Regulation of Sphingosine Kinase by Interacting Proteins

Tamara Marie Leclercq

School of Molecular and Biomedical Science, Discipline of Biochemistry

The University of Adelaide

February 2010

Declaration by Author

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 to Tamara Marie Leclercq and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis (listed below) resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Leclercq,T.M. & Pitson,S.M. (2006). Cellular signalling by sphingosine kinase and sphingosine 1-phosphate. IUBMB Life. 2006. *58*, 467-472. Review

Leclercq,T.M., Moretti,B.B., Vadas,M.A. and Pitson,S.M. (2008). Eukaryotic elongation Factor 1A interacts with sphingosine kinase and directly enhances its catalytic activity. J. Biol. Chem. 2008. *283*, 9606-9614

Tamara Leclercq

Acknowledgements

First thanks must go to my supervisor, Dr Stuart Pitson. Thank you for giving me the opportunity to work and study in your laboratory. Your role as my early career mentor will forever be appreciated and you tireless efforts towards my projects, and of course in this thesis, will not be forgotten.

Secondly to my wonderful parents. They say you can't choose your family, but I couldn't have chosen any better anyway. This thesis symbolizes the culmination of many years of study, through which your unwavering love and support have seen me through. Mum, thanks for all the dinners, and dad, thanks for all the scrabble and yahtzee challenges, they were welcome breaks. I love you both very much.

I would also like to thank all my family and friends. Although you may think you have not played a role in this achievement, time spent together having fun and relaxing have kept me sane! Particular thanks to the girls in the laboratory (you know who you are), I'm sure science is sending us mad, but working with you has been an absolute pleasure.

I could not complete this acknowledgements page without mentioning my wonderful husband. You proposed to me at the beginning of my Phd and I wonder if you knew what you were getting yourself into! Whenever I needed to talk or needed someone to encourage me to keep going, you were always there. Your ability to make me laugh in any situation has saved me from destroying my laptop on several occasions! You are truly my better half, and I thank and love you for loving me.

Last but by no means least, I would like to thank you, the examiners. I know examining this thesis must be an arduous task and I genuinely appreciate it. Regular red wine breaks are highly recommended!

Abstract

Sphingosine kinase 1 (SK1) is responsible for phosphorylating the lipid sphingosine, generating the bio-active phospholipid, sphingosine 1-phosphate (S1P). Cells possess basal SK1 activity which has been proposed to serve in a 'housekeeping' function to limit the levels of pro-apoptotic sphingosine and ceramide in the cell. In some circumstances, however, such as cell exposure to growth factors and cytokines this basal level of SK1 activity is increased, resulting in an increased production of S1P. As S1P is a pro-proliferative, pro-survival molecule, its increased production is associated with enhanced cell proliferation, survival and an oncogenic phenotype.

The Pitson laboratory has shown previously that one mechanism by which SK1 is activated is through phosphorylation at Ser-225 by ERK1/2. Here, my studies focused on alternative mechanisms of SK1 activation that arise through its interaction with two proteins, eukaryotic elongation factor 1A (eEF1A) and a relatively uncharacterised protein, SK activator molecule 1 (SKAM). eEF1A is able to directly increase the catalytic activity of SK1 *in vitro* and is also able to increase endogenous SK activity when over-expressed in quiescent cells that have reduced levels of endogenous eEF1A protein. Due to the abundance of eEF1A protein within a cell, I hypothesized that the effect of eEF1A on SK activity may be dynamically regulated. eEF1A contains a 'G protein-like' domain that enables it to bind GDP and GTP. When bound by GTP, eEF1A undergoes a large conformational change that enables it to bind aminoacyl-tRNA for transport to the ribosome. Similarly, just as the nucleotide-bound state of eEF1A regulates its role in protein synthesis, I found that the nucleotide-bound state of eEF1A also regulates its ability to activate SK1. Strikingly, it is only the translationally inactive eEF1A.GDP that can activate SK1. A truncated form of eEF1A named PTI-1 has been described that lacks the 'G protein-like' domain and thus can not bind guanine nucleotides, rendering it structurally analogous to eEF1A.GDP. In keeping with my finding that only eEF1A.GDP activates SK1, I found that PTI-1 also activates SK1 both *in vitro* and in cells. Importantly, PTI-1 has been previously characterized as an oncoprotein and for the first time my studies have shown a likely mechanism by which PTI-1 induces a tumourigenic phenotype. Expression of PTI-1 in NIH 3T3 cells induces neoplastic transformation, as measured by focus formation. Notably, this PTI-1-induced transformation is blocked when cells are treated with

SK inhibitors or when cells are co-transfected with PTI-1 and a dominant negative SK1, indicating that oncogenesis by PTI-1 is mediated through SK1.

