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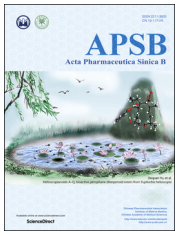

PERSPECTIVE

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

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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.

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

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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[1]. 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[2,3]. 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[4]. 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[5]. 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[6]. Both SGK2 and SGK3 produce two types of variants; however, the functional consequence of SGK2 and SGK3 variants are not yet understood[6]. SGK1 and SGK3 isoforms are ubiquitously expressed, and SGK2 expression is restricted to the liver, kidney, pancreas, and brain[7]. 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 C-terminal hydrophobic motif (Ser 486 in SGK3, Fig. 1), and phosphorylation of both sites are required for complete activation[5,7,8]. 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[9]. 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[9–11].

SGK3 endosomal membrane localization is required for complete kinase activity[9]. Mutation of the PX domain prevents phospholipid binding and endosomal localization, and subsequently results in decreased SGK3 activity[11]. Binding of PI(3)P to the PX domain promotes phosphorylation and activation of SGK3 by PDK1,

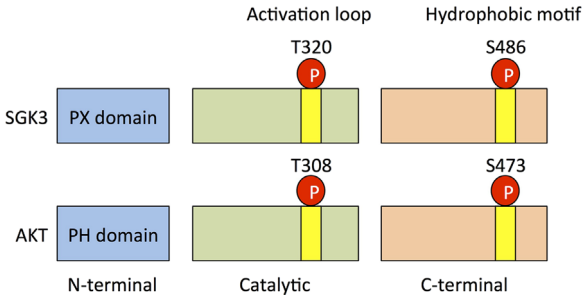


Figure 1 Domain organization of SGK3 and AKT. SGK3 and AKT share a common domain organization consisting of an N-terminal domain, a catalytic domain and a C-terminal domain. They also share similar phosphorylation sites: a Thr residue in the activation loop of the catalytic domain and a Ser residue in the hydrophobic motif of the C-terminal domain.

however this dependence is lost after phosphorylation of the hydrophobic motif at the C-terminal region[6], 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[7]. In addition, unlike AKT, association of SGK with the cell membrane is not essential for activation[9].

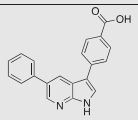
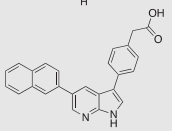
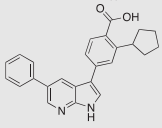
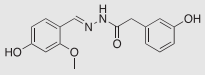
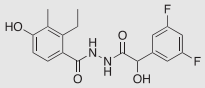
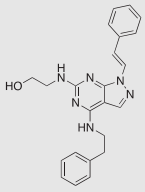
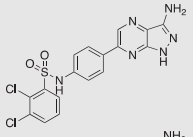
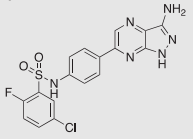
3. Structure and activation of AKT

The AKT family also has three isoforms: AKT1, AKT2, and AKT3[12,13]. All three isoforms share a conserved structure that includes three functional domains: an N-terminal pleckstrin homology (PH) domain, a central catalytic domain, and a C-terminal regulatory domain containing the hydrophobic motif (Fig. 1)[13,14]. SGK isoforms share the same substrate consensus phosphorylation motif and have similar structural and biological functions to that of the AKT family[6]. 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[6,7,13,15]. Given the similarity in structure and substrate specificity, the SGK family is also considered as a second AKT family in cancer signalling[6]. 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[16,17]. AKT is phosphorylated at Thr 308 by PDK1 and at Ser 473 by mTORC2[16]. AKT signals downstream of class 1A and 1B PI3K, which are activated by tyrosine kinase and G-protein-coupled receptors, respectively[18]. Once activated, PI3K phosphorylates the 3 hydroxyl group of the inositol ring of $PI(4,5)P_2$ to generate $PI(3,4,5)P_3$ at the lipid membrane[19,20]. AKT is then recruited to the plasma membrane when its PH domain binds to PIP_3, allowing phosphorylation at Thr 308 and partial activation of AKT by PDK1. AKT is fully activated when it is also phosphorylated by mTORC2 at Ser 473[15].

4. SGK and AKT in cancer

Despite the critical role of AKT in tumor development, the function of downstream effectors that signal independently

Table 1 Some small molecule SGK inhibitors reported in the literature.

Inhibitor	Structure	IC_{50} (nmol/L)			
		SGK1	SGK2	SGK3	AKT
1[31]		40	Not available	Not available	Not available
2[31]		63	Not available	Not available	Not available
GSK650394[33]		62	103	Not available	> 30-fold selectivity
3[34-38]		138	Not available	Not available	Not available
EMD638683[39]		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]		600	Not available	Not available	50,000
4[38]		3/442*	924*	23,300	Not available
5[38]		1/41*	128*	3,100	Not available

*Tested using 500 μmol/L of ATP.

(*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[6]. 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

involved in androgen-mediated prostate cancer cell proliferation[30]. Together, these point to the significance of SGK signalling 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[31]. 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[32], 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[33].

Another class of SGK1 inhibitors with a different scaffold reported by Merck[34–37] represented by **3** was predicted to interact with the linker *via* a *para*-phenol group and with the catalytic lysine *via* a carbonyl group[38], 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[39]. SI113 identified by Ortuso et al.[40] was able to decrease the growth of RKO colon cancer, MCF-7 breast cancer and A-172 brain cancer cells[41]. 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[40].

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.[38] 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 signalling. 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-dimentional 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.

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