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SGK1: The Dark Side of PI3K Signaling

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Abstract

Activation of the PI3K pathway is central to a variety of physiological and pathological processes. In these contexts, AKT is classically considered the de facto mediator of PI3K-dependent signaling. However, in recent years, accumulating data point to the existence of additional effectors of PI3K activity, parallel to and independent of AKT, that play critical and unique roles in mediating different developmental, homeostatic, and pathological processes.

In this review, I summarize and discuss our current understanding of the function of the serine/threonine kinase SGK1 as a downstream effector of PI3K, and try to separate targets and pathways validated as uniquely SGK1-dependent from those shared with AKT.

1. INTRODUCTION: THE GLUCOCORTICOIDREGULATED KINASE FAMILY

Serum- and glucocorticoid-regulated kinases (SGKs) are members of the AGC (PKA-, PKG-, PKC-related) family of serine/threonine kinases, one of the most evolutionarily conserved groups of protein kinases, represented in most eukaryotic organisms (Arencibia, Pastor-Flores, Bauer, Schulze, & Biondi, 2013). Well-known members of the AGC family are AKT, PDK1, S6K, PKC, and RSK. SGK kinases share greatest sequence homology with the AKT family (Pearce, Komander, & Alessi, 2010). The SGK family consists of three distinct but highly homologous isoforms (SGK1, SGK2, and SGK3) that are produced from three distinct genes localized on different chromosomes (Lang & Cohen, 2001).

Structurally, SGK kinases, as most AGC kinases, consist of three domains: an N-terminal variable region, a catalytic domain, and the C-terminal tail. SGKs are subject to tight spatial and temporal regulation, mainly through phosphorylation of two conserved residues, one in the activation loop contained in the kinase domain, and one in the hydrophobic motif within the C-tail, which is indispensable for full kinase activation (Pearce et al., 2010).

While the N-terminal region of some AGC kinases, such as AKT and PDK1, contains a phosphoinositide-binding pleckstrin homology (PH) domain, essential for kinase recruitment to membrane-bound phosphatidylinositol-3-phosphate, SGK1 and SGK2 have no recognizable N-terminal functional domain. On the other hand, unique in the family, SGK3 possesses an N-terminal phosphoinositide-binding Phox homology (PX) domain, which interacts with phosphatidylinositol-3-phosphate to mediate the endosomal association of SGK3, essential for its phosphorylation and activation (Tessier & Woodgett, 2006).

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2. SGK1: EXPRESSION AND STABILITY CONTROL

SGK isoforms are not equally expressed in all tissues. SGK2 expression is constitutive but restricted to the liver, pancreas, brain, and kidney proximal tubules (Kobayashi, Deak, Morrice, & Cohen, 1999; Pao et al., 2010). SGK3 is also constitutively expressed, but its expression is ubiquitous (Kobayashi et al., 1999).

On the other hand, expression of SGK1, while found in all tissues examined, is strictly transcriptionally and posttranscriptionally regulated. In fact, SGK1 was discovered as an immediate early gene, transcriptionally induced in rat mammary cancer cells by glucocorticoids and serum (Webster, Goya, Ge, Maiyar, & Firestone, 1993).

A multitude of stimuli, including growth factors (Mizuno & Nishida, 2001; Waldegger et al., 1999), mineralocorticoids (Naray-Fejes-Toth, Canessa, Cleaveland, Aldrich, & Fejes-Toth, 1999), cytokines (Fagerli et al., 2011), as well as various cellular stresses such as hyperosmotic cell shrinkage (Waldegger, Barth, Raber, & Lang, 1997), heat shock, ultraviolet irradiation, and oxidative stress (Leong, Maiyar, Kim, O'Keeffe, & Firestone, 2003), have been shown to induce SGK1 gene transcription. In addition, SGK1 mRNA has a short half-life, disappearing within 20 min from transcription (Waldegger et al., 1997).

A second level of tight control over SGK1 levels is represented by protein stability. SGK1 is polyubiquitinated and rapidly turned over, with a half-life of approximately 30 min (Brickley, Mikosz, Hagan, & Conzen, 2002).

