CELLULAR AND MOLECULAR MECHANISMS INVOLVED IN THE REPAIR OF THE INJURED GROWTH PLATE IN YOUNG RATS

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BY

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THESIS SUMMARY

The growth plate cartilage, which is located at the ends of children's long bones, is responsible for longitudinal growth of the skeleton. However, due to its cartilaginous composition and its location, the growth plate is commonly injured, mostly through fractures. An undesirable outcome to growth plate fracture is the bony repair of the injured cartilage at the fractured area. Consequently, children often incur skeletal angular deformities and growth arrest. Current corrective surgical treatments for these outcomes are highly invasive, and therapeutic interventions are not possible as little is known about the mechanisms and pathways that lead to bone bridge formation.

Using a rat model, previous studies have shown sequential inflammatory, fibrogenic, osteogenic and bone maturation responses involved in the bony repair of the injured growth plate. However, structural changes in the growth plate, at both the injury site and at the non-injured area, have not been closely examined previously, and little is known about the molecular mechanisms underlying the bony repair. Therefore, this PhD study, using a rat tibial growth plate injury model, aimed to examine the effects of growth plate injury on the structure and composition of the injured growth plate in a longitudinal study using micro-CT and histology. Microarray analysis of the injury site only, collected using laser capture microdissection was used to identify potential cellular and molecular mechanisms involved in bone bridge formation. In addition, Real Time RT-PCR on adjacent uninjured growth plate was used to examine potential cellular/molecular changes at the uninjured area and on whole growth plate scrapes to examine the potential involvement of Wnt signalling in bone bridge formation.

Micro-CT analysis revealed a significant increase in bone material within the injury site (when compared to normal) at 14 and 60 days post-injury, where 12% and 40% of the injury site was replaced by bone, respectively. Interestingly, although there were no changes in growth plate thickness between injured and normal rats at either day 14 or 60, at day 60, many small bone tethers formed at the adjacent growth plate outside the injury site but none were found in normal aged-matched control rats. Histological studies revealed dereased proliferation but increased apoptosis of chondrocytes at the adjacent growth plate cartilage, and RT-PCR analysis revealed differential expression of apoptosis-regulatory genes Bcl-2 and FasL (compared to normal), confirming the increase in apoptosis in the adjacent uninjured growth plate. Down-regulation of Sox-9 and IGF-1 on days 7 and 14 suggests that growth plate injury may slow down the rate of longitudinal growth by decreasing chondrocyte proliferation and/or differentiaiton soon after injury. Lastly, bone matrix protein osteocalcin was increased on day 60, suggesting degeneration and bone formation at the adjacent uninjured area.

Microarray analysis identified changes in several key BMP and Wnt signalling components across the time-course of bone bridge formation, including BMP-2, BMP-6, BMP-7, chordin, chordin-like 2, and Id-1, and β-catenin, Csnk2a1, SFRP-1 and SFRP-4, respectively, in early stages of bone bridge formation (day 4 and day 8). Osteocalcin expression was also prominent at day 8, supportive of osteoblast development and bone formation. During later stages (day 14), active bone formation and remodelling was prominent and was largely regulated by the BMP signalling pathway (increased BMP-1 and BMP-6 but decreased inhibitor chordin), as well as by Traf6, Fgfr1, osteopontin, Mmp9 and

Wnt signalling, where several genes were up and down-regulated. Expression levels of Wnt signalling inhibitors (SFRP-1, SFRP-4 and Wisp1) were increased at days 8 and 14 and may be negatively regulating bone formation during the osteogenic phases of the repair of the growth plate injury site. Findings were also suggestive of an overall increase in the canonical Wnt signalling pathway at days 4 and14, supported by increased expression of β-catenin and drecreased expression of Wnt inhibitors, and decreased expression of Fzd1 and Fzd2 and increased Lef1 expression, respectively. Overall, this study found a complex balance between the canonical and non-canonical Wnt pathways as well as an association with BMP signalling over the time-course of bone bridge formation.

