PERSPECTIVE

Serum and glucocorticoid inducible protein kinases (SGKs): a potential target for cancer intervention

Rajesh Basneta\textsuperscript{a,b},†, Grace Qun Gong\textsuperscript{a,c},†, Chenyao Li\textsuperscript{a,b}, Ming-Wei Wang\textsuperscript{a,b,d,*}

\textsuperscript{a}The National Center for Drug Screening and the CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences (CAS), Shanghai 201203, China
\textsuperscript{b}University of Chinese Academy of Sciences, Beijing 100049, China
\textsuperscript{c}Department of Molecular Medicine and Pathology, The University of Auckland, Auckland, New Zealand
\textsuperscript{d}School of Pharmacy, Fudan University, Shanghai 201203, China

Received 10 April 2018; received in revised form 3 June 2018; accepted 14 June 2018

Abstract The serum and glucocorticoid inducible protein kinase (SGK) family members share similar structure, substrate specificity and function with AKT and signal downstream of the phosphatidylinositol 3-kinase (PI3K) signalling pathway. They regulate a range of fundamental cellular processes such as cell proliferation and survival, thereby playing an important role in cancer development. This perspective intends to give an overview on the involvement of SGKs (particularly SGK3) in cancer progression, and compares the actions of SGK3 and AKT in cell cycle regulation, oncogenic signalling, and the potential as a therapeutic target for cancer.

© 2018 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Abbreviations: ER, estrogen receptor; mTORC2, mammalian target of rapamycin complex 2; PDK1, phosphoinositide-dependent kinase-1; PH, pleckstrin homology; PI3K, phosphatidylinositol 3-kinase; PX, Phox; SGK, serum and glucocorticoid inducible protein kinase

*Corresponding author at: The National Center for Drug Screening and the CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences (CAS), Shanghai 201203, China.
E-mail address: mwwang@simm.ac.cn (Ming-Wei Wang).
†These authors made equal contributions to this work.
Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

https://doi.org/10.1016/j.apsb.2018.07.001
2211-3835 © 2018 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1. Introduction

The phosphatidylinositol 3-kinase (PI3K) signalling pathway controls a range of fundamental cellular processes. The serum and glucocorticoid inducible protein kinase (SGK) family signals downstream of the PI3K pathway, and shares similar structure, substrate specificity and function with AKT. Like AKT, SGK is involved in the regulation of cell proliferation and survival. In addition, SGK also plays an important role in cancer development via an AKT-independent signalling pathway. In order to identify novel compounds capable of inhibiting SGK activities, a high-throughput screening campaign against one of the three SGK isoforms, namely SGK3, was carried out and a dozen of hits with IC_{50} values in the low micromolar to sub-micromolar range were subsequently discovered and characterized. Since SGK3 is less well-known among the scientific community, this perspective intends to give an overview on the role of SGK3 in cancer progression downstream of PI3K, and compares the potential roles of SGK3 and AKT in cellular regulation, oncogenic signalling, and the potential as a therapeutic target for cancer.

2. Structure and activation of SGKs

Dysregulation and hyperactivation of the phosphatidylinositol 3-kinase (PI3K) signalling pathway occurs frequently in many human cancers. It is one of the major pathways activated following growth factor stimulation; it activates a cascade of downstream signalling proteins and responses to control cell proliferation, survival, metabolism and migration. SGK is a family consisting of three isoforms: SGK1, SGK2, and SGK3 encoded by the genes SGK1, SGK2 and SGK3, respectively, and they are activated downstream of the PI3K pathway. The SGK isoforms are highly similar in structure, with almost 80% sequence identity within the catalytic domains and almost 50% within the C-terminus region. The major differences in structure between the isoforms are at the N-terminus. Specifically, SGK1 has four distinct variants which all differ in the N-terminal area. The presence of a six amino acid hydrophobic motif in the most abundant variant of SGK1 is responsible for its localization to the endoplasmic reticulum and degradation through the 26S proteasome. Both SGK2 and SGK3 produce two types of variants; however, the functional consequence of SGK2 and SGK3 variants are not yet understood. SGK1 and SGK3 isoforms are ubiquitously expressed, and SGK2 expression is restricted to the liver, kidney, pancreas, and brain. SGKs have two key regulatory sites: a Thr residue in the activation loop of the catalytic domain (Thr 320 in SGK3) and a Ser residue in the hydrophobic motif of the C-terminal domain.

