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TARGETING NF-κB AND NFATc1 SIGNALLING TO INHIBIT BONE RESORPTION IN PERI-PROSTHETIC OSTEOLYSIS

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TABLE OF CONTENT

TABLE OF ABBREVIATION

αvβ3	Alpha-v-beta-3 integrin
3D	Three dimensional
AnnVIII	Annexin 8
AP-1	Activator protein-1
ATPase	Adenosine triphosphatase
Atpv0d2	D2 isoform of vacuolar (H^+) atpase (V-atpase) V0 domain
BMD	Bone mass density
BMMs	Bone marrow macrophages
BMU	Basic multicellular unit
C	Carbon
CaMKs	Calcium/calmodulin-activated kinase
CAPE	Caffeic acid phenethyl ester
CathK	Cathepsin K
ChIP	Chromatin immunoprecipitation
CIA	Collagen-induced arthritis
CoCr	Cobalt chrome alloy
Co-Cr-Mo	Cobalt-chromium-molybdenum alloy
cpTi	Commercially pure titanium
CsA	Cyclosporin A
CSF-1	Colony stimulating factor 1
CTR	Calcitonin receptor
CTX-1	Serum type-1 carboxy-terminal collagen crosslinks
DAG	Diacylglycerol
DAP12	DNAX-activating protein 12kda
DC-STAMP	Dendritic-cell transmembrane protein
EU	Endotoxin units
FBGCs	Foreign-body giant cells
FcRγ	Fc receptor common gamma subunit
FK506	Tacrolimus
FKBP12	FK506-binding protein 12
GFP	Green fluorescent tagged
GIT	Gastrointestinal tract
GM-CSF	Granulocyte-macrophage colony-stimulating factor
H^+	Hydrogen ions
HCl	Hydrochloric acid
HMEECs	Human middle ear epithelial cells
ID2	Inhibitor of differentiation 2
Ig	Immunoglobulin
IL	Interleukin
INF-γ	Interferon-γ
IP3	Inositol-1,4,5-trisphosphate
ITAM	Immunoreceptor tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibitory motif
JNK	C-Jun terminal kinase

LPS	Lipopolysaccharides
LRC	Leukocyte receptor complex
MAGPHPVIVITGPHEE	Commercially available calcineurin inhibitory peptide motif VIVIT
M-CSF	Macrophage-colony stimulating factor
MITF	Microphtalmia transcription factor
MMPs	Lysosomal proteases matrix metalloproteinases
NC100	Natural compound 100
NFATc1	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1
O	Oxygen
OCIF	Osteoclasts inhibitory factor
ODF	Osteoclast differentiation factor
OPG	Osteoprotegerin
OPG-Fc	OPG-fusion protein
OPGL	Osteoprotegerin ligand
OSCAR	Osteoclast-associated receptor
OVX	Ovariectomized
PAR	Parthenolide
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate-buffered saline
P-C-P	Bisphosphonates
PD	Periodontal diseases
PE	Polyethylene particles
PGE2	Prostaglandin E2
PIAS3	Protein inhibitor of activated STAT 3
PIP2	Phospholipid phosphatidylinosotol-4,5-bisphosphate
PIR-A	Paired Ig receptor-A
PLCγ	Phospholipase C gamma
PLOSL	Polycystic lipomembranous osteodysplasia with sclerosis leukoencephalopathy
PMMA	Polymethylmethacrylate
P-O-P	Pyrophosphate
PTK	Src family protein tyrosine kinase
RA	Rheumatoid arthritis
RANK	Receptor activator of NF-κB
RANKL	Receptor activator of NF-κB ligand
RUNX-2	Runt-related transcription factor 2
SDF-1	Stromal-cell derived factor-1
SH2	Src homology 2
siRNA	Small interfering RNA
SS	Stainless steel
STA	Arthritic serum transfer
Syk	Spleen tyrosine kinase
Ti	Titanium alloy

TiAlV	Titanium-6-aluminium-4-vanadium alloy
TNF	Tumor necrosis factor
TRAF	TNF receptor activating factor
TRANCE	TNF-related induced cytokine
TRAP	Tartrate-resistant acid phosphatase
TREM2	Triggering receptor expressed in myeloid cells
TYROBP	Tyrosine kinase binding protein
UHMWPE	Ultra-high-molecular-weight PE
VIVIT	11R-VIVIT peptide
μCT	Micro-computed tomography

ABSTRACT

Peri-prosthetic osteolysis is a bone loss disease involving granulomatous inflammation in the soft tissues around prostheses characterised by excessive bone resorption adjacent to implants. Macrophages phagocytose particles of prosthetic material, thereby inducing persistent release of pro-inflammatory osteoclastogenic cytokines, such as, receptor activator of NF-κB ligand (RANKL). RANKL interacts with its receptor RANK, to activate key transcription factor in osteoclastogenesis, NFATc1. This activates an inflammatory response leading to bone erosion at the implant bone interface and subsequent prosthetic failure. Other bone cells are affected by particles. For example, osteoblasts have reduced bone formation activity, and osteocytes, undergo apoptosis, whereby in this process they release cytokines that stimulate bone loss.