Lastly, Real-Time PCR on Wnt signalling components revealed significant changes in gene expression of Wnt genes, receptors and inhibitors, but were inconclusive as the method of tissue isolation was not specific enough to represent true changes in gene expression.

DECLARATION

This work contains no material which has been accepted for the award of any other degrees or diplomas in any university or other tertiary institution to Carmen Elizabeth Macsai 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.

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Published works are:

Carmen E. Macsai, Bruce K. Foster, Cory J. Xian (2008). Roles of Wnt signalling in bone growth, remodelling, skeletal disorders and fracture repair. *J Cell Physiol*. Jun; 215(3):578-87.

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ABBREVIATIONS

Abbreviation	Full name
ALP	Alkaline phosphatase
APC	Adenomatous polyposis coli
ATF4	Activating transcription factor 4
Bglap2	Bone gamma-carboxyglutamate protein 2
BMPs	Bone morphogenic proteins
BMUs	Basic multicellular units
BSA	Bovine serum albumin
BSP	Bone sialoprotein
Camk2	Calmodulin dependent protein kinase 2
Cbfa1	Core-binding factor alpha 1
CBP	Cyclic AMP response element-binding protein
cDNA	complementary DNA
Ck1A1	Casein kinase 1, alpha 1
COX-2	Cyclo-oxygenase 2
cRNA	complementary RiboNucleic Acid
DMSO	Dimethyl sulfoxide
DNA	DeoxyriboNucleic Acid
DSH	Dishevelled
EDTA	Ethylenediaminetetraacetic acid
FGF	Fibroblast growth factor
FHL2	Four and half LIM domain 2

Fzd	Frizzled
GH	Growth hormone
GSK-3	Glycogen synthase kinase-3
H&E	Haematoxylin & Eosin
HGF	Hepatocyte growth factor
HSCs	Hematopoietic stem cells
5-HT	Serotonin/5-HydroxyTryptamine
IGFBPs	IGF binding proteins
Ihh	Indian hedgehog
iNos	inducible Nitric oxide synthase
Id-1	Inhibitor of DNA binding-1
LCM `	Laser capture microdissection
LEF1	Lymphoid enhancer-binding factor 1
LRP-5/-6	Low density lipoprotein receptor-related protein-5/6
Ltbp2	Latent transforming growth factor beta binding protein 2
MAPK	Mitogen activated protein kinase
M-CSF	Macrophage colony-stimulating factor
MES	2-(*N*-Morpholino) EthaneSulfonic Acid
mm, µm	millimetre, micrometre
Mmps	Matrix metalloproteases
MSCs	Mesenchymal stem cells
NFAT	Nuclear factor of activated T cells
°C	Degrees Celcius

OCT	Optimal cutting temperature
OPG	Osteoprotegrin
Osx	Osterix
PCP	Planar cell polarity pathway
PCR	Polymerase chain reaction
PDGF	Platelet derived growth factor
Ptc	Patched
PTHrP	Parathyroid hormone (PTH)-related peptide
%	Percent
RANK	Receptor activator of NF-κβ
RANKL	Receptor activator of nuclear factor-kappaB ligand
RNA	RiboNucleic Acid
rpm	revolutions per minute
RT	Reverse transcription
Runx2	Runt-related transcription factor 2
SFRP	Secreted frizzled related protein
Shh	Sonic hedgehog
Sox	SRY (sex determining region Y)-box
TCF	T-cell factor
TGF-β	Transforming growth factor-β
TH	Thyroid hormone
TNF-α	Transforming nuclear factor-α
Tph1	Tryptophan hydroxylase 1

Traf6	Tumour necrosis factor receptor-associated factor 6
TRAP	Tartrate resistant acid phosphatase
g, mg, µg, ng,	gram, milligram, microgram, nanogram
ml, ul	millilitre, microlitre,
M, mM	Mole, milliMolar
VEGF	Vascular endothelial growth factor
Wif-1	Wnt inhibitory factor-1
Wnt	Wingless-Int
Wisp	Wnt1 inducible signalling pathway protein