The signals required for SGK1 degradation reside in the first 60 amino acids (Brickley et al., 2002). More specifically, a six amino acid motif devoid of lysines is required for polyubiquitination and rapid degradation by the 26S proteasome (Bogusz, Brickley, Pew, & Conzen, 2006). This process appears to involve different E3 ubiquitin ligases: SGK1 has been in fact reported to associate with the stress-associated, chaperone-dependent, U-box E3 ubiquitin ligase CHIP (Belova et al., 2006), with the ER-associated, transmembrane E3 ubiquitin ligase HRD1 (Arteaga, Wang, Ravid, Hochstrasser, & Canessa, 2006), with the HECT domain E3 ubiquitin ligase NEDD4L (Zhou & Snyder, 2005), and more recently, with a new E3 complex that includes Rictor, Cullin-1, and Rbx1 (Gao et al., 2010).

3. SGK1 IS ACTIVATED IN A PI3K-DEPENDENT MANNER

It was not until several years after SGK1 identification and characterization that a number of studies reported that SGK1 phosphorylation and activation was controlled by the PI3K signaling cascade (Kobayashi & Cohen, 1999; Park et al., 1999). These studies stemmed from the observation that the catalytic and C-terminal domains of SGK1 are highly homologous to those of other AGC kinases such as AKT, PKC, and S6K1, which had just been discovered to be phosphorylated and activated by PDK1 on a conserved residue in the activation loop. In fact, the PI3K inhibitor LY294002 was found to completely abolish insulin- and IGF-1-induced SGK1 activity in HEK293 cells. Furthermore, these studies demonstrated that PDK1 is directly responsible for SGK1 phosphorylation on Thr256, and that Ser422 in the hydrophobic domain is phosphorylated in response to PI3K activation,

likely by the same kinase that phosphorylates and activates AKT on Ser473 (Kobayashi & Cohen, 1999; Park et al., 1999).

Strikingly, these experiments also showed that, contrary to what is observed with AKT, the Ser422-to-Asp and Thr256-to-Asp SGK1 double mutant did not gain constitutive activity, a still unexplained feature shared with S6K1, which has significantly hindered progress on the delineation of SGK1 activity and role in cell models.

The identity of the kinase responsible for the PI3K-dependent phosphorylation of SGK1 remained mysterious for several years, until 2008, when the Alessi group, using genetic and biochemical approaches, identified mTORC2 as the complex phosphorylating SGK1 on Ser422 (Garcia-Martinez & Alessi, 2008).

While this finding provided clear evidence that the rapamycin-insensitive mTORC2 complex, already associated with AKT phosphorylation on Ser473, is the bona fide kinase for SGK1 Ser422, the question of the mechanism governing its dependence on PI3K activity still remained open. Very recently, however, the Wei laboratory has proposed a mechanism of mTORC2 activation that depends on the availability of phosphatidylinositol (3,4,5)-triphosphate, PIP3, the product of PI3K enzymatic activity (Liu et al., 2015). The Wei lab data support a model in which the PH domain of the mTORC2 essential component, SIN1, binds to and inhibits mTOR in the absence of PIP3. However, when growth factor-receptor stimulation leads to PI3K activation and accumulation of PIP3, the binding of SIN1 PH domain to PIP3 relieves the inhibition on mTOR, leading to complex activation, and recruits mTORC2 to the membrane, in proximity of many of its substrates.

4. AKT AND SGK1: TARGET OVERLAP AND SELECTIVITY

As described earlier, AKT, the prototypical effector of the PI3K signaling cascade, and SGK1 share the essential features of their activation mechanism, with mTORC2 and PDK1 phosphorylating the hydrophobic motif and the activation loop, respectively, of these two kinases. There are, however, some important differences. One is that AKT activation requires binding of its PH domain to PIP3, which induces a conformational change facilitating phosphorylation on Thr308 (Calleja et al., 2007; Milburn et al., 2003). A second critical difference is that, in the case of AKT, the two activating phosphorylations, both necessary for full kinase activation, are largely independent of each other (Biondi, Kieloch, Currie, Deak, & Alessi, 2001). On the other hand, in the case of SGK1, mTORC2-mediated phosphorylation on Ser422 has an essential priming function for the subsequent PDK1-mediated phosphorylation on Thr256, since it allows the PIF pocket of PDK1 to bind to phospho-Ser422 on SGK1 (Biondi et al., 2001; Collins, Deak, Arthur, Armit, & Alessi, 2003).

