The role of serum and glucocorticoid inducible kinase 3 in the regulation of cell growth and malignant transformation

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Abstract

The most well established downstream effector of phosphatidylinositol 3-kinase (PI3-K) signalling is the v-akt murine thymoma viral oncogene homolog/protein kinase B (AKT/PKB) kinase family, with many studies highlighting the critical importance of this family in normal cell physiology, including cell growth, proliferation and survival, in addition to disease states such as cancer. However, more recently AKT-independent PI3-K signalling pathways have been reported, including signalling via serum and glucocorticoid inducible kinase (SGK) 3, a member of the SGK family of serine/threonine kinases. The three SGK kinases (SGK1, -2, -3) share 54% structural homology with the AKT kinases in the catalytic domain, and have shown to be similarly activated in a PI3-K-dependent manner via 3-phosphoinositide-dependent kinase 1 (PDK1). Moreover, the SGK and AKT families share many of the same downstream targets including glycogen synthase kinase 3 beta (GSK3β), forkhead transcription factor 3a (FOXO3a), and Bcl-2 associated death promoter (BAD). The level of similarity existing between these two kinase families suggests possible functional redundancy, however studies using single isoform AKT and SGK knockout mice suggests isoform specific signalling. Furthermore distinct differences in cellular localisation between these kinases make it more likely that they each have important and specific roles.

In addition to the recent studies demonstrating that SGK3 is likely to be an important factor involved in AKT-independent malignant cell transformation, earlier studies in our laboratory demonstrated that the SGK3 isoform revealed increased transcript expression in a panel of ovarian tumour cells compared with SGK1 and SGK2, making SGK3 an interesting candidate to further characterise in both normal cell physiology and tumourigenesis.

The AKT family has been widely reported as a key downstream effector of PI3-K signalling to cell growth, thus this thesis firstly aimed to examine a possible role for SGK3 in cell growth and proliferation. Using multiple SGK3 gain-of-function epithelial and fibroblast cell lines, studies presented here revealed a strong role for SGK3 in signalling to components of the cell growth pathway, regulating macromolecular (RNA and protein) content, cell size, and regulating ribosomal-DNA (rDNA) transcription. Furthermore, using the mammalian target of rapamycin complex 1 (mTORC1) inhibitor rapamycin, these studies were also able to demonstrate that SGK3 is able to regulate cell growth in a largely mTORC1-dependent manner. Moreover, studies presented herein were also able to explore the importance of SGK3's reported endosomal localisation, demonstrating SGK3 functions optimally to influence cell growth when localised at the endosomal compartment.

Following the identification of SGK3 as an important modulator of cell growth, the second aim was to investigate the influence of SGK3 on malignant cell transformation and tumourigenesis. These studies utilised multiple pre-tumourigenic genetically defined SGK3 gain-of-function cell lines to obtain functional readouts for a variety

of well-established hallmarks of tumourigenesis, including anchorage-independent growth, cell migration and chemoresistance. Moreover, these studies incorporated the use of AKT gain-of-function cell lines in order to make comparisons between the ability of SGK3 and AKT to promote tumourigenic hallmarks. Results from these studies revealed both SGK3 and the AKT isoforms to promote anchorage-independent growth, in addition to a role for AKT in promoting cell migration, suggesting that in addition to the AKT family, SGK3 is also an important effector of malignant transformation.

Finally, the third aim of this project was to extend functional studies to global gene expression analysis, in an attempt to further define mechanisms associated with SGK3 function, and identify possible novel associations existing between SGK3 and other factors. Global gene analysis was conducted using Affymetrix genechip microarrays of all SGK3 and AKT gain-of-function cell lines used for functional studies. Through the use of two different bioinformatic approaches, including the MetaCore™ platform by GeneGo, along with Gene Set Enrichment Analysis (GSEA) from the Broad Institute, all data was interrogated to determine novel associations with SGK3, in addition to determining possible mechanisms associated with readouts observed in earlier functional analyses. These studies revealed not only known associations with SGK3, but also novel associations including demonstrating a possible role for SGK3 in the immune response, in addition to possible involvement in lysophosphatidic acid (LPA) pathway signalling. Moreover, these gene expression studies enabled comparison of differential gene regulation between both SGK3 and the AKT isoforms.

In summary, studies presented in this thesis demonstrate for the first time an important role for SGK3 in regulating cell growth via regulation of rDNA transcription, which is likely to be largely dependent on mTORC1. Furthermore, SGK3 also appears to play a critical role in the regulation of malignant transformation, which is consistent with recent reports demonstrating a role for SGK3 in AKT-independent PI3-K oncogenic signalling. Additionally, global gene expression studies allowed for the detection of novel connections with SGK3 including a role for SGK3 in the immune response. In summary this thesis furthers our understanding of the role of SGK3 in health and disease and provides an important platform that can be used as a basis for future investigations into SGK3 function.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Maressa A Bruhn

Preface

This thesis acknowledges the attributions from the following people for the experiments listed below:

Figure 3.3 and Figure 3.5 was completed in collaboration with Megan Astle.

