Gene Regulation by Sphingosine kinase

Duyen H. Pham

Centre for Cancer Biology, SA Pathology and
Discipline of Biochemistry, School of Molecular and Biomedical Science

University of Adelaide

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ABBREVIATIONS

ABC;	ATP binding cassette
AC;	Adenylate cyclase
AD;	Alzheimer disease
ADP;	Adenosine-5'-diphosphate
ApoE;	Apolipoprotein E
ApoM;	Apolipoprotien M
AREs;	AU-rich mRNA destabilizing elements
ATP;	Adenosine-5'-triphosphate
AML;	Acute myeloid leukaemia
BAL;	Bronchoalveolar lavage
BH3;	Bcl-2 homology 3
BrdU;	5-bromo-2-deoxyuridine
BSA;	Bovine serum albumin
Cdc42;	Cell division cycle 42
CerS;	Ceramide synthase
CIB1;	Calcium and integrin binding protein 1
C1P;	Ceramide-1-phosphate
CML;	Chronic myeloid leukaemia
CNS;	Central nervous system
COX-2;	Cyclooxygenase 2
DAPI;	4',6-diamidino-2-phenylindole
DEPC;	Diethylpyrocarbonate
DMS;	*N,N*-dimethylsphingosine
Dox;	Doxycycline
DTT;	Dithiothreitol
EAE;	Encephalomyelitis
EC;	Endothelial cell
eEF1A;	Eukaryotic elongation factor 1A
EGF;	Epidermall growth factor
EGFP;	Enhanced green fluorescent protein

eNOS;	Endothelial nitric oxide synthase
ER;	Endoplasmic reticulum
ERK1/2;	Extracellular signal regulated kinase 1/ 2
eYFP;	Enhanced yellow fluorescent protein
FACS;	Fluorescence associated cell sorter
FBS;	Foetal bovine serum
FcϵRI;	High-affinity receptor for IgE
FDR;	False discovery rate
FHL-2;	Four and a half LIM domains protein 2
FLNa;	Filamin A
GAP43;	Growth associated protein 43
GC;	Germinal centre
GDNF;	Glial cell line-derived neurotrophic factor
HDAC;	Histone deactetylases
HDL;	High density lipoproteins
HEK293;	Human embryonic kidney 293 cells
HRP;	Horseradish peroxidase
HSPs;	Heat shock proteins
IRES;	Internal ribosome entry site
IRPs;	Iron regulatory proteins
LDL;	Low density lipoproteins
LPA;	Lysophosphatidic acid
LPP;	Lipid phosphate phosphatase
LPS;	Lipopolysaccharide
MAPK;	Mitogen-activated protein kinase
MiRNA;	microRNA
MMP;	Matrix metalloproteinase
MS;	Multiple sclerosis
MTs;	Metallothioneins
NES;	Nuclear export signal
NGF;	Nerve growth factor

NO;	Nitric oxide
OVA;	Ovalbumin
Ox-LDL;	Oxidised LDL
PA;	Phosphatidic acid
PBS;	Phosphate buffered saline
PCR;	Polymerase chain reaction
PDGF;	Platelet-derived growth factor
PECAM-1;	Platelet endothelial cell adhesion molecule
PGE2;	prostaglandin E2
PHB2;	Prohibitin 2
PI3K;	Phosphatidylinositol 3-kinase
PLC;	Phospholipase C
PMA;	Phorbol 12-myristate 13-acetate
PS;	Phosphatidylserine
PS2;	Presenilin 2
qPCR;	Quantitative real-time PCR
rtTA;	Reverse tetracycline-responsive transcriptional activator
siRNA;	Small interfering RNA
SDS;	Sodium dodecyl sulfate
SDS-PAGE;	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SKs;	Sphingosine kinases
SKIP;	SK1-interacting protein
SMP;	Skim milk powder
S1P;	Sphingosine 1-phosphate
$S1PR_{1-5}$;	Sphingosine 1-phosphate receptors 1-5
SPP1/2;	S1P phosphatases 1/ 2
SPT;	Serine palmitoyltransferase
SREBP;	Sterol regulatory element binding protein
SSC;	Saline-sodium citrate
Tet;	Tetracycline
TetR;	Tet-repressor

