

PKB/Akt

Key References

- Brazil, D.P., et al., Advances in protein kinase B signaling: AKTion on multiple fronts., *Trends Biochem. Sci.*, **29**, 233-242 (2004).
- Coghlan, M.P., et al., Selective small molecule inhibitors of glycogen synthase kinase-3 modulate glycogen metabolism and gene transcription., *Chem. Biol.*, **7**, 793-803 (2000).
- Davies, S.P., et al., Specificity and mechanism of action of some commonly used protein kinase inhibitors., *Biochem. J.*, **351**, 95-105 (2000).
- Downward, J., PI 3-kinase, Akt and cell survival., *Semin. Cell Dev. Biol.*, **15**, 177-182 (2004).
- Franke, T.F., et al., PI3K/Akt and apoptosis: size matters., *Oncogene*, **22**, 8983-8998 (2003).
- Harris, T.E., et al., TOR signaling., *Sci STKE*, **9**, re15 (2003).
- Jope, R.S. and Johnson, G.V., The glamour and gloom of glycogen synthase kinase-3., *Trends Biochem. Sci.*, **29**, 95-102 (2004).
- Li, Y., et al., TSC2: filling the GAP in the mTOR signaling pathway., *Trends Biochem. Sci.*, **29**, 32-38 (2004).
- Mora, A., et al., PDK1, the master regulator of AGC kinase signal transduction., *Semin. Cell Dev. Biol.*, **15**, 161-170 (2004).
- Pearce, D., SGK1 regulation of epithelial sodium transport., *Cell Physiol. Biochem.*, **13**, 13-20 (2003).
- Richardson, C.J., et al., PI3-kinase and TOR: PIKTIOR-ing cell growth., *Semin. Cell Dev. Biol.*, **15**, 147-159 (2004).
- Thomas, G., The S6 kinase signaling pathway in the control of development and growth., *Biol. Res.*, **35**, 305-313 (2002).

Overview

The PDK1–PKB/Akt axis represents one of the most actively researched cell signaling pathways. This protein kinase cascade is known to play a central role in mediating the actions of a range of stimuli including insulin, growth factors, integrins and GPCRs in addition to being involved in the regulation of cell survival, cellular metabolism (including insulin-stimulated glucose transport and glycogen synthesis), gene expression, cell cycle entry and protein synthesis.

All the kinases associated with this pathway lie in the protein serine/threonine kinase family and form a single highly branching protein kinase cascade (hence their grouping together). Several of these kinases contain pleckstrin homology (PH) domains that bind specific phosphoinositide lipids (e.g. phosphoinositide-3,4,5-trisphosphate; PIP₃) which are generated in the plasma membrane in response to agonist activity. As a result, the kinases are activated in a phosphoinositide 3-OH-kinase (PI3-kinase)-dependent manner.

3-Phosphoinositide-dependent protein kinase-1 (PDK1) stands at the head of this important signaling pathway. Whether extracellular stimuli directly activate PDK1 (perhaps via the generation of plasma membrane-localized PIP₃), or whether they simply induce the translocation of PDK1 to its substrate proteins within the plasma membrane, is not known. PDK1 activates a number of AGC-family protein kinases (named after their homology to protein kinases A, G and C) by phosphorylation, including protein kinase B (PKB or Akt) via phosphorylation of the T-loop residue Thr 308. The full activation of PKB/Akt also involves the binding of PIP₃ to the PH domain of PKB/Akt and the phosphorylation of an

additional residue, Ser 473, by an as yet unidentified kinase called “PDK2” (recently proposed to be DNA-dependent protein kinase: DNA-PK). There is a great deal of functional overlap between PKB/Akt isoforms; all phosphorylate the same RXRXXS/T motif and all are capable of transforming a cell when rendered constitutively active by the introduction of a myristoylation signal sequence.

Thr 308 and Ser 473 lie within regions of PKB/Akt that are conserved throughout the AGC family kinases. Hence, PDK1 also phosphorylates and activates several other AGC-family kinases, including the serum and glucocorticoid-induced kinases (SGK), atypical forms of protein kinase C (e.g. PKC ζ and PKC ι/λ), p70S6-kinase and p90RSK. PDK1 is therefore a central controller of multiple cell signaling pathways.

Once phosphorylated and activated, PKB/Akt phosphorylates and inhibits glycogen synthase kinase 3 (GSK3) leading to a decreased phosphorylation and activation of glycogen synthase. PKB/Akt also phosphorylates the mammalian target of rapamycin (mTOR, also known as FRAP and RAFT) although the role of this action is not yet known. GSK3 continues to grow in importance as it also plays a role in the regulation of β -catenin stability and thus gene expression.

mTOR, which can be phosphorylated by PKB/Akt, is unusual in that it possesses both serine/threonine protein kinase as well as lipid kinase activities. It is a large complex molecule that is a receptor for the immunosuppressant, rapamycin. mTOR, along with PDK1, then plays an as yet ill-defined role in the activation of p70S6K which is important in the control of protein synthe-

sis, development and growth control. Thus, at least in part, the immunosuppressive activity of rapamycin is due to its actions on mTOR.