In addition to phosphorylation, SGK1 expression can also be transcriptionally regulated and degraded by ubiquitination. SGK3 is phosphorylated at Thr 320 by phosphoinositide-dependent kinase-1 (PDK1), and mammalian target of rapamycin complex 2 (mTORC2) is proposed to phosphorylate SGK3 at Ser 486. SGK3 is distinct from the other two SGK isoforms and the AKT family: it has a Phox (PX) domain in the N-terminal region (amino acids 12–120) which is important for its protein kinase activity and responsible for targeting SGK3 to endosomal compartments and vesicle-like structures.

SGK3 endosomal membrane localization is required for complete kinase activity. Mutation of the PX domain prevents phospholipid binding and endosomal localization, and subsequently results in decreased SGK3 activity. Binding of PI(3)P to the PX domain promotes phosphorylation and activation of SGK3 by PDK1, however this dependence is lost after phosphorylation of the hydrophobic motif at the C-terminal region, suggesting that membrane binding via the PX domain is important to co-localize SGK3 and mTORC2, the kinase proposed to phosphorylate SGK3 at the hydrophobic motif. Activation of SGK3 is slower than AKT, implying that the endosomal location of SGK3 causes a delay in the activation process compared with activation of AKT at the plasma membrane. In addition, unlike AKT, association of SGK with the cell membrane is not essential for activation.

3. Structure and activation of AKT

The AKT family also has three isoforms: AKT1, AKT2, and AKT3. All three isoforms share a conserved structure that includes three functional domains: an N-terminal pleckstrin homology (PH) domain, a catalytic domain, and a C-terminal regulatory domain containing the hydrophobic motif (Fig. 1). AKT isoforms share the same substrate consensus phosphorylation motif and have similar structural and biological functions to that of the AKT family. AKT and SGK3 substrates control a range of cellular responses to growth factors and other extracellular stimuli including cell proliferation, survival, migration, metabolism, and angiogenesis. Given the similarity in structure and substrate specificity, the SGK family is also considered as a second AKT family in cancer signalling. AKT has two key regulatory sites, Thr 308 in the activation loop of the catalytic domain and Ser 473 in the C-terminal hydrophobic motif, and similar to SGK, both sites require phosphorylation for complete activation. AKT is phosphorylated at Thr 308 by PDK1 and at Ser 473 by mTORC2. AKT signals downstream of class 1A and 1B PI3K, which are activated by tyrosine kinase and G-protein-coupled receptors, respectively. Once activated, PI3K phosphorylates the 3 hydroxyl group of the inositol ring of PIP(3,4,5)P_3 to generate PI(3,4,5)P_3, which is important for its protein kinase activity and responsible for targeting SGK3 to endosomal compartments and vesicle-like structures.

4. SGK and AKT in cancer

Despite the critical role of AKT in tumor development, the function of downstream effectors that signal independently
(i.e., not mediated by AKT) has also emerged. AKT signalling is clearly diminished in many tumor cell lines, and instead, these cell lines are dependent on other signalling proteins such as SGK3\(^{21}\). Interestingly, there is mounting evidence to show the importance of other signalling factors downstream of PI3K that act independently of AKT to mediate crucial cell processes involved in malignant transformation\(^{1}\). The over expression of activated AKT is not enough to restore malignant phenotypes in PDK1 knockdown cells, suggesting there is a subset of tumors that are PI3K/PDK1-dependent but AKT independent\(^{22}\). The expression levels of SGK proteins have a key role in growth and development of tumors that are resistant to AKT inhibition, as Sommer et al.\(^{23}\) demonstrated that breast cancer cell lines expressing high levels of SGK1 were resistant to inhibition of AKT. Prolonged treatment with AKT inhibitors and class I PI3K inhibitors have been shown to upregulate SGK3, and dual treatment with AKT and SGK inhibitors reduces tumor growth in BT-474 xenograft model\(^{24}\). SGK3 is essential to cell viability in PIK3CA mutant cell lines with low AKT activation, indicating a functional dependency on SGK3 in these cells. Amplification and overexpression of SGK3 is more common than AKT in hepatocellular carcinoma, and forced expression of SGK3 was able to mediate increased cell growth, as well as anchorage independent growth in hepatocellular carcinoma\(^{25}\). Another study showed that microRNA miR-144-3p was able to inhibit cell proliferation, migration and angiogenesis by targeting SGK3 in hepatocellular carcinoma, further implicating the role of SGK3 in cancer development\(^{26}\). In addition, PDK1 knockdown decreased phosphorylation of SGK3 at Thr 320 in MCF-7 cells with low activation of AKT, but this effect was less in T47D cells with high activation of AKT\(^{21}\). Furthermore, estrogen receptor (ER) positive breast tumors display a positive correlation between SGK3 expression levels and tumor prognosis\(^{27,28}\), and SGK3 contributes to the resistance against aromatase inhibitors in ER positive breast cancer by maintaining endoplasmic reticulum homeostasis\(^{29}\). In addition, SGK3 is also

