DOCTORAL THESIS

Role of CKIP-1 in suppression of osteoblast mediated bone repair in a collagen induced non-human primate arthritis model

Shaikh, Atik Badshah

Date of Award:
2017

Abstract

Rheumatoid arthritis (RA) is a systemic, inflammatory disease, which predominantly affects multiple joints. RA is characterized by swollen joints and peri-articular bone erosion. Conventional RA treatments have shown to reduce inflammation and slow down bone erosion, but repair of bone erosion is limited. Additionally, failure to repair for bone erosion in rheumatoid arthritis (RA) is caused by inadequate osteoblast-mediated bone formation resulting from focal inflammatory environment. Hence, there is immediate need to facilitate greater insight and develop a new therapeutic strategy to aid osteoblast -mediated repair of bone loss in RA. CKIP-1 is an intracellular inhibitor, that can negatively regulate osteoblast lineage cells differentiation and activity. CKIP-1 levels were found to be aberrantly over expressed in bone specimens from RA patients and arthritis mice, which was associated with reduced bone formation and increased disease severity. By genetic approach, overexpressed CKIP-1 in osteoblast exacerbated bone erosion and articular inflammation in CIA mice, whereas deficiency of CKIP-1 in osteoblast alleviates bone erosion in CIA mice. By pharmacological approach, RNA interference-based silencing of osteoblastic CKIP-1 led to bone formation-mediated reparative process at erosive site and reduced articular inflammation in arthritis induced mice. To extend above findings to a more relevant species that more closely

resemble humans, we aimed to investigate the role of osteoblastic Casein kinase-2 interacting protein-1 (CKIP-1) in failure to repair bone erosion in non-human primate (NHP) arthritis model in this study. We found that CKIP-1 mRNA expression in osteoblasts of arthritic NHP was significantly suppressed by CKIP-1 siRNA treatment. Moreover, silencing of CKIP-1 in osteoblast of arthritis monkey improved clinical signs such as reduction in arthritis score, joint swelling and increase in body weight. Similarly, suppression of osteoblastic CKIP-1 resulted in better organized bony and articular structure with, fewer bone erosion sites as observed in x ray and micro CT images. Moreover, we found increase in bone mass, bone formation parameters such as BFR/BS and MAR and histological examination revealed attenuation of peri articular bone erosion and intraarticular inflammation in CKIP-1 siRNA treated arthritis monkeys.

Taken together, these data strongly suggest that highly expressed osteoblastic CKIP-1 plays an important role in failure to repair articular bone erosion by inhibiting bone formation in RA. Targeting osteoblastic CKIP-1 could serve as a new therapeutic strategy by bone repair augmentation in RA.

Table of Content

Declaration i
Abstract ii
Acknowledgement iv
Table of Content v
List of figures and tables ix
List of Abbreviation xi
Chapter 1. Background 1
1.1 Introduction 1
1.2 Clinical Challenge 2
1.3 Molecular mechanism underlying failure of osteoblast –mediated bone repair. 3
1.3.1 Role of local inflammation microenvironment 3
1.3.2 Role of TNF-α and BMP signaling inhibitors 4
1.3.3 Role of Wnt signaling inhibitors 5
1.4 Role of CKIP-1 5
1.5 Novel targeted delivery system 7
1.6. Need for non-human primate model (NHP) 7
1.7 Hypothesis 8
1.7.1 Aim of study 8
Chapter 2. Materials and Methods 9
2.1 Animal 9
2.2 Collagen-induced arthritis (CIA) non-human primate model 10
2.3 Preparation of $(DSS)_6$-liposome encapsulating siRNA 11
2.4 Treatment. 12
2.5 Evaluation of arthritic severity 13
2.6 Radiographic Examination 14
2.7 Micro CT analysis 15
2.8 Laser captured micro-dissection (LCM) 15

2.9 Total RNA extraction, real-time quantitative reverse transcription-PC....17
2.10 Dynamic bone histomorphometry 18
2.11 Histology evaluation 19
2.12 Routine blood tests and blood biochemistry. 20
2.13 Inflammatory cytokine measurement by multiplex bead array kits 21
2.14 Statistical analysis....22
Chapter 3. Established collagen-induced non-human primate arthritis model. 23
3.1 Aim 23
3.2 Experimental design 23
3.3 Results....24
3.3.1 Collagen-induced arthritis (CIA) in non-human primates. 24
3.4 Conclusion 25
Chapter 4. CKIP-1 siRNA suppresses osteoblastic CKIP-1 expression and prevent weight loss in non-human primate arthritis model 26
4.1 Aim 26
4.2 Experimental design 26
4.3 Results....27
4.3.1 Therapeutic suppression of osteoblastic CKIP-1 expression in non-human primate arthritis model. 27
4.3.2 Increase in body weight after CKIP-1 suppression in non-human primate arthritis model. 28
4.4 Conclusion 31
Chapter 5. Inhibition of osteoblastic CKIP-1 attenuates articular inflammation in NHP arthritis model 32
5.1 Aim 32
5.2 Experimental design 32
5.3 Results....33
5.3.1 RNA interference-based silencing of CKIP-1 reduces arthritis score. 33
5.3.2 Reduced joint swelling observed after silencing of CKIP-1 in

osteoblasts in non-human primate arthritis model 34

5.4 Conclusion 37

Chapter 6. Down-regulation of osteoblastic CKIP-1 attenuates articular bone erosion and joint destruction in NHP arthritis model 38

6.1 Aim 38

6.2 Experimental design 38

6.3 Results 38

6.3.1 Preservation of joint and bone integrity, with no radiological changes of joint destruction after siRNA treatment. 38

6.4 Conclusion 41

Chapter 7. Suppression of CKIP-1 in osteoblasts improves microarchitecture and increases bone mass in NHP arthritis model 42

7.1 Aim 42

7.2 Experimental design 42

7.3 Results 42

7.3.1 Improved PIP joint trabecular architecture and preserved bony articular integrity in arthritic monkeys after CKIP-1 siRNA treatment 42

Chapter 8. Suppression of CKIP-1 in osteoblasts prevents joint damage and lowers inflammatory process in NHP arthritis model 46

8.1 Aim 46

8.2 Experimental design 46

8.3 Results 47

8.3.1 Attenuation of articular inflammatory process in siRNA treated arthritis monkeys. 47

6.4 Conclusion 49

Chapter 9. Reduced levels of CKIP-1 within osteoblasts promotes bone formation in NHP arthritis model 50

9.1 Aim 50

9.2 Experimental design 50

9.3 Results 50

9.3.1 Improved bone formation parameters in arthritic monkey treated by

siRNA. 50
9.4 Conclusion 52
Chapter 10. Silencing of osteoblast specific CKIP-1 does not effect systemic inflammatory cytokines in NHP arthritis model 53
10.1 Aim 53
10.2 Experimental design 53
10.3 Results 53
10.3.1 No changes in systemic inflammatory cytokine. 53
10.4 Conclusion 55
Chapter 11. Routine blood tests to evaluate safety. 56
11.1 Aim 56
11.2 Experimental design 56
11.3 Results 56
11.3.1 Routine blood tests. 56
11.4 Conclusion 60
Chapter 12. Discussion 61
12.1 Summary 61
12.2 Conclusion 69
Bibliography 71
CURRICULUM VITAE 82