Figure 3.9 was completed in collaboration with Karen Sheppard

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Figure 4.7 was completed in collaboration with Rob Southgate

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Publications arising from this thesis

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Bruhn MA, Riddell K, Abbott C, Pearson RB, Hannan RD, Sheppard KE. *A potential role for SGK3, an AKT related kinase, in promoting cell transformation.* 3rd Barossa Meeting Science Amongst the Vines 2007.

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Abbreviations

Amino acid abbreviations

Ala	A	Alanine
Arg	R	Arginine
Asn	N	Asparagine
Asp	D	Aspartic acid (Aspartate)
Cys	C	Cysteine
Gln	Q	Glutamine
Glu	E	Glutamic acid (Glutamate)
Gly	G	Glycine
His	H	Histidine
Ile	I	Isoleucine
Leu	L	Leucine
Lys	K	Lysine
Met	M	Methionine
Phe	F	Phenylalanine
Pro	P	Proline
Ser	S	Serine
Thr	T	Threonine
Trp	W	Tryptophan
Tyr	Y	Tyrosine
Val	V	Valine
Asx	B	Aspartic acid or Asparagine
Glx	Z	Glutamine or Glutamic acid
Xaa	X	Any amino acid

Other abbreviations

-/-	knockout
+/-	heterozygous
∞	affinity
Ω	ohms
μF	microfarad
μg	micrograms
μl	microlitres
μM	micromolar
4EBP1	eukaryotic initiating factor 4E (eiF4E)-binding protein 1
5'ETS	5' externally transcribed spacer region
5'TOP	5' oligopyrimidine tract
AGC	Cyclic AMP-dependent protein kinase, cyclic GMP-dependent protein kinase and protein kinase C family
AIP4	atrophin-1 interacting protein 4
AKT	v-akt murine thymoma viral oncogene homolog
AKTi	AKT inhibitor
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
APC	adenomatous polyposis coli
APOD	apolipoprotein D
AR	androgen receptor
ASCT2	sodium-dependent neutral amino acid transporter type 2
ATF6	activating transcription factor 6
ATM	ataxia telangiectasia mutated

ATP	adenosine triphosphate
ATR	ataxia telangiectasia and Rad3 related
BAD	Bcl-2 associated death promoter
BBC3	bcl-2-binding component 3
Bcl-2	B-cell leukemia/lymphoma 2
BH3	Bcl-2 homology domain 3
BIM	bcl-2-like protein 11
BMK-1	big MAP kinase
BMP7	bone morphogenetic protein
B-Raf	v-raf murine sarcoma viral oncogene homolog B1
BSA	bovine serum albumin
C3	complement component 3
CA	constitutively active
$CaCl_2$	calcium chloride
CaMKK	calmodulin-dependent protein kinase kinase
cAMP	cycline adenosine monophosphate
CCND1	cyclin D1
CD36	cluster of differentiation 36
CDC42	cell division cycle 42 (GTP binding protein, 25kDa)
CDK	cyclin dependent kinases
cDNA	complementary DNA
CFTR	the cystic fibrosis transmembrane conductance regulator Cl^- channel
Chk1	checkpoint kinase 1
CHIP	C-terminus of Hsc (heat shock cognate protein) 70-interacting protein
CIP	cyclin dependent kinase (CDK)-inhibitor
CISK	cytokine independent survival kinase
ClC-Ka	renal and inner ear Cl^- channel
ClC2	ubiquitous Cl^- channel
CLDN1	caludin-1
CO_2	carbon dioxide
COL14A1	collagen type XIV alpha 1
CPM	counts per minute
CRAC	Orai1 the pore-forming unit of Ca^{2+} -release-activated- Ca^2 channel
CREB	camp response element binding protein
c-Rel	reticuloendotheliosis viral oncogene homolog
CST	cell signaling technology
C-terminal	carboxyl-terminal
CXCL12	chemokine (C-X-C motif) 12
CXCR4	chemokine (C-X-C motif) receptor 4
dATP	deoxyadenosine triphosphate
DAPI	4',6-diamidino-2-phenylindole
DDIT4	DNA-damage-inducible transcript 4
DEPC	diethyl pyrocarbonate
DISC	death-inducing signalling complex
DMEM	Dulbecco's modified eagle medium
DMSO	dimethyl sulphoxide
DN	dominant negative
DNA	deoxy ribonucleic acid
dNTPs	deoxyribonucleotide triphosphates
DSP	desmoplakin
DTT	dithiothreitol
EAAT1/2/3/4/5	excitatory amino-acid transporters
ECM	extracellular matrix