A key feature of these two kinases is that they share the same optimal target motif, Arg-X-Arg-X-X-Ser/Thr (Alessi, Caudwell, Andjelkovic, Hemmings, & Cohen, 1996; Kobayashi et al., 1999; Park et al., 1999). While AKT prefers a bulky hydrophobic residue immediately following the phosphorylation site, this characteristic does not seem to be shared by SGK1 (Kobayashi et al., 1999; Murray, Cummings, Bloomberg, & Cohen, 2005; Park et al., 1999).

Thus, although the identity of surrounding amino acids, or the kinase subcellular localization, or even interacting adapter proteins might dictate some level of target specificity, AKT and SGK1 appear to display a high level of promiscuity, often complicating the attribution of specific biological functions to either of them (Fig. 1).

Nevertheless, to date, few of the classical AKT targets have been convincingly shown to be phosphorylated by SGK1 in physiological conditions. As an example, some of AKT best-known bona fide targets, such as GSK3 β and FOXO3, were initially shown to be phosphorylated by SGK1 in vitro or in transfection-based systems (Brunet et al., 2001; Kobayashi & Cohen, 1999). However, the analysis of cells in which SGK1 cannot be activated because of a point mutation that was introduced in the PIF pocket of PDK1 failed to show changes in GSK3 α/β and FOXO3 phosphorylation levels, strongly suggesting that SGK1 is not a primary driver of GSK3 α/β and FOXO3 phosphorylation in physiological conditions (Collins et al., 2003).

Along the same line, early reports showing that both AKT (Gratton et al., 2001; Guan et al., 2000) and SGK1 (Chun et al., 2003; Zhang et al., 2001) could phosphorylate and negatively regulate BRAF and MEKK3 have never been conclusively validated.

On the other hand, a large amount of data from independent groups strongly supports the notion that the E3 ubiquitin ligase NEDD4L, which regulates internalization and turnover of a number of membrane proteins, can be negatively regulated by both AKT and SGK1 (Boehmer, Okur, Setiawan, Broer, & Lang, 2003; Caohuy et al., 2014; Lee, Dinudom, Sanchez-Perez, Kumar, & Cook, 2007), although gain- and loss-of-function experiments support SGK1 as more relevant for this function (Andersen et al., 2013).

Another shared target with related biological functions appears to be PIKFYVE (phosphatidylinositol-3-phosphate 5-kinase type III), which controls endomembrane homeostasis and protein trafficking by facilitating the progression of early endosomes toward multivesicular bodies (Shisheva, 2012). Both AKT (Berwick et al., 2004) and SGK1 (Seeböhm et al., 2007) appear to be able to phosphorylate and activate PIKFYVE, thus regulating the localization and activity of diverse transporters, including the K⁺ channel complex KCNQ1/KCNE1 (Seeböhm et al., 2007), the Na⁺/monocarboxylate transporter SLC5A1 (Shojaiefard, Strutz-Seeböhm, Tavaré, Seeböhm, & Lang, 2007), the creatine transporter SLC6A8 (Lopez-Barradas et al., 2016), the Ca²⁺-permeable cation channel TRPV6 (Sopjani et al., 2010), as well as the amino acid transporters EAAT2, -3, -4 (Gehring et al., 2009). In all these cases, experimental validation of the relative role and relevance of AKT and SGK1 is still lacking.

The list of validated SGK1-specific phosphorylation targets is rather limited. NDRG1 and -2 (see later) are the best-known and most credible SGK1-specific targets identified so far (Murray et al., 2004). Notably, they are inefficiently phosphorylated by AKT (Murray et al., 2005). Although SGK1-mediated phosphorylation appears to prime them for subsequent hyperphosphorylation by GSK3 (Murray et al., 2004), the role of these modifications is still elusive. It has been proposed that GSK3-mediated phosphorylation of NDRG1 leads to its ubiquitination and degradation (Gasser et al., 2014); thus, it is possible that this function is

also shared by SGK1 and AKT indirectly, although experimental validation of this inference is still lacking.

Two additional bona fide exclusive targets of SGK1 activity are NHE3 (Sandu et al., 2006; Wang, Sun, Lang, & Yun, 2005) and ENAC (Arteaga & Canessa, 2005; Lu et al., 2010). SGK-mediated phosphorylation of NHE3 (Na^+/H^+ exchanger 3) increases its activity toward the transcellular reabsorption of Na^+ and HCO_3^- in the kidney and intestine (Wang et al., 2005). Phosphorylation of the epithelial sodium (Na^+) channel (ENaC), instead, activates Na^+ transport in the distal nephron.