Apart from the RANKL/RANK-NFATc1 system, immunoreceptor tyrosine-based activation motif (ITAM)-dependent pathway has been identified as a co-stimulatory pathway in osteoclasts. Osteoclast-associated receptor (OSCAR) and TREM2 are ITAM-containing receptors pairing with adaptor molecules FcRγ and DAP12, respectively. Our group has demonstrated the increased expression of NFATc1 and ITAM-related molecules adjacent to sites of bone loss in human peri-prosthetic tissues and polyethylene (PE) particle-stimulated osteoclasts *in vitro*. Soluble OSCAR has been proposed as a potential regulator of osteoclast activity in osteolysis. Considering the importance of NF-κB and NFATc1 we hypothesize that their inhibition of will abrogate osteoclast bone resorption.

The data presented here showed that inhibition of NFATc1 by calcineurin-NFAT inhibitors, FK506 and VIVIT, and inhibition of both NFATc1 and NF-κB by NC100, significantly suppressed osteoclast formation and activity in normal human-derived RANKL-induced osteoclast differentiation *in vitro*. mRNA expression analysis showed OSCAR was inhibited by FK506, VIVIT and NC100 at later stages of osteoclastogenesis. Together, this demonstrates potential benefits of targeting NFATc1 and NF-κB to suppress osteoclastogenesis and modulate ITAM-containing molecules in human osteoclasts.

In the murine calvarial model of PE-induced peri-prosthetic osteolysis, live animal micro-computed tomography analyses showed that PE particles significantly induced localised osteolysis in mice implanted with PE particles compared to controls. PAR strongly reduced surface bone resorption but not local bone volume. However, CAPE

treatment reduced local PE-induced calvarial osteolysis at both surface resorption and volumetric change. Additionally, PE particles significantly increased serum levels of bone resorptive marker CTX-1 and soluble OSCAR. However, neither PAR nor CAPE regulated CTX-1 and OSCAR in PE-implanted mice. Neither PE nor CAPE affected the gastrointestinal tract, a potential side effect of their treatments.

Interestingly, PE particles strongly enhanced osteocyte death, supporting previous reports that osteocytes undergo apoptosis in response to PE particles. PAR significantly decreased osteocyte apoptosis in PE-implanted mice, as assessed by reduced number of osteocytes with empty lacunae within the calvarial tissues. It could be important to determine the possible mechanism by which PE particles activate NF-κB in osteocytes as this could be an important mechanism inducing osteolysis in response to prosthetic particles.

Overall the results demonstrate that targeting NFATc1 and NF-κB signalling suppresses osteoclast differentiation and resorption induced by PE particles. Future studies are necessary to fully understand this pathology.

STUDENT 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 in my name and, to the best of my knowledge and belief, contains no material published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this will, in the future, be used in a submission in my name, for any other degree or diploma in any university without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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LIST OF JOINTLY AUTHORED PAPERS INCLUDED IN THESIS

CHAPTER 2

Regulation of ITAM adaptor molecules and their receptors by inhibition of calcineurin-NFAT signalling during late stage osteoclast differentiation

***M.S.F. Zawawi**, A.A.S.S.K. Dharmapatni, M.D. Cantley, K.P. McHugh, D.R. Haynes, T.N. Crotti.*

Biochemical and Biophysical Research Communications 2012; 427(2):404-9.

CHAPTER 3

Molecular mechanisms and therapeutic effects of NC100 on osteoclastic bone resorption and osteoporosis

*Qian Liu, Tao Wang, **Muhamad Syahrul Fitri Zawawi**, Tania Crotti, An Qin, Zhen Lin, Jinbo Yuan, Huagang Liu, Minghao Zheng, Jiake Xu, Jinmin Zhao.*

Journal of Cellular Physiology (manuscript in preparation).

CHAPTER 4

Parthenolide inhibits osteocyte apoptosis and osteoclastic bone surface resorption induced by polyethylene particles in a murine calvarial model of peri-implant osteolysis

***Muhamad S F Zawawi**, Victor Marino, Egon Perilli, Melissa D Cantley, Jiake Xu, P. Edward Purdue, Anak A S S K Dharmapatni, David R Haynes, Tania N Crotti.*

Journal of Biomedical Materials Research Part A – accepted April 2015 as "Parthenolide reduces empty lacunae and osteoclastic bone surface resorption induced by polyethylene particles in a murine calvarial model of peri-implant osteolysis"

CHAPTER 5

Caffeic acid phenethyl ester abrogates bone resorption in a murine calvarial model of polyethylene particle-induced osteolysis

***M.S.F. Zawawi**, E. Perilli, R.L. Stansborough, V. Marino, M.D. Cantley, J. Xu, A.A.S.S.K., Dharmapatni, D.R. Haynes, R.J. Gibson, and T.N. Crotti.*

Calcified Tissue International - accepted March 2015.