EDTA	ethylenediaminetetraacetic acid
EEA1	early endosomal antigen 1
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
eIF4E	eukaryotic initiating factor 4E
eiF4G	eukaryotic initiation factor 4G
EMT	epithelial-mesenchymal transition
ENac	epithelial sodium channel
ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase family member 2/Autotaxin
ER	endoplasmic reticulum
ERK	extracellular signal-related kinase
ES	embryonic stem cells
ETS	externally transcribed spacer
FACS	fluorescence-activated cell sorting
FAK	focal adhesion kinase
FasL	Fas Ligand
FAST	forkhead activin signal transducer
FBS	foetal bovine serum
Fbw7	of F-box/WD repeat containing protein 7
FC	fold change
FDR	false discovery rate
FGF	fibroblast growth factor
fL	femtolitres
FLI-I	flightless-I
FOXO3a	Forkhead transcription factor
FSH	follicle stimulating hormone
FXR	farnesoids
FYVE	Fab1/YOTB/2K632.12/Vac1/EEA1
GβL	G protein β-subunit-like protein
GFP	green fluorescent protein
GILZ1	glucocorticoid-induced leucine zipper protein-1
GJA5	connexin 40
GLUT	glucose transporter
GM-CSF	granulocyte/macrophage colony stimulating factor
GPCR	G protein coupled receptors
GR	glucocorticoid receptor
GRE	glucocorticoid response element
GSEA	gene set enrichment analysis
GSK3β	glycogen synthase kinase β
GTC	guanidine thiocyanate
HA	haemagglutinin
HD	Huntington's disease
HDAC	histone deacetylase
HEK293	human embryonic kidney 293 cells
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HER2	human epidermal growth factor receptor 2
HERG	human ether-a-go-go
HGF	hepatic growth factor
HIF	hypoxia inducible factor
HM	hydrophobic motif
HMEC	human mammary epithelial cells
H-Ras	v-Ha-ras Harvey rat sarcoma viral oncogene homolog
HOSE	human ovarian surface epithelial
HPV	human papilloma virus

Hr	hour
HRP	horseradish peroxidase
HSF	heat shock factor
Hsp90	heat shock protein 90
hVps34	human vacuolar sorting protein 34
IFI6	interferon alpha-inducible protein 6
IFN	interferon
IGF	insulin-like growth factor
IGFR	insulin growth factor receptor
IKKβ	I kappa B kinase beta
IL-1	interleukin 1
IL-2	interleukin 2
IL-3	interleukin 3
IL-6	interleukin 6
ILK	integrin-linked kinase
IOSE523	immortalised ovarian surface epithelial 523
IRES	internal ribosome entry site
ISGF3	interferon-stimulated gamma factor 3
ITS	internally transcribed spacer
JNK	c-Jun N-terminal kinase
JUP	plakoglobin
K^+	potassium
KCNQ1/KCNE	cardiac and epithelial K^+ channels
KCNQ4	inner ear K^+ channels
KD	kinase dead
KO	knockout
kV	kilovolts
KV1.3, KV1.5	voltage-gated K^+ channels and Kv4.3
kDa	kilodalton
KIP	kinase inhibitor proteins
LAMC1	laminin 1
LB	luria broth
LIPC	hepatic triacylglycerol lipase
LIPE	endothelial lipase
LKB1	liver kinase B1
LPA	lysophosphatidic acid
LPAR3	lysophosphatidic acid receptor 3
LPXN	Leupaxin
LT	simian virus 40 large T antigen
LTR	long terminal repeat
M	Molar (with respect to solution concentrations)
MAPK	mitogen activated protein kinase
MAPK3	mitogen-activated protein kinase 3
MBD2	methy-CpG binding domain protein 2
Mdm2	murine double minute 2
MEFs	mouse embryonic fibroblasts
MEK1/2	mitogen-activated protein kinase kinase 1/2
MEKK3	mitogen-activated protein kinase kinase kinase 3
Min	minute
MKK3	mitogen-activated protein kinase kinase 3
ml	millilitres
MLLT11	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog Drosophila) translocated to 11
mLST8	mammalian lethal with sec thirteen