One particularly relevant feature of SGK1 and AKT is the ability to engage in opportunistic compensation when one of the two kinases is genetically repressed or pharmacologically inhibited. This notion might explain, for example, the reported ability of SGK1 to phosphorylate, in vitro or in overexpression conditions, AKT substrates such as GSK3 β and FOXO3 (Brunet et al., 2001; Kobayashi & Cohen, 1999), and that of AKT to phosphorylate NDRG2 (Burchfield et al., 2004).

More importantly, breast cancer cells with high Akt activity and low SGK1 expression display significant NDRG1 phosphorylation that is suppressed by Akt inhibitors (Sommer et al., 2013). Furthermore, in the presence of a PI3K inhibitor that completely blocks AKT activity, SGK1 can directly phosphorylate TSC2, a bona fide AKT substrate, thus activating mTORC1 (Castel et al., 2016) (see later).

5. ROADBLOCKS TO DEFINING SPECIFIC SGK1 FUNCTIONS

The reported target promiscuity of AKT and SGK1 and the presence of three SGK genes, which could theoretically partially compensate each other in genetic ablation or depletion approaches, have represented major barriers to defining and validating SGK1 molecular targets and biological functions.

Highly selective inhibitors would be invaluable tools to mitigate these problems. However, only a handful of SGK inhibitors have been described, and most of them have fallen short of showing the required specificity, bioavailability, or cellular permeability.

The best-known and most used SGK1 inhibitor, GSK650394 (Sherk et al., 2008), has an IC_{50} of 64 nM for SGK1. Off-target effects are notable, with less than 10-fold selectivity for SGK1 over Aurora kinase, JNK1, and JNK3. This inhibitor has rather poor cell permeability (Zapf et al., 2016), and a 10 μM concentration is necessary to observe reduced phosphorylation of NEDD4L (Sherk et al., 2008) and NDRG1 (Mansley & Wilson, 2010).

Another commonly used inhibitor is EMD638683 (Ackermann et al., 2011). While its in vitro IC_{50} toward SGK1 was not specified, it showed better selectivity than GSK650394 (>30-fold for PKA and MSK1). However, once again, at least 10 μM was needed to abolish NDRG1 phosphorylation, underlining its poor cell permeability. Along the same line, when tested in vivo in mice, the effective dose was very high (600 mg/kg) (Ackermann et al., 2011).

The most recently described inhibitor, SGK-inh (Castel et al., 2016; Halland et al., 2015), has an IC_{50} of 4.8 nM for SGK1. Interestingly, the only other kinase inhibited with selectivity lower than 10-fold is p70S6K. Again, limited permeability resulted in the need of at least a 10 μ M concentration to achieve significant inhibition of NDRG1 phosphorylation (Castel et al., 2016).

These data underline the critical need for better SGK1 inhibitors, in particular with improved pharmacodynamic and pharmacokinetic characteristics that allow in vivo application.

6. SGK1 IN DEVELOPMENT AND DIFFERENTIATION

Cell line-based data linking SGK1 activation to specific developmental or differentiation pathways are rather limited and often subjected to a number of caveats, including the use of overexpression systems and the absence of a physiological multicellular context. Using these approaches, for example, it has been proposed that SGK1 might regulate adipocyte differentiation, at least in part through the phosphorylation and relocalization of FOXO1, a canonical AKT target (Di Pietro et al., 2010).

On the other hand, some of these cell-based analyses have been designed to overcome most common caveats by using shRNA-mediated approaches as well as cells with targeted inactivation of *Sgk1*. As an example, a number of recent studies have uncovered a key role for SGK1 in promoting differentiation of specific T cell subsets under high salt conditions, which induce an increase of SGK1 expression. Two studies have found that high salt-induced SGK1 leads to increased differentiation of IL17-producing helper T cells, a CD4⁺ population involved in inflammation and autoimmunity (Kleinewietfeld et al., 2013; Wu et al., 2013). Importantly, the involvement of PI3K in the differentiation of TH17 cells was already well established (Kim et al., 2005), and an AKT-mTORC1 pathway has been identified as essential for the differentiation of TH17 cells in normal conditions (Lee et al., 2010).

