The Role of Sphingosine Kinases and SKAM1 in Cutaneous Wound Healing

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A Thesis submitted for the degree of

Doctor of Philosophy

September 2014

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Chan, H., and Pitson, S.M. (2013). Post-translational regulation of sphingosine kinases. Biochim Biophys Acta *1831*, 147-156.

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Sept 2014

Statement of Authorship

Chan H and Pitson SM (2013). Post-translational regulation of sphingosine kinases.

Biochim Biophys Acta *1831*, 147-156.

Huasheng Chan: Wrote the article.

I hereby certify that the statement of contribution is accurate

Signed: *Date:*

Stuart M. Pitson: Prepared figures and edited contribution from the other author.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of this paper in the thesis

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Acknowledgement

I would like to thank Prof. Stuart Pitson for the opportunity to work on this project and for all his invaluable advice, support and patience over the years. I truly appreciate all his efforts and assistance throughout this journey. I would also like to thank Dr Briony Gliddon for her guidance and support. I am grateful for her efforts and assistance. Thanks to Prof. Allison Cowin, Dr Zlatko Kopecki and Mr Damian Adams for their advice and assistance with some of the wound healing work. I would also like to thank Dr Jo Woodcock and Dr Claudine Bonder for their suggestions during our combined lab meetings throughout my candidature.

To the members within the Pitson group, thank you all for the help and support over the years. I appreciate the company and laughters during my time in the laboratory. It is my pleasure to have worked with all members of the Pitson group. I am particularly grateful to all the intelligent conversations I had over the years. Special thank goes to Paul Moretti and Julia Dobbins for their assistance in my studies. I would also like to thank all the staff and students whom I have the honour to meet at the Centre for Cancer Biology. All the mutual support we gave each other will be solely missed. Thanks should also go to the staff at the animal care facility for their help with looking after the animals.

I would like to thank my friends and family for all their support. Thanks for all the encouragement and support over the years. I would also take this opportunity to express my sincerest gratitude and biggest thank you to my parents, Hing Chan and Boon Hwa Tan, sister Yeesend Chan and brother Huater Chan. I am eternally grateful and honoured to be part of this wonderful family. Without them I would not be who I am today and could not have come this far. A special thank you to Pei Shan Lee who

has provided unconditional support in this journey. I am thankful for her care and concern over the years.

Finally, I would also like to thank the University of Adelaide and CRC for Biomarker Translation for providing financial support throughout my study.

Abstract

Sphingosine kinases (SKs) phosphorylate sphingosine to generate the bioactive lipid sphingosine 1-phosphate (S1P). SKs and S1P regulate a diverse range of cellular processes, including cell proliferation, survival, differentiation, migration, smooth muscle cell contraction, inflammation, cytoskeleton reorganisation and angiogenesis, mainly via the engagement of S1P to a family of five S1P-specific G protein-coupled receptors (GPCRs). As such, the SKs and S1P are involved in regulating a plethora of cellular processes that are known to be fundamental to wound healing.

The role of SK and S1P in cancer and other diseases including asthma, hypertension, atherosclerosis and allergy are well established. Notably, however, the direct role of the SKs and S1P in wound healing has not been previously examined in any detail. My studies sought to fill this gap in knowledge. Using a well-established mouse model of incisional wound healing, I have shown that $SK1^{-/-}$, $SK2^{-/-}$, $SK1^{-/-}$ $SK2^{+/-}$ mice healed at a slower rate compared to wildtype mice. This may be attributed to a decrease in cellular proliferation in the early steps of wound repair. These studies highlight the importance of SKs in the very complex process of wound healing. My studies also examined the role of a relatively uncharacterised protein, fibroblast growth factor receptor-1 oncogenic partner 2 (FGFR1OP2), in wound healing. FGFR1OP2 is a protein that was identified from a yeast two-hybrid screen for SK1 interacting proteins. Unpublished work performed from the Pitson laboratory has shown that FGFR1OP2 can interact and activate SK1 in cells and *in vitro*. As such, we have more appropriately named this protein SKAM1 (Sphingosine Kinase Activating Molecule 1). SKAM1 has previously been reported to be upregulated following tooth extraction in rat oral mucosa. The Pitson laboratory has shown that

overexpression of SKAM1 induced collagen matrix contraction, an *in vitro* model of wound contraction, was mediated by SK1 and the S1P receptors, $S1P_{1/3}$.

Using a number of classical *in vitro* models of wound healing, I found that overexpression of SKAM1 in NIH3T3 fibroblasts did not affect cellular migration and proliferation. Notably, however, NIH3T3 fibroblasts overexpressing SKAM1 were resistant to serum deprivation-induced apoptosis. We also generated SKAM1 transgenic mice, where SKAM1 was ubiquitously expressed, to study the role of this protein in wound healing *in vivo*. We found no observable phenotypical difference between SKAM1 transgenic and wildtype mice at 12 and 48 weeks of age. Primary mouse embryonic fibroblasts (MEFs) isolated from SKAM1 transgenic embryos showed enhanced ability to contract collagen matrix compared with the wildtype. Somewhat surprisingly, the rate of wound healing following incisional wounding was similar between SKAM1A transgenic and wildtype mice. Notably, however, SKAM1A transgenic mice showed enhanced wound resolution compared with the wildtype following full-thickness excisional wounding. In addition, SKAM1 gene-trap mice with conditional potential have also been successfully generated and provide a tool for the study of the effect of SKAM1A knockout in wound healing *in vivo*.

