

09PH
26371



ROLES OF TNF- α SIGNALLING AND p38 MAP
KINASE ACTIVATION IN THE RESPONSES TO
GROWTH PLATE INJURY IN YOUNG RATS

A THESIS SUBMITTED IN TOTAL FULFILMENT
OF THE REQUIREMENTS OF
THE DEGREE OF DOCTOR OF PHILOSOPHY

BY

Fiona Huan-huan Zhou

Bachelor of Biotechnology (Honours)

Department of Orthopaedic Surgery,

Women's and Children's Hospital;

Discipline of Paediatrics and Reproductive Medicine,

Faculty of Health Sciences, University of Adelaide;

Adelaide, South Australia

4 October 2006

TABLE OF CONTENTS

THESIS SUMMARY.....	IV
DECLARATION	VI
ABBREVIATIONS.....	VIII
CHAPTER 1	2
LITERATURE REVIEW & PROJECT AIMS	
1.1 Introduction.....	3
1.2 Immature Long Bone Structure	4
1.2.1 Epiphysis.....	6
1.2.2 Growth Plate.....	7
1.2.3 Woven and Lamellar Bone	7
1.2.4 Metaphysis	8
1.2.5 Diaphysis.....	9
1.3 Growth Plate Structure.....	10
1.3.1 Resting Zone	11
1.3.2 Proliferative Zone.....	12
1.3.3 Hypertrophic Zone.....	14
1.3.4 Groove of Ranvier and Perichondrial Ring of LaCroix.....	14
1.4 Cells and Matrix Proteins of Cartilage and Bone	15
1.4.1 Chondrocytes.....	16
1.4.2 Osteoblasts	18
1.4.3 Osteocytes	19
1.4.4 Bone Lining Cells.....	20
1.4.5 Osteoclasts	21
1.4.6 Bone Marrow Cells.....	22
1.4.6.1 Mesenchymal Stromal Cells.....	22
1.4.6.2 Hematopoietic Stem Cells	24
1.4.7 Matrix Proteins in Cartilage and Bone	25
1.4.7.1 Collagen -II.....	25
1.4.7.2 Collagen -X.....	27
1.4.7.3 Collagen -I.....	28
1.4.7.4 Osteocalcin	30
1.5 Bone Formation.....	31

1.5.1	Endochondral Ossification.....	31
1.5.2	Intramembranous Ossification	35
1.5.3	Bone remodelling	35
1.6	Bone and Growth Plate Fracture Repair	41
1.6.1	Bone Fracture Healing Responses.....	41
1.6.1.1	<i>Inflammation Phase</i>	42
1.6.1.2	<i>Reparative Phase</i>	45
1.6.1.3	<i>Remodelling Phase</i>	49
1.6.2	Growth Plate Injury Responses.....	50
1.6.3	Current Treatments For Growth Plate Injury-Induced Bone Growth Defects..	57
1.6.4	Regeneration of Injured Cartilage	59
1.7	Expression and Roles of Regulating Factors in Fracture Repair	62
1.7.1	Inflammatory Factors	62
1.7.1.1	<i>Pro-inflammatory Cytokines</i>	62
1.7.1.2	<i>p38 MAP Kinase</i>	64
1.7.2	Growth Factors.....	66
1.7.2.1	<i>TGF-β1</i>	67
1.7.2.2	<i>FGF-2</i>	69
1.7.2.3	<i>PDGF-B</i>	71
1.7.2.4	<i>IGF-I</i>	73
1.7.2.5	<i>BMPs</i>	75
1.7.3	Transcription factors.....	77
1.7.3.1	<i>Sox9</i>	77
1.7.3.2	<i>cbfal</i>	80
1.8	Project Rationale, Hypothesis and Aims	83
1.8.1	Project Rationale and Overview.....	83
1.8.2	Project Hypothesis.....	86
1.8.3	Project Aims.....	87
CHAPTER 2	88
EXPRESSION OF PROINFLAMMATORY CYTOKINES AND GROWTH FACTORS		
AT THE INJURED GROWTH PLATE CARTILAGE IN YOUNG RATS		
CHAPTER 3	98
TNF-α MEDIATES p38 MAP KINASE ACTIVATION AND NEGATIVELY		
REGULATES BONE FORMATION AT THE INJURED GROWTH PLATE IN RATS		
CHAPTER 4	113