Similarly, the differentiation of FOXP3⁺ Treg cells is the default outcome of T cell activation in mTORC1- and mTORC2-deficient mice under physiological conditions (Delgoffe et al., 2011), but not in T cell-specific *Sgk1*^{-/-} mice, indicating a critical suppressive role for a linear AKT-mTORC1 pathway in physiological conditions. However, under high salt conditions, SGK1 can inhibit Treg generation (Hernandez et al., 2015).

Thus, in physiological conditions, AKT appears to be the primary driver of differentiation of activated T cells. However, under stress conditions such as high salt-induced *Sgk1* upregulation (conditions that do not alter AKT expression and activity), SGK1 appears to directly control the development of TH17 and the repression of Treg cells. Consequently, it is not implausible to extend this notion to other acute or nonphysiological stimuli that can increase SGK1 levels and activity.

The generation of isoform-specific *Sgk* mutant mice has been a critical driver in clarifying SGK1 roles in a physiological context.

Given the high degree of homology, it is theoretically conceivable that the other two SGK isoforms, which are constitutively expressed, could compensate for SGK1 absence, at least under physiological conditions. The same notion could apply to additional kinases with overlapping target specificity, such as AKT, which could engage in opportunistic compensation.

6.1 Ion Transport

The initial characterization of whole-body *Sgk1* knockout mice suggested, unexpectedly, that SGK1 is completely dispensable for basic, steady-state physiological functions (Wulff et al., 2002). Mutant mice and organs appeared physiologically normal, and only under conditions of sodium deprivation they showed a reduced ability to maintain Na^+ levels by activating distal-tubular resorption, despite the presence of appropriately increased aldosterone levels. Along the same line, *Sgk1* mutant mice did not display alterations in Na^+ -coupled intestinal glucose transport under normal conditions, but, contrary to wild-type mice, they were unable to increase glucose transport upon glucocorticoid stimulation (Grahammer, Henke, et al., 2006).

Thus, based on in vivo data, it seems likely that, in normal conditions, SGK1 is not the primary regulator of those physiological processes to which it has been associated by in vitro studies, but is instead involved in their fine-tuning upon specific acute stresses.

Unexpectedly, the use of compound mutants has shown that functional compensation between isoforms is very limited, and that several functions ascribed to the whole SGK family appear to be quite isoform-specific, as clearly exemplified by renal Na^+ handling. Compound *Sgk1* and *Sgk3* mutants, in fact, show simple coexistence of the different phenotypes observed in single mutants (Na^+ handling in *Sgk1*^{-/-} mice, hair growth delay in *Sgk3*^{-/-} mice), without any exacerbation of these defects (Grahammer, Artunc, et al., 2006). Along the same line, while *Sgk2*^{-/-} mice do not display any Na^+ handling problem in normal or even salt-deprivation conditions, compound *Sgk1*, *Sgk2* mutants further escalate the Na^+ resorption deficit seen in *Sgk1* knockouts upon NaCl deprivation (Schnackenberg et al., 2007), suggesting that SGK2 controls some aspects of Na^+ handling under acute stress, but in lesser measure compared to SGK1.

6.2 Implantation and Pregnancy

Following up on the finding that endometrial SGK1 levels rapidly increase upon progesterone stimulation, it recently has been shown that SGK1 is involved in implantation and pregnancy maintenance (Salker et al., 2011). Forced expression of SGK1 in the uterine luminal epithelium after conception in a time window that is normally characterized by a transient loss of SGK1 expression, dramatically impairs the rate of embryo implantation. This finding strikingly correlates with the reduced litter size and impaired trophoblast invasion in mice with decidual-specific loss of *Pten*, which constitutively activates PI3K in this compartment (Lague et al., 2010). Thus, it is likely that tight regulation of the PI3K-PTEN-SGK1 axis is essential during embryo implantation.

Sgk1^{-/-} female mice experience a high rate (>30%) of pregnancy loss, associated with uterine bleeding, dramatic fetal growth restriction, and deregulation of reactive oxygen

species scavengers in the decidua (Salker et al., 2011), further underlining a critical role for SGK1 in the maintenance of pregnancy. A similar growth restriction and pregnancy loss phenotype was also observed in *Akt1*^{-/-} mice (Kent, Ohboshi, & Soares, 2012; Yang et al., 2003), suggesting that both AKT and SGK contribute to placental function.

