

The Role of Sphingosine Kinases and SKAM1 in Cutaneous Wound Healing

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Statement of Authorship

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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 Unpublished work performed from the Pitson laboratory has shown that FGFR1OP2 can interact and activate SK1 in cells and *in vitro*.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, SKAM1⁷¹⁻¹⁰⁵, 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-SKAM1⁷⁶⁻¹⁰⁵, 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-SKAM1⁷⁶⁻¹⁰⁵ showed enhanced collagen contraction, and more

importantly, intradermal injection of TAT-SKAM1A⁷⁶⁻¹⁰⁵ 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.

Table of Contents

Declaration.....	2
Acknowledgement	4
Abstract.....	6
List of tables.....	15
List of abbreviations	16
CHAPTER 1 General Introduction	20
1. General introduction	21
1.1 Impaired wound healing: a significant health burden.....	21
1.2 The process of cutaneous wound healing	22
1.2.1 Inflammatory phase	22
1.2.2 Proliferative phase	24
1.2.3 Remodeling and resolution phase	25
1.3 Sphingosine kinase and sphingosine 1-phosphate: new potential mediators of wound healing.....	27
1.3.1 SK and S1P in cellular signalling	27
1.3.2 S1P signalling	28
1.3.3 SK1 and S1P in wound healing	35
1.3.4 SK/S1P in tissue fibrosis.....	39
1.4 Post-translation modification of SKs	41
1.5 Fibroblast growth factor receptor-1 oncogenic partner 2 (FGFR1OP2): A novel protein involved in wound healing	52
1.5.1 FGFR1OP2	52
1.5.2 FGFR1OP2 in wound healing.....	53
1.6 Biochemistry of FGFR1OP2	54
1.7 Aims and hypothesis of the thesis.....	61
1.7.1 Hypothesis.....	61
1.7.2 Aims.....	61
CHAPTER 2 Materials and Methods	63
2. Materials and methods	64
2.1 Cell culture and transfection	64

2.2 Protein assays.....	64
2.3 <i>In vitro</i> scratch wound assay.....	65
2.4 <i>In vitro</i> fibroblast-populated collagen contraction assay.....	65
2.5 Cell proliferation and apoptosis assays.....	66
2.6 RNA preparation and cDNA synthesis.....	67
2.7 Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)	67
2.8 Western blotting.....	68
2.9 Protein extraction from mouse tissues.....	68
2.10 Sphingosine kinase assays.....	69
2.11 Animal ethics.....	70
2.12 Full-thickness incisional wound healing study in mice.....	70
2.13 Full-thickness excisional wound healing study in mice.....	71
2.14 Generation of SKAM1A gene-trap mice.....	71
2.15 Generation of transgenic mice.....	72
2.15.1 Generation of <i>pCX-FLAG/SKAM1A-IRES-EGFP</i> construct.....	72
2.15.2 Generation of SKAM1A transgenic mice.....	73
2.15.3 Screening for SKAM1A transgenic mice.....	74
2.16 Focus formation assay.....	74
2.17 Isolation of primary mouse embryonic fibroblasts (MEFs).....	74
2.18 Extraction of S1P from plasma for high performance liquid chromatography (HPLC).....	75
2.19 Histology, immunohistochemistry and image analysis.....	76
2.20 Histological analysis of SKAM1A transgenic mice.....	77
2.21 Effect of cell permeable SKAM peptides on SK1 activity <i>in vitro</i>	77
2.22 Delivery of TAT fusion peptides into NIH3T3 cells.....	78
2.23 Effect of cell permeable SKAM peptides on NIH3T3 fibroblast-mediated collagen matrix contraction.....	78
2.24 Delivery of SKAM peptides into full-thickness excisional wounds.....	78
CHAPTER 3 The Role of Sphingosine Kinases in Wound Healing.....	80
3. The role of sphingosine kinases in wound healing.....	81
3.1 Abstract.....	81
3.2 Introduction.....	82
3.3 Preliminary data leading to the project.....	83
3.3.1 SK1 overexpression enhances fibroblast-mediated collagen contraction...83	
3.3.2 Genetic ablation or chemical inhibition of SK1 results in reduced collagen contraction.....	83
3.3.3 The S1P _{1/3} antagonist VPC23019 blocks SK1-induced collagen contraction.....	83
3.4 Results.....	88
3.4.1 SK knockout mice and relative plasma S1P levels.....	88
3.4.2 SK deficiency impairs wound healing.....	90
3.4.3 Proliferation is impaired in SK deficient wounds.....	95
3.4.4 Number of proliferating fibroblasts is decreased in SK1 ^{-/-} wounds.....	98
3.4.5 Collagen I expression is not affected in SK deficient wounds.....	101
3.4.6 Myofibroblasts numbers are not affected in SK deficient wounds.....	104