Tf;	Transferrin
TFR1;	Transferrin receptor 1
TGFβ;	Transforming growth factor-β
TIMP;	Tissue inhibitor of metalloproteinase
TNF-α;	Tumour necrosis factor-α
TRAF2;	TNF receptor-associated factor 2
TRE;	Tet-responsive element
VEGF;	Vascular endothelial growth factor
VCAM;	Vascular cell adhesion molecule
VSMC;	Vascular smooth muscle cells

ABSTRACT

Sphingosine kinases (SKs) are lipid kinases that catalyse the phosphorylation of sphingosine to form sphingosine-1-phosphate (S1P), a bioactive phospholipid that plays important roles in a wide variety of cellular processes, including calcium mobilisation, proliferation, apoptosis, angiogenesis, inflammatory responses and cytoskeletal rearrangement. Two SK isoforms exist in mammals, termed SK1 and SK2, which originate from different genes, but possess a high degree of sequence similarity. Although the two enzymes utilise the same substrate, sphingosine, to generate S1P, surprisingly, studies have suggested that SK1 and SK2 may have opposing cellular functions, with SK1 inducing cell survival and SK2 appearing to promote apoptosis. However, the molecular mechanisms mediating these apparently divergent roles for the two SKs have not been extensively examined at present. Furthermore, mouse knockout studies have suggested the two enzymes may have at least some overlapping functions.

There is strong evidence implicating SK1 in crucial role(s) in the development and progression of tumourigenesis. However, the mechanism whereby this enzyme induces tumourigenic processes is less clear and remains an important question to be answered in the field. Although high levels of intracellular S1P appears to have a role in regulation of cell proliferation and survival, various observations also suggest a role for extracellular S1P in cell surface G protein-coupled receptor-mediated cell proliferation and survival. However, the specific downstream pathways mediating this oncogenic signalling by SK1 are still poorly defined.

In attempts to answer these questions, studies to date have mainly focused on elucidating the cellular signalling pathways that are transiently modulated following SK1 activation. Considerable evidence suggests that SK1 is transcriptionally upregulated in many human cancers and also that its product, S1P, can induce activation of various transcription factors to regulate transcription of other genes. While this type of cellular regulation by SK1 is likely to play an important role in tumourigenesis, no studies have yet been published that systematically examined the molecular mechanisms whereby enhanced SK1 levels lead to oncogenesis. Thus, the main aim of the studies outlined in this thesis was to elucidate the genes regulated by increased cellular SK activity that may be important for normal and pathological cellular regulation.

In order to do this, we generated cell lines with tight doxycycline-inducible expression of SK1 and SK2 via a novel approach that involves the incorporation of AU-rich mRNA destabilizing elements (AREs) into the 3' untranslated regions of the tetracycline-inducible constructs. Use of these tightly controlled SK inducible systems allowed us to perform DNA microarrays and microRNA arrays to elucidate genes and microRNAs regulated soon after a moderate increase in cellular SK levels (approximately 10- and 6-fold overexpression of SK1 and SK2, respectively). This was done to maximise the likelihood of observing direct downstream effects of physiologically relevant increased SK expression that may have been missed by very high constitutive SK expression. While no microRNA regulation was observed following SK1 expression, screening of the Compugen human 19,000-oligonucleotide library, lead to the identification of various genes that were regulated by either SK1 or SK2 or by both enzymes. Of the various SK-regulated genes identified, transferrin receptor 1 (TFR1) was examined in greater detail in this study since its upregulation has been reported in various human cancers, and implicated in tumourigenic progression.

Here, we demonstrate a novel mechanism whereby SK1 regulates cell survival, proliferation and neoplastic transformation through upregulation of TFR1 expression. We show that elevated levels of SK1 enhanced total as well as cell-surface TFR1 expression resulting in increased transferrin (Tf) uptake into the cells. We also found that SK1 phosphorylation and/or translocation to the plasma membrane, which have been shown previously to be critical for SK1-mediated oncogenic effects, are necessary for regulation of TFR1 expression. Furthermore, we also demonstrated that S1P receptor 2 ($S1P_2$) is essential for SK1-induced TFR1 expression through the use of a $S1P_2$-specific inhibitor and siRNA knock-down of $S1P_2$. Finally, we show that blocking TFR1 function with a neutralizing antibody attenuated SK1-induced cell proliferation, survival and transformation. Together, these findings suggest that TFR1 plays an important role in oncogenesis mediated by SK1.

DECLARATION

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 Duyen Hong Pham 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.

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