mm	millimetre
MMP	matrix metalloproteinase
MQ	millique
mRNA	messenger RNA
mSin1	mammalian stress activated protein kinase-interacting protein
MSCV	murine stem cell virus
MSigDB	Molecular signatures database
MTM1	myotubularin 1
mTOR	mammalian target of rapamycin
mTORC1	mammalian target of rapamycin complex 1
mTORC2	mammalian target of rapamycin complex 2
MTT	thiazolyl blue tetrazolium bromide
Myr	myristolated
Na^+	sodium
NaAc	sodium acetate
NaCl	sodium chloride
NaDC-1	Na^+-dicarboxylate cotransporter
Na_2HPO_4	disodium hydrogen phosphate
NaPiIIb	Na^+ dependent Pi cotransporter
NFκB	nuclear factor κB
NHE3	sodium-hydrogen antiporter 3
NHERF2	Na^+/H^+ exchanger regulating factor 2
NLS	nuclear localisation signal
nM	nanomolar
NP-40	nonyl phenoxypolyethoxylethanol
Nt	nucleotide
N-terminal	amino-terminal
P	phosphorylated
PAS	phospho-AKT substrate
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDGF	platelet derived growth factor
PDGFR	platelet derived growth factor receptor
PDK1	3-phosphoinositide-dependent kinase 1
pg	picograms
PH	Pleckstrin homology
PI	propidium iodide
PI3-K	phosphatidylinositol 3-kinase
PIF	PDK1 interacting fragment
PIKK	phosphoinositide 3-kinase-related kinase
PIP2	phosphatidylinositol-3,4-biphosphate
PIP3	phosphatidylinositol-3,4,5-triphosphate
PIPES	piperazinethanesulfonic acid
PKA	protein kinase A
PKB	protein kinase B
PKC	protein kinase C
PKG	protein kinase G
PLA2	phospholipase A2
PLAU	plasminogen activator urokinase
PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1
PMCC	peter maccallum cancer centre
pMIG	MSCV-IRES-GFP
pMIC	MSCV-IRES-Cherry
PMSF	phenylmethylsulfonyl fluoride
Pol I	RNA polymerase I

Pol II	RNA polymerase II
Pol III	RNA polymerase III
PP2A	protein phosphatase 2A
PPARγ	peroxysome proliferator activator receptor gamma
PRAS40	proline rich AKT substrate of 40 kDa
PRICKLE2	prickle homolog 2 (Drosophila)
PTEN	phosphatase and tensin homolog deleted on chromosome 10
PtdIns	inositol-containing lipids
PtdIns(3)P	phosphatidylinositol 3-phosphate
PtdIns(4)P	PtdIns-4-phosphate
$PtdIns(4,5)P_2$	PtdIns-4,5-bisphosphate
$PtdIns(3,4,5)P_3$	phosphatidylinositol 3,4,5-trisphosphate
p-val	P-value
PVDF	polyvinylidene fluoride
PX	phox homology
Rac1	Ras-related C3 botulinum toxin substrate 1
Rb	retinoblastoma protein
rDNA	ribosomal DNA
RFP	cherry fluorescent protein
Rheb	Ras homolog enriched in brain
RLB	rac lysis buffer
RNA	ribonucleic acid
RNAi	interfering RNA
r-proteins	ribosomal proteins
ROMK1	renal outer medullary K^+ channel
RPA	ribonuclease protection assay
rpS6	ribosomal protein S6
rRNA	ribosomal RNA
RTK	receptor tyrosine kinases
RXR	retinoids
S6K1	S6 kinase 1
SAPK	stress-activated protein kinase
SCN5A	cardiac voltage gated Na^+ channel
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate – polyacrylamide gel
SEM	standard error of the mean
SERPINE2	serpin peptidase inhibitor clade E (nexin plasminogen activator inhibitor type 1) member 2
SGK	serum and glucocorticoid inducible kinase
SGLT1	Na^+-glucose cotransporter
SH2	src homology domain
SILAC	stable isotope labelling by amino acids in cell culture
siRNA	small interfering RNA
SLC14A1	solute carrier family 14 (urea transporter) member 1 (Kidd blood group)
SLC6A8	sodium- and chloride-dependent creatine transporter 1
SLC6A15	solute carrier family 6 (neutral amino acid transporter) member 15
SMAD	mothers against decapentaplegic homolog
SMIT1	hypertonicity-activated myo-inositol transporter
SN1	system N1
SOC	super optimal broth with catabolite repression
SOX4	SRY (sex determining region Y)-box 4
SPARC	osteonectin
SREBP	sterol regulatory element binding protein
ST	simian virus 40 small T antigen

STAT	signal transducers and activators of transcription
SV40	simian virus 40
TBS	tris buffered saline
TE	tris-EDTA
TGF-β	transforming growth factor beta
TRPV5	renal epithelial Ca^{2+} channel
TSC1	tuberous sclerosis complex 1
TSC2	tuberous sclerosis complex 2
UBF	upstream binding factor/upstream binding transcription factor RNA polymerase I
UTR	untranslated region
UVB	ultraviolet B
VDR	vitamin D receptor
VEGF	vacular endothelial growth factor
WNK	with no lysine K
WSB	western solubilisation buffer