6.3 Myocardial Injury

Recent in depth analysis of *Sgk1*^{-/-} mice has revealed subtle cardiac phenotypes, including a smaller heart, reduced heart rate, and reduced cardiomyocyte size, compared to wild-type controls (Zarrinpashneh et al., 2013). *Sgk1*^{-/-} endothelial cells display defective tube formation, which translates into increased cardiac fibrosis after ischemic insult in vivo due to inefficient capillary formation (Zarrinpashneh et al., 2013), a phenotype that contrasts the protection from fibrosis observed in *Akt1*^{-/-} mice subjected to the same ischemic insult (Ma, Kerr, Naga Prasad, Byzova, & Somanath, 2014). These results underline the presence of clearly differential functions of SGK1 and AKT downstream of PI3K in the heart.

6.4 Muscle Mass

PI3K activity has long been known to regulate skeletal muscle atrophy and hypertrophy (Glass, 2010). Recent studies have shown that skeletal muscle-specific *Sgk1* inactivation leads to moderate atrophy and decreased muscle strength, despite a small increase in activated AKT (Andres-Mateos et al., 2013). Correspondingly, overexpression of SGK1 protects against muscle atrophy induced by disuse and starvation. Interestingly, also *Akt1*^{-/-} and *Akt2*^{-/-} mice display a similar muscle phenotype (Goncalves et al., 2010), and SGK1 expression is slightly increased in the muscle of *Akt1*^{-/-} mice (Andres-Mateos et al., 2013). The similar phenotypes of *Sgk* and *Akt* mutant muscles, and the fact that loss of one gene induces increased expression of the other in the absence of a complete rescue strongly suggest that SGK1 and AKT cannot completely compensate for the loss of each other, and that both AKT and SGK1 are necessary to ensure normal muscle size and function.

6.5 T Cell Activation

Earlier genetic data had linked mTOR activity to the differentiation of CD4⁺ T cells into both TH1 and TH2 populations, and had specifically identified a linear AKT-mTORC1 pathway leading to the TH1 phenotype (Delgoffe et al., 2011; Lee et al., 2010). More specifically, constitutively active AKT was shown to rescue the TH1 but not the TH2-impairment in *riCTOR* mutant mice, which cannot activate mTORC2 (Lee et al., 2010). By using CD4⁺ T cell-specific *Sgk1* mutants, SGK1 has recently been identified as essential for the differentiation of CD4⁺ T cells into TH2 helper cells, while being dispensable for TH1 differentiation (Heikamp et al., 2014). Mechanistically, SGK1-dependent inhibition of NEDD4L relieved posttranscriptional control of JunB, whose activity is essential for TH2 differentiation (Li, Tournier, Davis, & Flavell, 1999). The fact that AKT and SGK isoforms are coexpressed in CD4⁺ T cells and that they are both activated upon TH1 and TH2 conditions underlines the notion that the differential requirement of AKT and SGK1 for TH1 vs TH2 differentiation is likely determined by specific substrate availability, which might be dictated by the polarizing stimulus.

6.6 Macrophage Motility and Function

The importance of PI3K-dependent signaling, in particular PI3K- γ , in the response of monocytes and macrophages to atherogenic mediators is well established (Chang et al., 2007).

Deletion of *Sgk1* in a mouse model of atherosclerosis led to a 50% reduction in both lesion size and macrophage infiltration (Borst et al., 2015). The defect in macrophage migration was further validated in a mouse model of thioglycollate-induced peritoneal inflammation. *Sgk1* mutant macrophages displayed reduced NFkB-dependent MMP9 expression, which likely contributes to the migration defect. A different study examined the effect of SGK1 deficiency on hypertensive cardiac remodeling associated with activation of the renin-angiotensin system and found that *Sgk1* mutant mice were protected from cardiac inflammation and fibrosis after angiotensin II infusion (Yang et al., 2012). Attenuation of the infiltration of proinflammatory cells in the heart was associated with the prevention of activation of STAT3 and reduced polarization to M2 macrophages.

In a similar mouse model of cholesterol-induced atherosclerosis, specific deletion of *Akt1* in hematopoietic cells did not affect the size and macrophage content of atherosclerotic lesions (Babaev et al., 2014). Conversely, deletion of *Akt2* drastically reduced atherosclerotic lesion size and macrophage content. Interestingly, *Akt1*^{-/-} blood monocytes and macrophages were found to be skewed to the M1 phenotype, while *Akt2*^{-/-} monocyte and macrophages displayed the M2 phenotype (Arranz et al., 2012; Babaev et al., 2014).