Other than rapamycin, there are few if any highly specific pharmacological inhibitors of this collection of protein kinases. This has made understanding the role of these protein kinases in mediating the effects of extracellular stimuli very difficult to ascertain. Furthermore, while in some cases kinase-dead derivatives of these kinases have been reported to act as dominant-negatives, this has often been highly controversial, largely because of their complex domain structures and abilities to interact with other proteins and signaling lipids. However, given the central importance of these protein kinases in numerous disease states (e.g. cancer and diabetes), the identification of specific inhibitors remains a very important goal.

PKB/Akt

FAMILY MEMBERS	PDK1	PKB/Akt	SGK
OTHER NAMES	3-Phosphoinositide protein kinase-1	RAC-protein kinase	Serum and glucocorticoid-induced kinase, CISK (SGK3)
MOLECULAR WEIGHT/ STRUCTURAL DATA	63 kDa 556 aa Monomer	56 kDa (All isoforms) PKB α : 480 aa PKB β : 481 aa PKB γ : 479 aa All monomers	SGK1: 49 kDa SGK2 α : 48 kDa SGK2 β : 41 kDa SGK3: 57 kDa SGK1: 431 aa SGK2 α : 367 aa SGK2 β : 427 aa SGK3: 496 aa All monomers
ISOFORMS	Not known	PKB α /Akt1, PKB β /Akt2, PKB γ /Akt3	SGK1, SGK2 α /2 β (splice variants), SGK3
SPECIES	All eukaryotes	All metazoans (kinases orthologous to PKB and SGK exist in fungi, plasmodium, dictyostelium)	All metazoans (kinases orthologous to PKB and SGK exist in fungi, plasmodium, dictyostelium)
DOMAIN ORGANIZATION	1 PH domain binds PtdIns(3,4,5)P ₃	1 PH domain binds PtdIns(3,4,5)P ₃ , PtdIns(3,4)P ₂	Protein kinase domain
PHOSPHORYLATION SITES	Ser ²⁴¹ (autophosphorylation)	PKB α sites, but conserved: Thr ³⁰⁸ , Ser ⁴⁷³ , Thr ⁴⁵⁰ (constitutive), Tyr ³¹⁵ , Tyr ³²⁶	SGK1 sites, but conserved: Thr ²⁵⁶ , Ser ⁴⁴² , Ser ⁷⁸
TISSUE DISTRIBUTION	Ubiquitous	Ubiquitous	Ubiquitous
SUBCELLULAR LOCALIZATION	Cytosolic, membranes	Cytosolic, membranes	Cytosolic, membranes (SGK3)
BINDING PARTNERS/ ASSOCIATED PROTEINS	Not known PKC ζ , IMPDH, Ft1, TCL1,	CTMP, APPL, Periplakin, POSH, HSP90, PKC θ , Brk,	Not known
UPSTREAM ACTIVATORS	Complex-see Overview	Phosphorylation by PDK1 and "PDK2" and binding of PIP3	Phosphorylation by PDK1 (SGK1 expression induced by glucocorticoids)

FOOTNOTES

^a Inhibitors in parentheses are non-selective or yet unproven to be highly specific.

PKB/Akt

DOWNSTREAM ACTIVATION	PKA (P5511, C8482, P2645), PKG, PKB/Akts, MSKs, atypical PKCs, p70S6Ks, p90RSKs (all are protein kinases)	GSK3, mTOR, Raf, IKK, BAD, eNOS, hCaspase-9 (C1099), hTERT, BRCA1, IRS-1, PFK2, FKHR, PRAS40, TSC2, AS160, PIKfyve, hdm-2, WNK1	FKHR, NDRG1, NDRG2, Nedd4-2, B-Raf
ACTIVATORS	Not known	Not known	Not known
INHIBITORS^a	(UCN-1)	(SH5), (API-2), (AKT-I-1; Akt-I-1.2)	Not known
SELECTIVE ACTIVATORS	Not known	Not known	Not known
PHYSIOLOGICAL FUNCTION	Cell proliferation, growth, apoptosis and metabolism	Cell proliferation, growth, apoptosis and metabolism	Regulation of Na ⁺ and Cl ⁻ channels
DISEASE RELEVANCE	Cancer, inflammation	Cancer, inflammation	Hypertension

FOOTNOTES

^a Inhibitors in parentheses are non-selective or yet unproven to be highly specific.