3.5 Discussion	107
CHAPTER 4 Characterisation of the Role of SKAM1 in Wound Healing	112
4. Characterisation of the role of SKAM1 in wound healing.....	113
4.1 Abstract	113
4.2 Introduction.....	115
4.3 Preliminary data: Targeting SK or S1P _{1/3} blocks SKAM1-induced collagen contraction.....	116
4.4 Results.....	119
4.4.1 SKAM1 is upregulated during wound healing <i>in vivo</i>	119
4.4.2 SKAM1 expression appears to be regulated by TGFβ1 and PDGF	122
4.4.3 SKAM1 overexpression does not affect cell migration.....	124
4.4.4 SKAM1 overexpression prevents serum-deprivation-induced apoptosis.	124
4.4.5 SKAM1 overexpression does not affect cellular proliferation	128
4.4.6 SKAM1 overexpression does not result in neoplastic transformation.....	128
4.4.7 Generation of mouse models to study the role of SKAM1A in wound healing.....	131
4.4.8 Analysis of SKAM1A transgenic mice.....	145
4.5 Discussion	166
CHAPTER 5 Effect of a Cell Permeable Peptide, TAT-SKAM1A⁷⁶⁻¹⁰⁵, on Wound Healing.....	175
5. Effect of a cell permeable peptide, TAT-SKAM1A⁷⁶⁻¹⁰⁵, on wound healing ..	176
5.1 Abstract	176
5.2 Introduction.....	178
5.3 Results.....	184
5.3.1 Generation of a cell-permeable version of the SKAM1A ⁷⁶⁻¹⁰⁵ peptide	184
5.3.2 TAT-SKAM1A ⁷⁶⁻¹⁰⁵ directly increases SK1 activity <i>in vitro</i>	184
5.3.3 TAT-SKAM1A ⁷⁶⁻¹⁰⁵ treatment enhances cellular SK1 activity.....	189
5.3.4 TAT-SKAM1A ⁷⁶⁻¹⁰⁵ treatment enhances collagen contraction by NIH3T3 fibroblasts.....	189
5.3.5 TAT-SKAM1A ⁷⁶⁻¹⁰⁵ treatment enhances wound resolution in mice.....	195
5.4 Discussion	199
CHAPTER 6 General Discussion	207
Chapter 6: General discussion.....	208
6.1 SK/S1P: New players in wound healing.....	208
6.1.1 SK1 and SKAM1 in wound healing	212

6.2 FGFR1OP2/SKAM in wound healing	217
6.3 SKAM1A transgenic and gene-trap mice	218
6.4 Topical application of a cell-permeable SKAM peptide as a treatment for aberrant wound healing.....	223
6.5 Murine models of cutaneous wound healing: Incisional and excisional models of wound healing.....	226
6.6 Differences between murine and human wound healing.....	228
6.7 Wound healing versus cancer: SK1 and SKAM1	230
6.8 Conclusion, significance and future directions	231
References.....	233
Appendix 1.....	257

List of Figures

CHAPTER 1

Fig. 1.1 Signalling of S1P ₁₋₅	34
Fig. 1.2 SKAM1 isoforms.....	56
Fig. 1.3 All naturally occurring SKAM1 isoforms interact with SK1.....	58
Fig. 1.4 All isoforms of SKAM1 activate SK1 in cells and <i>in vitro</i>	60

CHAPTER 3

Fig. 3.1 SK1 enhances fibroblast-mediated collagen contraction.....	86
Fig. 3.2 SK1-induced contraction is mediated by S1P _{1/3}	87
Fig. 3.3 Plasma S1P levels of wildtype, SK1 ^{-/-} , SK2 ^{-/-} and SK1 ^{-/-} SK2 ^{+/-} mice.....	89
Fig. 3.4 SK deficient mice have impaired wound healing.....	94
Fig. 3.5 Proliferation is impaired in SK deficient wounds	97
Fig. 3.6 Number of proliferating fibroblast is reduced in SK1 ^{-/-} wounds.....	100
Fig. 3.7 Collagen I expression is not affected in SK deficient wounds.....	103
Fig. 3.8 Myofibroblasts expression is not affected in SK deficient wounds	106