The current study also investigated the regulation of SK1 activity by its interaction with SKAM1. Previous studies have shown that SKAM1, like eEF1A, can directly increase the catalytic activity of SK1 *in vitro* and in cells. My studies have determined the minimal region of interaction of SKAM1 that is still able to interact with and activate SK1. Remarkably, a 35 amino acid SKAM1 peptide retained the ability to activate SK1. The physiological relevance of the SK1-SKAM1 interaction was also examined and I have shown that knock-down of SKAM1, and the related protein SKAM2, in HEK 293T cells resulted in decreased cell proliferation coupled with increased susceptibility to apoptosis. Results presented here, also suggest that phosphorylation of SKAM1 at Tyr-46 acts as a negative regulator for SKAM1-induced SK1 activation.

In summary, the current study presents two novel SK1 interacting proteins that directly increase the catalytic activity of this enzyme, and investigates mechanisms by which their effects on SK1 activity are regulated. While the guanine nucleotide bound state of eEF1A1 determines its effects on SK1 activity, the phosphorylation status of SKAM1 appears to determine its ability to activate SK1.

Table of Contents

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Tables

Chapter 2

Chapter 4

List of Abbreviations

AAD	adult atopic dermatitis
AC	adenylyl cyclase
AML	acute myeloid leukaemia
aa-tRNA	aminoacyl-transfer ribonucleic acid
Acy1	aminoacylase 1
BMMC	bone marrow-derived mast cells
BrdU	bromodeoxyuridine
CDP	calcium dependent protein
Cer	ceramide
CHO	Chinese hamster ovarian
CIB1	calcium and integrin binding protein 1
DAPI	4'-6-Diamidino-2-phenylindole
DMS	dimethyl-sphingosine
eEF1A	eukaryotic elongation factor 1A
eEF1B	eukaryotic elongation factor 1B
EGF	epidermal growth factor
EPG	ethanolamine-phosphoglycerol
ER	endoplasmic reticulum
ERK	extracellularly regulated kinase
FBS	foetal bovine serum
FGFR1	fibroblast growth factor receptor 1
FGFR1OP2	fibroblast growth factor receptor 1 oncogenic partner 2
FHL2	four-and-a-half LIM only protein-2
FLNa	Filamin A
GAP	GTPase-activating protein
GDP	guanine di-phosphate
GDI	guanine nucleotide dissociation inhibitor

GEF	guanine nucleotide exchange factor
GPCR	G-protein coupled receptors
GSH	glutathione
GST	glutathione-*s*-transferase
GTP	guanine tri-phosphate
HA	haemmaglutinin
HDAC1/HDAC2	histone deacetylase 1 / histone deacetylase 2
HDL	high density lipoprotein
HEK	human embryonic kidney
Hrg	heregulin
HuMC	human peripheral blood-derived mast cells
FcεRI	immunoglobulin E
MS	mass-spectrometry
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
PAK1	p21-activated kinase 1
PECAM-1	platelet endothelial cell adhesion molecule 1
PI4K	phosphatidylinositol 4-kinase
PKC	protein kinase C
PKD	protein kinase D
PLCγ	phospholipase Cγ
PMS	phenazine methosulfate
PP2A	protein phosphatase 2A
Prdx-I	peroxiredoxin I
PTI-1	prostate tumour inducer-1
rSK or rec-SK	purified recombinant SK
TCTP	translationally controlled tumour protein
TNF-α	tumour necrosis factor α
TRAF2	TNFα receptor associated factor 2

SK	sphingosine kinase
SKAM	sphingosine kinase activator molecule
SKIP	sphingosine kinase interacting protein
S1P	Sphingosine 1-phosphate
$S1P_{1-5}$	sphingosine 1-phosphate receptors 1-5
S1PL	sphingosine 1-phosphate lyase
S1PP	sphingosine 1-phosphate phosphatase
Sph	sphingosine
VECAM-1	vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor
VEGFR2	vascular endothelial growth factor receptor 2
Wit3.0	wound inducible transcript 3.0