SCIENTIFIC COMMUNICATIONS

INTERNATIONAL:

2015

Work presented to Prof Heinz Redl at the Ludwig Boltzman Institute, Vienna, Austria.

2014

The 2nd Asia-Pacific Bone & Mineral Research (APBMR) Meeting in conjunction with the 26th Spring Scientific Congress of Korean Society for Bone and Mineral Research and the 2nd Seoul Symposium on Bone Health, Seoul, South Korea – Oral.

2013

American Society for Bone Mineral Research (ASBMR) Annual Meeting – Poster.

Hospital for Special Surgery (HSS), New York – Oral.

2011

International Osteoporosis Federation (IOF) 2nd Asia-Pacific Osteoporosis and Bone Meeting: Australian & New Zealand Bone & Mineral Society (ANZBMS) Annual Scientific Meeting with Japanese Society for Bone and Mineral Research (JSBMR), Queensland, Australia – Poster.

NATIONAL AND LOCAL:

2014

Florey International Postgraduate Research Conference, Faculty of Health Sciences (FHS) Postgraduate Research Conference 2014, The University of Adelaide. Adelaide, Australia – Poster.

20th Annual Conference of Australian & New Zealand Orthopaedic Research Society (ANZORS), Adelaide, Australia – Oral.

Invited presentation and laboratory visit. School of Pathology and Laboratory Medicine, The University of Western Australia. Sir Charles Gairdner Hospital, The University of Western Australia, Perth. – Oral.

School of Medical Sciences, The University of Adelaide. PhD Progress Seminar – Oral.

1st Australasian Bruker Skyscan User Meeting. SA, Australia – Oral.

2013

Australian & New Zealand Bone & Mineral Society (ANZBMS) 23rd Annual Scientific Meeting, Melbourne, Australia - Poster.

19th Annual Conference of Australian & New Zealand Orthopaedic Research Society (ANZORS), Sydney, Australia – Oral.

Faculty of Health Sciences (FHS) Postgraduate Research Conference 2013, Adelaide, Australia - Poster.

Australian Society for Medical Research (ASMR) South Australia Annual Scientific Meeting – Oral.

1st Malaysian Postgraduate Student Symposium of South Australia 2013, Adelaide, Australia – Oral.

Australian Rheumatology Association (ARA) South Australia Annual Scientific Meeting, Adelaide, Australia – Oral.

2012

Australian & New Zealand Bone & Mineral Society (ANZBMS) 22nd Annual Scientific Meeting in conjunction with 1st Asia-Pacific Bone and Mineral Research meeting, Perth, Australia – Poster.

Faculty of Health Sciences (FHS) Postgraduate Research Conference 2012, Adelaide, Australia - Poster.

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ADDITIONAL ACHIEVEMENTS DURING CANDIDATURE

2014

Winner of MASCA-MONASH University Science Excellence Award (National Level), Australia. Malaysian Summit of Australia (MASA) 2014, Malaysian Students' Council of Australia (MASCA) National, Australia.

Travel award of The School of Medical Sciences Postgraduate Travel Award (International Conference Attendance). Faculty of Health Science (FHS), The University of Adelaide, SA, Australia.

Invited speaker (panelist). Postgraduate Forum, organized by Malaysian Postgraduate Student Association of South Australia Incorporated (MyPSA SA Inc.) and Adelaide University Malaysian Students Association (AUMSA), SA, Australia.

Role-Model Exemplary Excellence Award 2014 (Anugerah Murabbi Terbaik 2014). Australian-Malaysian Muslim Solidarity, South Australia Chapter (ISMA SA). South Australia, Australia.

2013

A Faculty Prize (Best Poster Presentation). Faculty of Health Sciences (FHS) Postgraduate Research Conference 2013. The University of Adelaide, SA, Australia.

A Travel Grant Award to attend a national conference. The 19th Annual Conference of Australian & New Zealand Orthopaedic Research Society (ANZORS). New South Wales, Australia.

Invited Main Speaker for an Academic Motivational Talk. Title "From Zero to Hero." Pursuit of Knowledge (Program Kembara Ilmu) by Australian-Malaysian Muslim Solidarity, South Australia Chapter (ISMA SA), SA, Australia.

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Finalist for the Adelaide Research & Innovation Pty Ltd (ARI) Award. Faculty of Health Sciences (FHS) Postgraduate Research Conference 2012. The University of Adelaide, SA, Australia.

Academic and Character Excellence Award 2012 (Anugerah Kecemerlangan Akademik dan Sahsiah (AKASIA)). Australian-Malaysian Muslim Solidarity, South Austalia Chapter (ISMA SA). South Australia, Australia.

2011

RLKA Scholarship (Full), School of Medical Sciences, Universiti Sains Malaysia (USM).

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