B</i> promoter activity.....	84
3.2.5	<i>TWIST-1</i> binds directly to <i>WNT2</i> and <i>WNT2B</i> promoters.....	85
3.2.6	Generation of <i>WNT2</i> and <i>WNT2B</i> overexpressing MSC by Retroviral Transduction.....	86
3.2.7	Flow cytometric analysis of <i>WNT2</i> and <i>WNT2B</i> overexpressing MSC	87
3.2.8	Effects of <i>WNT2</i> and <i>WNT2B</i> on known differentiation markers	87
3.2.9	<i>WNT2</i> overexpression had no effect on MSC multi-differentiation.....	88
3.2.10	<i>WNT2B</i> overexpression enhanced adipogenesis of MSC but did not affect osteogenesis or chondrogenesis.....	89
3.2.11	MSC proliferation and senescence are not impacted by enforced expression of <i>WNT2</i> or <i>WNT2B</i>	90
3.3	DISCUSSION	92
4	HETEROZYGOUS <i>TWIST-1</i>^{+/-} MUTANT MICE DISPLAY ENHANCED OSTEOBLASTIC DIFFERENTIATION AND ACCELERATED LONG BONE FRACTURE REPAIR.....	97
4.1	INTRODUCTION	97
4.2	RESULTS	100
4.3	PILOT STUDY ASSESSING THE VIABILITY OF INTERNAL FRACTURE STABILIZATION WITH A CARBON ROD..	100
4.3.1	Carbon rod does not interfere with X-ray imaging	100
4.3.1.1	Analysis of the Heterozygous <i>Twist</i> ^{+/-} Phenotype	101
4.4	FRACTURE EXPERIMENTAL PLAN AND ANALYSIS.....	102
4.4.1	<i>TWIST-1</i> ^{+/-} mice have reduced callus size at two four and eight weeks post fracture.....	102
4.4.2	<i>TWIST-1</i> ^{+/-} mice display increased callus mineralization at one and two weeks post fracture	102
4.4.3	<i>TWIST-1</i> ^{+/-} and WT mice display comparable cartilage and fibrous tissue formation.....	103
4.4.4	Mechanical properties restored to baseline in fractured limbs of <i>TWIST-1</i> ^{+/-} mice at eight weeks post fracture	103
4.4.1	<i>TWIST-1</i> ^{+/-} mice display increased osteoblast numbers at eight weeks post fracture	104
4.4.2	<i>TWIST-1</i> ^{+/-} mice display increased osteoclast numbers at one week post fracture	104
4.4.3	<i>TWIST-1</i> ^{+/-} mice exhibit a higher proportion of osteogenic committed CFU-F.....	104
4.5	DISCUSSION	107
5	HETEROZYGOUS <i>TWIST-1</i>^{+/-} KNOCKOUT MICE EXHIBIT REDUCED BONE LOSS AFTER OVARIECTOMY	112
5.1	INTRODUCTION	112
5.2	RESULTS	115
5.2.1	Determination of optimal time point for analysis following ovariectomy	115
5.2.2	Ovariectomy experimental plan and analysis.....	115
5.2.3	<i>TWIST-1</i> ^{+/-} mice maintain trabecular bone volume following ovariectomy	116
5.2.4	<i>TWIST-1</i> ^{+/-} mice maintain total CFU-F numbers following ovariectomy.....	117
5.2.5	<i>TWIST-1</i> ^{+/-} mice have decreased osteoblast numbers following ovariectomy	117

5.2.6	<i> Twist-I^{+/-}</i> mice display no increase in osteoclast number following ovariectomy	117
5.2.7	Cortical bone strength is not affected at eight weeks post ovariectomy	118
5.2.8	<i> Twist-I^{+/-}</i> mice display increased adipocyte number and area following ovariectomy	118
5.3	DISCUSSION	120
6	GENERAL DISCUSSION AND FUTURE DIRECTIONS.....	124
6.1	FUTURE DIRECTIONS.....	129
6.1.1	Isolation and characterisation of mouse embryonic fibroblasts from homozygous <i> Twist^{-/-}</i> embryos .	129
6.1.1	Isolation and characterisation of MSC from B6;129S7- <i> TwistI^{tm2Bhr}/Mmnc</i> mice.....	129
6.1.2	Isolation and genetic profiling of pure osteoclasts and osteoblasts from <i> Twist^{+/-}</i> mice.....	130
7	REFERENCES.....	131

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

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the copy of my thesis, when deposited at the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Signed

Lachlan Cooper

Date:

Acknowledgements

First I would like to thank my supervisors Professor Stan Gronthos and Professor Andrew Zannettino. Thanks to Stan for always making time for me when I needed it and for knowing what to say to keep me motivated even when I didn't know I needed motivation. I very much appreciate Andrew's honesty, professionalism, wisdom and ability to connect with everyone on a personal level within a work setting. Thank you both for creating an environment that inspires personal growth and enables everyone in the laboratory to achieve and maintain a high standard of research.