4.1	General Summary	114
4.2	The Pathway to Understanding Bone Bridge Formation	115
4.3	Thesis Conclusion	122
4.4	Future Directions.....	122
APPENDICES.....		125
Appendix 1.	Experimental Protocols Used in Chapter 2 and 3.....	126
Appendix 1.1	Total RNA Isolation from Growth Plate Cartilage	126
Appendix 1.2	Reverse transcription of total RNA.....	129
Appendix 1.3	Immunohistochemistry using rat proximal tibia sections.....	130
Appendix 1.4	Western Blotting Analysis	132
Appendix 2	Personal Publications and Conference Presentations	137
Appendix 2.1	Personal Publications (2003-2006).....	137
Appendix 2.2	Conference Presentations (Talks and Posters).....	140
BIBLIOGRAPHY		142

THESIS SUMMARY

Growth plate cartilage is responsible for bone lengthening in children and yet it has limited abilities to regenerate. After injury, a bone bridge often forms at the injury site, which may lead to growth arrest or angulation of the involved bone. Although our previous study has demonstrated inflammatory, fibrogenic, and osteogenic cellular responses after growth plate injury, the underlying molecular mechanisms remain unclear. Pro-inflammatory cytokine TNF- α inhibits osteoblast differentiation *in vitro* and yet TNF signalling is essential for bone fracture healing. In addition, the production and signal transduction of TNF- α and IL-1 β require activation of p38 MAP Kinase, which is often induced by stress and tissue injury. Currently roles of TNF- α signalling and p38 activation in the bony repair of injured growth plate cartilage are unknown.

In this study, firstly, the changes in expression of pro-inflammatory cytokines, growth factors and chondrogenic/osteogenic markers, and patterns of p38 activation were examined in injured proximal tibial growth plate of young rats. Results showed up-regulated expression in cytokines TNF- α , IL-1 β and TGF- β 1, but down regulated expression in cartilage marker Col-2a, osteogenic markers cbfa1 and osteocalcin during the immediate inflammatory phase. Consistent with TNF- α and IL-1 β expression pattern, up-regulation of p38 activation was also seen during the inflammatory response. While Col-2a and chondrogenic factor Sox9 mRNA levels were not altered during the subsequent fibrogenic response, fibrogenic growth factors FGF-2 and PDGF-B expression were up-regulated. In addition, expression of osteocalcin as well as bone remodelling regulatory factors IGF-I, TNF- α , FGF-2 and TGF- β 1 was induced during bone bridge formation and maturation stage. Changes in expression of these cytokines, p38 and growth factors suggest their possible roles in regulating the inflammatory, fibrogenic, osteogenic, and remodelling events for bony repair of the injured growth plate. Furthermore, we also found a significant inverse correlation between TNF- α and cbfa1 expression levels, suggesting a negative

relationship between TNF- α and cbfal in this *in vivo* model. Therefore these observations encourage us to further examine the roles of TNF- α signalling in p38 activation and in the subsequent bony repair of the injured growth plate.

In the second study, effects of TNF- α inhibitor on growth plate injury responses, and the regulatory effects of TNF- α and p38 signalling on proliferation and migration of cultured rat bone marrow mesenchymal cells (rBMMC) were examined. We are the first to identify that TNF- α signalling is required to mediate p38 activation induced by growth plate injury. TNF- α inhibition reduced mesenchymal infiltration and cell proliferation and FGF-2 expression at the injured growth plate. Consistently, TNF- α increased proliferation and migration of rBMMC *in vitro*, which required the action of p38. On the other hand, TNF- α inhibition up-regulated expression of cbfal and osteocalcin, and increased trabecular bone formation at the injury site. In conclusion, TNF- α signalling is required to induce mesenchymal cell proliferation and migration at the growth plate injury site and in cell culture through the p38 MAP kinase pathway; on the other hand, TNF- α signalling suppresses bony repair of the injured growth plate by inhibiting bone cell differentiation.