| Table 1 Some small molecule SGK inhibitors reported in the literature. |
|---|---|---|---|---|
| Inhibitor | Structure | GI50 (μmol/L) | SGK1 | SGK2 | SGK3 | AKT |
| I\(^{31}\) | ![Structure](image1) | 40 | Not available | Not available | Not available | Not available |
| 2\(^{31}\) | ![Structure](image2) | 63 | Not available | Not available | Not available | Not available |
| GSK650394\(^{33}\) | ![Structure](image3) | 62 | 103 | Not available | > 50-fold selectivity |
| 3\(^{33}\) | ![Structure](image4) | 138 | Not available | Not available | Not available | Not available |
| EMD638683\(^{39}\) | ![Structure](image5) | 85% inhibition at 1 μmol/L | 71% inhibition at 1 μmol/L | 75% inhibition at 1 μmol/L | 5% inhibition at 1 μmol/L |
| SI113\(^{40,41}\) | ![Structure](image6) | 600 | Not available | Not available | Not available | Not available |
| 4\(^{31}\) | ![Structure](image7) | 3/442* | 924* | 23,300 | Not available |
| 5\(^{31}\) | ![Structure](image8) | 1/41* | 128* | 3,100 | Not available |

*Tested using 500 μmol/L of ATP.
involved in androgen-mediated prostate cancer cell proliferation. Together, these point to the significance of SGK signaling independent of AKT in cancer pathogenesis and the potential application of SGKs as targets for cancer intervention.

5. Currently available SGK inhibitors

Given the implications of SGKs in cancer, a handful of SGK inhibitors have been discovered (Table 1) and a majority of them were tested against SGK1. These inhibitors bind to the ATP-site, hence inhibit the kinase activity of SGKs by competing with ATP and preventing its binding. Among them, 1 (PDB: 3HDM) and 2 (PDB: 3HDN) have been co-crystallized with SGK1, and this provided important insights into the critical protein-ligand interactions responsible for inhibitory activity. The azaindole core forms hydrogen bond donor-acceptor interactions with Asp 177 and Ile 179 in the linker. This is where the adenosine group of ATP interacts with the protein—a key site of the linker that is structurally conserved in many other kinases. Another important conserved residue is the catalytic Lys 127, which is responsible for interacting with the β phosphate of ATP, and both 1 and 2 interact with this catalytic lysine. GSK650394, analogous in structure to 1 and 2, is proposed to make similar interactions at the active site capable of reducing androgen-mediated LNCaP prostate cancer cell growth.

Another class of SGK inhibitors with a different scaffold reported by Merck represented by 3 was predicted to interact with the linker via a para-phenol group and with the catalytic lysine via a carbonyl group, respectively. A similar compound, EMD638683, was suggested to be a potential therapeutic agent for hypertension as it could decrease blood pressure in mice with hyperglycemia and salt excess. SI113 identified by Ortuso et al. was able to decrease the growth of RKO colon cancer, MCF-7 breast cancer and A-172 brain cancer cells. It is predicted to make hydrogen bond donor-acceptor interactions with Asp 177 and Ile 179 in the linker via a phenol group, and make π-stacking interactions with the catalytic lysine.

Due to the high homology between the SGK isoforms, especially in the catalytic domain, these inhibitors are not expected to be strongly selective for any of the SGK isoforms. Indeed, of those that have been tested in more than one isoform, only compounds 4 and 5 identified by Halland et al. were selective for SGK1 over SGK3, but not over SGK2 (Table 1). The number of SGK inhibitors available is rather limited, and information on their selectivity remains scarce. Therefore, there is a high demand for further characterization of available tool compounds, exploring both selectivity and key protein-ligand interactions, and the development of new SGK inhibitors.