I feel privileged to have worked with and learnt from Sandra Isenmann, one of the most passionate and technically talented scientists I will ever have the pleasure of working with. Thanks for believing in me, pushing me, gently reminding me, always being a great friend and passing on the reasons behind what works and what doesn't, in every protocol.

Thanks to everyone in the Bone and Cancer Laboratories for being such a great group of people who look out for one another and help each other succeed. Romana Panagopoulos, Mary Matthews, Dr Danijela Menicanin, Dr Agnes Arthur, Kris Mrozik, Catherine Gan, Sharon Paton, Kate Pilkington, Dr Peter Psaltis, Dr Peter Diamond, Dr Steve Fitter, Dr Sally Martin, Sarah Hemming, Dr Tony Cambareri, Jenny Drew, Dr Kate Vandyke, Dr Jim Cakouros, Thao Nguyen, Dr Kim Hynes, Dr Duncan Hewett, Dr Jacqueline Noll, Vicki Wilczek and Dr Sharon Hampton-Smith; I have received help and guidance from all of you and have developed friendships that I value greatly.

Stan and Agnes need to be singled out for their help teaching me the surgical techniques related to this project, Kate Vandyke for her amazing help and expertise with all of my histological and μ CT analysis, Mary for teaching me how to scan bones using μ CT, Steve, Peter and Sandra for help with all of my molecular cloning and infection techniques, Romana for keeping me sane while we were working long days together on fractures and ovariectomies, Kate Pilkington for early starts and the occasional all-nighter sorting, Sharon Paton for feeding my cells whenever I was sick, Sarah for helping out with population doubling assays when there weren't enough hours in the days and Jim for teaching me ChIP protocols.

Thanks to my partner Bec for making sure I was eating even if it meant bringing food into the lab at midnight, for picking me up in the rain, for dealing with my thesis getting the majority of my attention, and for being there for me through the journey that is a PhD.

Thanks to Mum and Dad for always encouraging, but never pressuring, me to do the things I love. Thanks to my little sister Jade for all of your chats and for being a great friend. Thanks to Grandma and Grandpa for always being proud of me and helping me want to be a good person.

I dedicate this thesis to my Grandpa who was always there for me, loved me unconditionally, and showed me what it is to be a good person.

Thank you.

Abbreviations

3D	Three dimensional
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 sialoprotein
<i>C/EBPα</i>	CCAAT/Enhancer Binding Protein alpha
<i>CBFA1</i>	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
<i>COL2a1</i>	Collagen type II alpha 1
CSA	chondroitin sulphate A
DAVID	Database for Annotation, Visualization and Integrated Discovery
DEPC	Diethylpyrocarbonate
<i>DERMO</i>	Dermis-expressed protein 1
<i>DLX5</i>	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
<i>EZH2</i>	Enhancer of zeste homolog 2
FACS	Fluorescence activated cell sorting
FCS	Foetal calf serum
<i>FGFR2</i>	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
<i>LDB2</i>	Lim domain binding 2
MACS	Magnetic cell sorting
<i>MAF</i>	Musculoaponeurotic fibrosarcoma
M-CSF	Macrophage colony-stimulating factor
MEM	Minimum essential medium
MPP	Multipotent progenitor cells
mRNA	Messenger ribonucleic acid

MSC	Mesenchymal stem cell
<i>MSX2</i>	Muscle segment homeobox 2
NBF	Neutral buffered formalin
NK	Natural killer
NOD	Normal osteoblast donor
<i>OCN</i>	Osteocalcin
<i>OPN</i>	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
<i>PGP-2</i>	Prostaglandin E2
PTH	Parathyroid hormone
PTHrP	Parathyroid hormone-related protein
<i>PTTG1</i>	Pituitary tumor-transforming 1
Q-PCR	Real-time polymerase chain reaction
<i>RANK</i>	Receptor activator of nuclear factor kappa-B
<i>RANKL</i>	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
<i>RUNX2</i>	Run related transcription factor
<i>SDF-1/CXCL12</i>	Stromal cell derived factor 1
SDS	Sodium dodecyl sulfate
<i>SMAD</i>	SMAD protein

SOC	Super optimal broth with cotabolite repression
<i>STRO-1</i>	Stromal precursor antigen - 1
TAE	Buffer containing Tris base, Acetic acid and EDTA
<i>TAZ</i>	Tafazzin protein
TBS	Tris buffered saline
TE	Buffer containing Tris and EDTA
<i>TGF-β</i>	Transforming growth factor beta
TRAP	tartrate-resistant acid phosphatase 5
Tris HCL	Tris Hydrochloride
Tween 20	Polyethylene glycol sorbitan monolaurate
<i>TWIST-1</i>	Transcription factor Twist-1
<i>TWIST-2</i>	Transcription factor Twist-2 (<i>DERMO-1</i>)
uCT	micro-computed tomography
UV	Ultra violet
<i>VSVG</i>	Vesicular stomatitis virus G-protein
Wnt	Wnt family of signalling molecules
<i>WNT2</i>	Wingless-type MMTV integration site family, member 2
<i>WNT2B</i>	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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