Thus, it appears that SGK1 and AKT2 exert similar positive effects on macrophage recruitment, while SGK1 regulates macrophage polarization in a manner more similar to AKT1.

6.7 Insulin Sensitivity

Analysis of liver-specific *Sgk1* mutant mice has revealed a role for SGK1 in the control of insulin sensitivity. Mutant mice displayed a slight elevation in fasting blood glucose as well as a mild reduction in both glucose clearance and insulin sensitivity (Liu et al., 2014). The mechanisms for this impaired response to insulin seem to include reduced insulin-induced phosphorylation of IRS1. Notably, phosphorylation of the insulin receptor was not altered upon loss of SGK1, which suggests that SGK1 might negatively regulate a phosphatase that controls IRS1 phosphorylation.

It must be underlined that the insulin resistance observed in *Sgk1*^{-/-} mice is significantly less severe than that observed in the overtly diabetic *Akt2*^{-/-} mice (Cho et al., 2001). On the other hand, *Akt1*^{-/-} mice do not display any insulin sensitivity phenotype (Chen et al., 2001). Thus, it is likely that SGK1 mediates only a minor part of the insulin-induced cellular uptake of glucose, which is instead mainly controlled by AKT2.

7. SGK1 AND CANCER

As discussed earlier, SGK1 expression is promptly induced by a variety of stimuli, especially stress-related ones. Furthermore, while being often dispensable for basal pathway

activity, SGK1 is instead involved in signaling under acute stimulation. Taken together, these notions support the possibility that SGK1 plays a critical role in mediating neoplastic transformation as an effector of PI3K.

Lack of highly specific reagents (antibodies and small molecule inhibitors), its originally postulated exclusive role in ion transport, and the apparent promiscuity with AKT isoforms have historically limited the interest in SGK1 as a mediator of the transformed phenotype. However, the past few years have witnessed a significant increase in the number of reports showing that indeed SGK1 has a defined AKT-independent role in cellular transformation, downstream of activated PI3K signaling.

Similar to AKT isoforms, SGK1 does not appear to be frequently mutated in human tumors, although a recent study has found frequent SGK1 mutations (approximately 50% of samples) in nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) (Hartmann et al., 2016). The functional significance of these alterations remains anyway to be defined. *SGK1* amplification has been observed in 31% of breast cancer patients in one study (Eirew et al., 2015) and overexpression in 48% of breast cancer patients in another study (Sahoo, Brickley, Kocherginsky, & Conzen, 2005).

The most compelling data linking SGK1 and tumor development come from studies employing genetically targeted *Sgk1* alleles.

The first in vivo demonstration that activation of SGK1 is important for neoplastic transformation came from the analysis of intestinal carcinogenesis in *Sgk1*^{-/-} mice (Nasir et al., 2009). Wild-type mice subjected to a chemical carcinogenesis protocol developed on average 12 colonic tumors per mouse, while *Sgk1*^{-/-} mice had a reduction of over 50% in the tumor load. Mechanistically, *Sgk1*^{-/-} tissues displayed increased levels of FOXO3A and BIM, two molecules that may affect tumor growth by increasing apoptosis levels.

Along the same line, generation of *Apc*^{Min/+}/*Sgk1*^{-/-} mice identified a clear protumorigenic role of SGK1 (Wang et al., 2010). *Apc*^{Min/+} mice develop colonic adenomatous polyps, which eventually progress to colon carcinoma. Deletion of *Sgk1* resulted in approximately 75% reduction in the average number of tumors per mouse.

It is important to underline that these studies, performed with whole-body *Sgk1* mutants, do not clarify whether the observed reduction in tumor development is cell autonomous.

In fact, T cell-specific deletion of *Sgk1* resulted in a dramatic reduction in the number of lung metastases from mouse melanoma cells injected in the tail vein (Heikamp et al., 2014), strongly suggesting that deletion of *Sgk1* in T cells leads to enhanced antitumor immunity.

The recent availability of a conditional *Sgk1* allele will be instrumental in defining cell autonomous vs nonautonomous SGK1 functions associated with neoplastic transformation and tumor progression.

A critical issue is the identity of the pathways through which SGK1 activation contributes to PI3K-dependent tumorigenesis. While it is possible that increased SGK1 expression and

activity impinge upon classical AKT targets, contributing to their phosphorylation, other proteins known to be primarily controlled by SGK1 might also play an important role.