6. Summary

The PI3K/AKT/mTOR signalling pathway is a major target for cancer therapy, especially those bearing PIK3CA mutations. SGK is a less explored target in the pathway and is suggested to play a major role in malignant transformation. SGK is activated downstream of PI3K and shares similar substrates with AKT, and is considered a second AKT in cancer signaling. SGK can also signal downstream of PI3K independent of AKT, contributing to resistance against AKT inhibition in cancer cell lines. The importance of SGKs in cancer development and the scarcity of potent and selective SGK inhibitors support the urgent need for discovery and development of small molecules inhibitors targeting SGK for PIK3CA mutant cancers, and especially those that are resistant to AKT inhibition. In order to achieve this goal, both conventional high-throughput screening campaigns against structurally diverse chemical libraries and computational biology-based virtual screens using available SGK 3-dimensional structure models are required. Cross-studies on the existing small molecule AKT inhibitors with the SGK inhibitors reported in the literature may deepen our understanding of the signalling mechanisms involved in the pathogenesis of various types of cancer and provide critical insights into the development of potent and selective SGK inhibitors.

Acknowledgments

This work was partially supported by grants from the Ministry of Science and Technology of China (2014DFG32200), Shanghai Science and Technology Development Fund (15DZ2291600) and the Thousand Talents Program in China ([2011]166). The funders had no role in study design, data collection and analysis, decision to publish, or manuscript preparation.

References

1. Gong GQ, Wang K, Dai XC, Basnet R, Yi Chen, Yang DH, et al. Identification, modification and characterization of potential small molecule SGK inhibitors with novel scaffolds. Acta Pharmacol Sin 2018. Available from: http://dx.doi.org/10.1038/s41401-018-0087-6.
2. Samuels Y, Ericson K. Oncoplastic PI3K and its role in cancer. Curr Opin Oncol 2006;18:77–82.
3. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science 2004;304:554.
4. Sheppard K, Kimross KM, Solomon B, Pearson RB, Phillips WA. Targeting PI3 kinase/AKT/mTOR signaling in cancer. Crit Rev Oncog 2012;17:69–95.
5. Firestone GL, Giampaolo JR, OKFee BA. Stimulus-dependent regulation of serum and glucocorticoid inducible protein kinase (SGK) transcription, subcellular localization and enzymatic activity. Cell Physiol Biochem 2003;13:1–12.
6. Bruhn MA, Pearson RB, Hannan RD, Sheppard KE. Second AKT: the rise of SGK in cancer signalling. Growth Factors 2010;28:394–408.
7. Tessier M, Woodgett JR. Serum and glucocorticoid-regulated protein kinases: variations on a theme. J Cell Biochem 2006;98:1391–407.
8. Kobayashi T, Deak M, Morrice N, Cohen P. Characterization of the structure and regulation of two novel isoforms of serum- and glucocorticoid-induced protein kinase. Biochem J 1999;344:189–97.
9. Tessier M, Woodgett JR. Role of the Phox homology domain and phosphorylation in activation of serum and glucocorticoid-regulated kinase-3. J Biol Chem 2006;281:23978–89.
10. Ellson CD, Andrews S, Stephens LR, Hawkins PT. The PX domain: a new phosphoinositide-binding module. J Cell Sci 2002;115:1099–105.
11. Liu D, Yang X, Songyang Z. Identification of CISK, a new member of the SGK kinase family that promotes IL-3-dependent survival. Curr Biol 2000;10:1233–6.
12. Brand Y, Levano S, Radojevic V, Nalid AM, Setz C, Ryan AF, et al. Akt isoforms (Akt1, Akt2, Akt3) are involved in normal hearing, but only Akt2 and Akt3 are involved in auditory
hair cell survival in the mammalian inner ear. *PLoS One* 2015;10:e0121599.

13. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. *Cell* 2007;129:1261–74.

14. Handa M, Feng J, Hemmings BA. Structure, regulation and function of PKB/AKT—a major therapeutic target. *Biochim Biophys Acta* 2004;1697:3–16.

15. Song G, Ouyang G, Bao S. The activation of Akt/PKB signaling pathway and cell survival. *J Cell Mol Med* 2005;9:59–71.