CHAPTER 4

Fig. 4.1 SKAM1-induced collagen contraction is mediated by SK1.....	117
Fig. 4.2 Targeting S1P _{1/3} blocked SKAM1-induced collagen contraction by NIH3T3 cells	118
Fig. 4.3 SKAM1 was upregulated during wound healing <i>in vivo</i>	121
Fig. 4.4 SKAM1 expression appears to be regulated by TGFβ1 and PDGF.....	123
Fig. 4.5 SKAM1 overexpression does not affect cell migration.....	127
Fig. 4.6 SKAM1A overexpression protects NIH3T3 fibroblasts against serum-deprivation-induced apoptosis but does not affect cellular proliferation.....	129
Fig. 4.7 NIH3T3 cells stably expressing SKAM1A did not acquire a neoplastic transformation phenotype.	130
Fig. 4.8 Schematic of the 'knockout-first' conditional allele.	134
Fig. 4.9 Identification of three SKAM1A gene-trap mice by PCR	135
Fig. 4.10 Restriction map of pCX-FLAG/SKAM1A-IRES-EGFP	137
Fig. 4.11 pCX-FLAG/SKAM1A-IRES-EGFP is highly expressed in transiently transfected NIH3T3 fibroblasts.....	139

Fig. 4.12 SalI-BamHI DNA fragment is expressed in NIH3T3 fibroblasts.....	141
Fig. 4.13 Three transgenic positive founder lines were identified by PCR.....	143
Fig. 4.14 Germline transmission was observed in the F1 progeny.....	144
Fig. 4.15 SKAM1A is ubiquitously expressed in transgenic mice.....	146
Fig. 4.16 SKAM1A transgenic mice display normal phenotype.....	155
Fig. 4.17 SKAM1A transgenic MEFs have enhanced collagen contraction.....	159
Fig. 4.18 SKAM1A transgenic mice showed slightly reduced S1P levels in plasma	160
Fig. 4.19 Wildtype and SKAM1 transgenic mice healed at a similar rate following incisional wounding.....	163
Fig. 4.20 Excisional wound healing is enhanced in SKAM1A transgenic mice.....	165

CHAPTER 5

Fig. 5.1 SKAM1 peptides retain the ability to interact with and enhance SK1 activity <i>in vitro</i>	181
Fig. 5.2 Sequence alignment of SKAM1A and SKAM2.....	182
Fig. 5.3 NIH3T3 cells overexpressing EGFP-SKAM1A ⁷¹⁻¹⁰⁵ , but not EGFP- SKAM1A ^{71-105(Y104F)} , enhance collagen contraction.....	183
Fig. 5.4 TAT-SKAM1A ⁷⁶⁻¹⁰⁵ and TAT-SKAM1A ^{76-105(Y104F)} are cell permeable.....	187
Fig. 5.5 TAT-SKAM1A ⁷⁶⁻¹⁰⁵ , but not TAT-SKAM1A ^{76-105(Y104F)} , directly activates recombinant SK1 <i>in vitro</i>	188
Fig. 5.6 TAT-SKAM1A ⁷⁶⁻¹⁰⁵ enhances endogenous SK1 activity in NIH3T3s and MEFs.....	191
Fig. 5.7 SKAM1A mRNA expression appears to be lower in NIH3T3 fibroblasts compared with MEFs.....	192
Fig. 5.8 TAT-SKAM1A ⁷⁶⁻¹⁰⁵ , but not TAT-SKAM1A ^{76-105(Y104F)} , enhances NIH3T3 fibroblast-mediated collagen contraction <i>in vitro</i>	194
Fig. 5.9 Intradermal injection of TAT-SKAM1A ⁷⁶⁻¹⁰⁵ , but not TAT-SKAM1A ^{76- 105(Y104F)} into mouse excisional wounds enhanced wound resolution.....	197

List of tables

CHAPTER 4

Table 4.1 Whole blood analysis of wildtype and SKAM1A transgenic mice. 161

CHAPTER 5

Table 5.1 Cell-permeable peptides of SKAM1..... 198

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	<i>N, N</i> -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-(<i>p</i> -hydroxyanilino)-4-(<i>p</i> -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