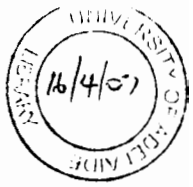

ROLES OF TNF-α SIGNALLING AND p38 MAP KINASE ACTIVATION IN THE RESPONSES TO GROWTH PLATE INJURY IN YOUNG RATS

A THESIS SUBMITTED IN TOTAL FULFILMENT

OF THE REQUIREMENTS OF

THE DEGREE OF DOCTOR OF PHILOSOPHY

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4 October 2006

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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-1β 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-1β and TGF-β1, 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-1β 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-β1 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 cbfa1 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 cbfa1 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.