16. Arcaro A, Guerreiro AS. The phosphoinositide 3-kinase pathway in human cancer: genetic alterations and therapeutic implications. *Curr Genom* 2007;8:271–306.

17. Luo J, Manning B, Cantley L. Targeting the PI3K pathway: rationale and promise. *Cancer Cell* 2003;4:257–62.

18. Fruman DA, Meyers RE, Cantley LC. Phosphoinositide kinases. *Rev Biochem* 1998;67:481–507.

19. Cantley L. The phosphoinositide 3-kinase pathway. *Science* 2002;296:1655–7.

20. Katso R, Okkenhaug K, Ahmadi K, White S, Timms J, Waterfield MD. Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. *Annu Rev Cell Dev Biol* 2001;17:615–75.

21. Vasudevan KM, Barbie DA, Davies MA, Rabinovitch RS, McNear CJ, Kim H, et al. AKT-independent signaling downstream of oncogenic PIK3CA mutations in human cancer. *Cancer Cell* 2009;16:21–32.

22. Gagliardi PA, di Blasio L, Oro F, Scano G, Sessa R, Taverna D, et al. 3-phosphoinositide-dependent kinase 1 controls breast tumor growth in a kinase-dependent but AKT-independent manner. *Neoplasia* 2012;14:719–31.

23. Sommer EM, Dry H, Cross D, Guichard S, Davies BR, Alessi DR. Elevated SGK1 predicts resistance of breast cancer cells to Akt inhibitors. *Biochem J* 2013;452:499–508.

24. Bago R, Sommer E, Castel P, Crafter C, Bailey FP, Shpiro N, et al. The hVps34-SGK3 pathway alleviates sustained PI3K/AKT inhibition by stimulating mTORC1 and tumour growth. *EMBO J* 2016;35:1902–22.

25. Liu M, Chen L, Chan TH, Wang J, Li Y, Li Y, et al. Serum and glucocorticoid kinase 3 at 8q13.1 promotes cell proliferation and survival in hepatocellular carcinoma. *Hepatology* 2012;55:1754–65.

26. Wu M, Huang C, Huang X, Liang R, Feng Y, Luo X. MicroRNA-144-3p suppresses tumor growth and angiogenesis by targeting SGK3 in hepatocellular carcinoma. *Oncol Rep* 2017;38:2173–81.

27. Xu J, Wan M, He Q, Bassett Jr. RL, Fu X, Chen AC, et al. SGK3 is associated with estrogen receptor expression in breast cancer. *Breast Cancer Res Treat* 2012;134:531–41.

28. Wang Y, Zhou D, Phung S, Warden C, Rashid R, Chan N, et al. SGK3 sustains ERα signaling and drives acquired aromatase inhibitor resistance through maintaining endoplasmic reticulum homeostasis. *Proc Natl Acad Sci U S A* 2017;114:E1500–8.

29. Wang Y, Zhou D, Chen S. SGK3 is an androgen-inducible kinase promoting prostate cancer cell proliferation through activation of p70 S6 kinase and up-regulation of cyclin D1. *Mol Endocrinol* 2014;28:935–48.

30. Halland N, Schmidt F, Weiss T, Saas J, Li Z, Czech J, et al. Discovery of N-[4-(1H-pyrazolo[3,4-b]pyrazin-6-yl)-phenyl]-sulfonamides as highly active and selective SGK1 inhibitors. *ACS Med Chem Lett* 2015;6:73–8.

31. Ackermann TF, Boini KM, Beier N, Scholz W, Fuchtbauer T, Lang F. EMDF638683, a novel SGK inhibitor with antihypertensive potency. *Cell Physiol Biochem* 2011;28:137–46.

32. D’Antona L, Amato R, Artese A, D’Antona L, Costa G, Talarico C, et al. Identification and biological evaluation of novel selective serum/glucocorticoid-inducible kinase 1 inhibitors based on the pyrazolo-pyrimidine scaffold. *J Chem Inf Model* 2014;54:1828–32.

33. D’Antona L, Amato R, Talarico C, Ortuso F, Mennti M, Dattilo V, et al. SI113, a specific inhibitor of the SGK1 kinase activity that counteracts cancer cell proliferation. *Cell Physiol Biochem* 2015;35:2006–18.