PKB/Akt

FAMILY MEMBERS	GSK3	mTOR	p70S6K (S6K)
OTHER NAMES	Glycogen synthase kinase 3 (G1663)	FRAP, RAFT, mammalian target of rapamycin	S6K1
MOLECULAR WEIGHT/ STRUCTURAL DATA	GSK3 α : 51 kDa GSK3 β : 48 kDa 483 aa β : 420 aa All monomers	289 kDa 2549 aa Monomer	α 1: 59 kDa α 2: 56 kDa β 1: 55 kDa β 2: 54 kDa α 1: 525 aa α 2: 502 aa β 1: 495 aa β 2: 482 aa All monomers
ISOFORMS	GSK3 α GSK3 β	Not known	p85 S6K α 1 = α 1, p70 S6K α 2 = α 2, (splice variants of the S6K1 gene), p60 S6K β 1 = β 1, p54 S6K β 2 = β 2, (splice variants of the S6K2 gene)
SPECIES	All eukaryotes	All eukaryotes	All metazoans
DOMAIN ORGANIZATION	Not known	16 HEAT domains, 1 PI3-kinase homology domain, 1 FKBP/rapamycin binding domain	1 autoinhibitory domain, 1 nuclear localization signal sequence on α 1, β 1 and β 2
PHOSPHORYLATION SITES	GSK3 α , but conserved in GSK3 β : Ser ²¹ (PKB and others), Tyr ²⁹⁶ (constitutive)	Ser ²⁴⁴⁸ , Ser ²⁴⁸¹ (autophosphorylation)	Sites α 2, but conserved: Thr ²²⁹ , Ser ³⁷¹ , Ser ³⁸⁹ , Ser ⁴¹¹ , Ser ⁴¹⁸ , Thr ⁴²⁴ , In α 2, conserved in α 1 only: Thr ⁴²¹
TISSUE DISTRIBUTION	Ubiquitous	Ubiquitous	Ubiquitous
SUBCELLULAR LOCALIZATION	Cytosolic, nuclear	Cytosolic	Cytosolic (α 2), Nuclear (α 1, β 1 and β 2)
BINDING PARTNERS/ ASSOCIATED PROTEINS	Frat-1/2/3, Presenilin, Axin, Axil, DLP, 14-3-3	FKBP12, Raptor, Rictor, Rheb, LST8	PP2A, Neurabin
UPSTREAM ACTIVATORS	Tyrosine phosphorylation (constitutive) Phosphorylation by PKB/Akt	Complex, but may include phosphorylation by PKB and regulation by TSC2/Rheb	Phosphorylation by PDK1, mTOR, and atypical PKCs

FOOTNOTES

a Inhibitors in parentheses are non-selective or yet unproven to be highly specific.

PKB/Akt

DOWNSTREAM ACTIVATION	Glycogen synthase (G1399), ATP-citrate lyase, eIF-2B, c-Jun, Myc, Myb, PKA (P5511 , C8482 , P2645), CREB, IκB, PP1 (P7937), tau (T7675 , T9392), NDRG1	4E-BP1, p70S6K	Ribosomal S6 subunit, SKAR
ACTIVATORS	Not known	Not known	Not known
INHIBITORS	SB-216763 (S3442), SB-415286 (S3567), (LiCl) (L0505), CT99021, AR-A014418	Rapamycin (R0395)	(H89) (B1427)
SELECTIVE ACTIVATORS	Not known	Not known	Not known
PHYSIOLOGICAL FUNCTION	Glycogen metabolism, gene expression, development	Protein synthesis, cell growth	Protein synthesis, cell growth
DISEASE RELEVANCE	Diabetes, metabolism	Cancer, immunosuppression	Cancer

Abbreviations

AR-A014418: N-(4-Methoxybenzyl)-N'-(5-nitro-1,3-thiazol-2-yl)urea

Brk: Breast tumor-related kinase

CTMP: C-terminal modulatory protein

CT 99021: 6-[2-[4-(2,4-dichlorophenyl)-5-(4-methyl-1H-imidazol-2-yl)-pyrimidin-2-ylamino]ethylamino]nicotinonitrile

DLP: Dynamin-like protein

DNA-PK: DNA-dependent protein kinase

FKBP12: FK502 binding protein

FRAP: FKBP12-rapamycin-associated protein

GSK3: Glycogen synthase kinase 3

H89: N-(2-[p-Bromocinnamylamino]ethyl)-5-isoquinolinesulfonamide

IMPDH: Inosine-5' monophosphate dehydrogenase

MSK: Mitogen- and stress-activated kinase

MTOR: Mammalian target of rapamycin

PIKfyve: FYVE domain containing phosphatidylinositol 3-phosphate 5-kinase

PKA: Protein kinase A

PKC: Protein Kinase C

PKG: Protein kinase G

POSH: Plenty of SH3 domains

PtdIns(3,4)P2: Phosphatidylinositol 3,4-bisphosphate

PtdIns(3,4,5)P3: Phosphatidylinositol 3,4,5-trisphosphate

RAFT: Rapamycin and FKBP12 target

Ro 31-8220: 2-[1-[3-Amidinothio]propyl]-1H-indol-3-yl]-3-(1-methylindol-3-yl)-maleimide

SB-216763: 3-(2,4-Dichlorophenyl)-4-(1-methyl-1H-indole-3-yl)-1H-pyrrole-2,5-dione

SB-415286: 3-(3-Chloro-4-hydroxyphenylamino)-4-(2-nitrophenyl)-1H-pyrrole-2,5-dione

SGK: Serum and glucocorticoid regulated kinase

TCL: T-cell leukemia

TSC2: Tuberous Sclerosis Complex-2

UCN-1: 7-Hydroxystaurosporine

FOOTNOTES

^a Inhibitors in parentheses are non-selective or yet unproven to be highly specific.