The Pitson laboratory previously showed that a 35 amino acid peptide of SKAM1, $SKAM1^{71-105}$, can surprisingly still activate SK1 and enhance collagen matrix contraction. Further to this I have shown that a 30 amino acid cell-permeable peptide of SKAM1, TAT-$SKAM1^{76-105}$, was able to directly activate recombinant SK1 *in vitro* and when applied to cells. Notably, this effect was blocked by a mutant version of this peptide Tyr104→Phe mutation. Furthermore, NIH3T3 fibroblasts treated with TAT-$SKAM1^{76-105}$ showed enhanced collagen contraction, and more

importantly, intradermal injection of TAT-SKAM1A$^{76\text{-}105}$ into full-thickness excisional wounds resulted in enhanced wound resolution in mice. Neither of these effects was observed with the mutant peptide. Taken together, my findings suggest a potential therapeutic use of this peptide for the enhancement of wound repair.

In summary, my findings have demonstrated for the first time a novel role of SK and SKAM1 in wound healing. Knowledge gained from this study will be valuable for the development of potential new therapeutics for the improvement of wound healing.

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List of abbreviations

ABC	ATP binding cassette
ALX	Alloxan
AML	Acute myeloid leukaemia
Ang1	Angiopoietin 1
APN	Australian phenomics network
BrdU	5-bromo-2-deoxyuridine
BSA	Bovine serum albumin
BVDV	Bovine viral diarrhoea virus
CIB1	Calcium- and integrin-binding protein
CMV	Cytomegalovirus
CPP	Cell permeable peptides
CTGF	Connective tissue growth factor
DAPI	4', 6-diamidino-2-phenylindole
DMEM	Dulbecco's modified eagle medium
DMS	*N*, *N*-dimethylsphingosine
DTT	Dithiothreitol
EB	Extraction buffer
ECL	Enhanced chemiluminescence
ECM	Extracellular matrix
eEF1A	Eukaryotic elongation factor 1A
EGF	Epidermal growth factor
EGFP	Enhanced green fluorescent protein
ER	Endoplasmic reticulum
ERK	Extracellular regulated kinase

ES	Embryonic stem
FCS	Fetal calf serum
FDA	Food and drug administration
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
FGFR1OP2	Fibroblast growth factor receptor-1 oncogenic partner 2
FITC	Fluorescein isothiocyanate
GAPDH	Glyceraldehyde phosphate dehydrogenase
GPCR	G protein-coupled receptor
GST	Glutathione-S-transferase
H/E	Haematoxylin and eosin
HDAC	Histone deacetylase
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
IGF	Insulin-like growth factors
IGTC	International gene trap consortium
IL	Interleukin
IP	Intra-peritoneal
IRES	Internal ribosomal entry site
JNK	c-Jun N-terminal kinase
MEF	Mouse embryonic fibroblast
MMP	Matrix metalloproteinases
NOD	Non-obese diabetic
OPA	O-phthalaldehyde
PA	Phosphatidic acid

PBS	Phosphate buffered saline
PCNA	Proliferating cell nuclear antigen
PDGF	Platelet-derived growth factor
PHB	Prohibitin
PI3P	Phosphatidylinositol-3-phosphate
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
PP2A	Protein phosphatase 2A
PS	Phosphatidylserine
PTD	Protein transduction domain
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
PTI-1	Prostate tumour inducer-1
PTK	Protein tyrosine kinase
rSK	Purified recombinant SK
S1P	Sphingosine 1-phosphate
$S1P_{1-5}$	Sphingosine 1-phosphate receptors 1-5
S1PP	Sphingosine 1-phosphate phosphatase
SDS	Sodium dodecyl sulphate
SIKE	Suppressor of IKappa kinase ε
SK	Sphingosine kinase
SKAM	SK activator molecule
SKi	2-(*p*-hydroxyanilino)-4-(*p*-chlorophenyl)thiazole
SKIP	SK interacting protein

SMA	Smooth muscle actin
SMP	Skim milk powder
Sph	Sphingosine
STZ	Streptozotocin
TASQ	Transgenic animal service of Queensland
TAT	Transactivator of transcription
TF	Tissue factor
TGF	Transforming growth factor
TIMP	Tissue inhibitor of metalloproteinases
TLC	Thin-layer chromatography
TNF	Tumour necrosis factor
TRAF	TNF receptor-associated factor
VCAM	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor
vWF	Von Willebrand factor
Wit3.0	Wound inducible transcript of 3.0kb