One class of possible SGK-controlled players in neoplastic transformation is represented by the Ca^{2+} release-activated Ca^{2+} channel (I_{CRAC}), composed of the pore-forming subunits ORAI1, -2, and -3 and their regulatory subunits STIM1 and -2. SGK1 can strongly upregulate these channels through two mechanisms, repression of the ubiquitin ligase NEDD4L, leading to increased protein levels of ORAI1, and transcriptional induction of both *ORAI1* and *STIM1*, by increasing the activity of NF κ B, which directly interacts with their promoter (Lang & Shumilina, 2013). Store-operated calcium channels such as the ORAI/STIM complexes regulate key aspects of the cancer cell phenotype, including tumor growth, angiogenesis, and metastasis formation (Xie, Pan, Yao, Zhou, & Han, 2016).

At the same time, SGK1-controlled potassium channels, such as Kv1.3, mediate plasma membrane hyperpolarization, which provides increased driving force for Ca^{2+} entry, thus further fostering Ca^{2+} -dependent tumor growth (Urrego, Tomczak, Zahed, Stühmer, & Pardo, 2014).

Additionally, SGK1-controlled proton exchangers such as NHE3 have been associated with the control of lysosome trafficking in prostate cancer cells (Steffan, Williams, Welbourne, & Cardelli, 2010), and ENAC activity has been shown to mediate a variety of processes, including cancer cell proliferation and migration (Liu, Zhu, Xu, Ji, & Li, 2016).

Finally, it is important to underline that SGK1 is likely to phosphorylate a number of still unknown targets, some of which may have critical roles in neoplastic transformation either directly, or by controlling additional downstream players. In fact, novel SGK1-mediated pathways are being discovered on a regular basis, although the specific mechanisms at play are still undefined. For example, SGK1 has been recently shown to regulate *RANBP1* gene transcription in colon carcinoma cells, thus affecting mitotic spindle function as well as cell sensitivity to taxanes (Amato et al., 2013). Furthermore, a more recent study has proposed that SGK1 mediates an increase in rRNA synthesis under conditions of PI3K activation by regulating the nucleolar localization of the histone demethylase KDM4A (Salifou et al., 2016).

As mentioned earlier, an additional mechanism through which SGK1 contributes to transformation is through opportunistic compensation under conditions of PI3K/AKT pharmacological inhibition. As an example, in breast cancer cells expressing high levels of SGK1 and treated with a PI3K inhibitor, SGK1 stimulates residual mTORC1 activity through direct phosphorylation and inhibition of TSC2, thus contributing to resistance to PI3K inhibition (Castel et al., 2016). These data confirm and extend those presented in a previous report, in which high levels of SGK1 were found to predict resistance of breast cancer cells to AKT inhibitors (Sommer et al., 2013). A key corollary to this notion is that a certain level of SGK1 activation is still possible under condition of PI3K inhibition. Whether it is mediated by residual PI3K activity, or by a PI3K-independent pool of activated mTORC2, remains to be addressed.

8. CONCLUDING REMARKS

After spending two decades under the shadow of AKT, SGK1 is finally claiming its own spot as an important mediator of PI3K signaling activity, in particular in conditions where growth factor- or stress-related inputs increase SGK1 levels. Recent data support a major role for SGK1 that extends well beyond what has always been considered SGKs primary function, the control of ion transport across membranes.

Current and future efforts, in particular those employing genetically engineered mouse models and tightly controlled shRNA-based cell systems, will need to clarify the extent of both physiological and opportunistic target overlap between AKT and SGK1, and to define the signaling molecules and pathways that are directly and specifically controlled by SGK1.

Finally, the notions (i) that SGK1 is actively involved in the control of pathways that are altered during inflammation, neoplastic transformation, and tumor response to cytotoxic and targeted therapy, and (ii) that SGK1 loss, in vivo, has minimal consequences for normal homeostasis should drive a renewed effort to develop selective SGK1 inhibitors with pharmacodynamic and pharmacokinetic properties allowing in vivo preclinical and clinical applications.

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Fig. 1. Schematic representation of the pathways leading to AKT and SGK1 activation, and of the bona fide targets and physiological processes specifically associated with each kinase or coregulated by AKT and SGK1. See text for details.