SGK3 (CISK) may induce tumor angiogenesis (Hypothesis)

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Abstract. Serum- and glucocorticoid-inducible protein kinase 3 (SGK3), also known as cytokine-independent survival kinase (CISK), encoded by chromosome 8q12.2, is a downstream mediator of phosphatidylinositol 3-kinase (PI3K) oncogenic signaling. As a downstream target of PI3K, SGK3 has been reported to mediate pivotal roles in oncogenic progress in various cancers, including breast cancer, ovarian cancer and hepatocellular carcinoma. Functionally parallel to v-akt murine thymoma viral oncogene homolog (AKT)/protein kinase B, SGK3 serves as a hallmark mediating glycogen synthase kinase-β (GSK3-β), B-cell lymphoma (Bcl)-2-associated death promoter, forkead family of transcription factors, Bcl-extra large, Bcl-2, mammalian target of rapamycin, C-X-C chemokine receptor type 4 (CXCR4) and numerous other molecules in cell proliferation, growth, survival, migration and even tumor angiogenesis. Tumor angiogenesis is recognized as an essential step for tumor growth, invasion and metastasis, and it has become an intriguing target for anticancer drug development for tumor investigators worldwide. An abundance of experiments have been performed to investigate the role of the phosphoinositide 3-kinase (PI3K)/AKT pathway in regulating tumor angiogenesis. The mechanism of angiogenesis regulated by the PI3K/AKT pathway is, to a certain extent, clear. Although a number of SGK3 target molecules, including CXCR4 and GSK3β, have demonstrated potential roles in promoting angiogenesis, the exact association between angiogenesis and SGK3 remains unclear. Thus, we hypothesize that SGK3, parallel to AKT, may also be important in mediating angiogenesis. Identifying the role of SGK3 in tumor angiogenesis will certainly present a novel perspective on the malignant transformation of tumors, as well as a target for tumor therapy.

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Abbreviations: PI3K, phosphoinositide 3-kinase; PDK1, 3-phosphoinositide-dependent kinase 1; AKT, v-akt murine thymoma viral oncogene homolog; SGK3, serum- and glucocorticoid-inducible protein kinase 3; CISK, cytokine-independent survival kinase; Bcl-xL, B-cell lymphoma-extra large; FKHRs, forkhead family of transcription factors; BAD, Bcl-2-associated death promoter; GSK3-β, glycogen synthase kinase-β; CXCR4, C-X-C chemokine receptor type 4; VEGF, vascular endothelial growth factor

Key words: GSK-3β, PI3K, VEGF, angiogenesis

Contents

1. Introduction

V-akt murine thymoma viral oncogene homolog [*SGK3*; also known as cytokine-independent survival kinase (*CISK*)], as a downstream target of the phosphoinositide 3-kinase (PI3K) cascade and a member of the AGC family of kinases, has certain similar substrates and shares certain target-signaling molecules with v-akt murine thymoma viral oncogene homolog (AKT) in cell proliferation, growth and survival (1-4). SGK3 is a serine/threonine protein kinase, and its key phosphorylation sites are Thr^{256} and Ser^{422} (5). The residues of SGK3 (also known as CISK) equivalent to those of AKT are targeted by 3-phosphoinositide-dependent kinase 1 (PDK1) and PDK2 respectively (5). Functionally parallel to AKT, SGK3 is involved in the malignant transformation of tumors by regulating cell proliferation, cell growth, cell survival and cell migration (4,6,7). The oncogenic effect of *SGK3* in tumors has been demonstrated by *in vitro* and *in vivo* functional assays (2,3). Xu *et al* (2) reported that the positive correlation between SGK3 expression and tumor prognosis varies with tumor grade and lymph node status. Angiogenesis is a key process in tumor malignant transformation, which involves blood vessel endothelial cell proliferation and migration. The signaling pathways of the cellular processes that SGK3 mediates, particularly in cell survival, have been well studied. However, angiogenesis, another tumor malignant transformation process, is seldom reported in comparison to AKT. Therefore, the present study aims to find evidence that SGK3 may be involved in angiogenesis.

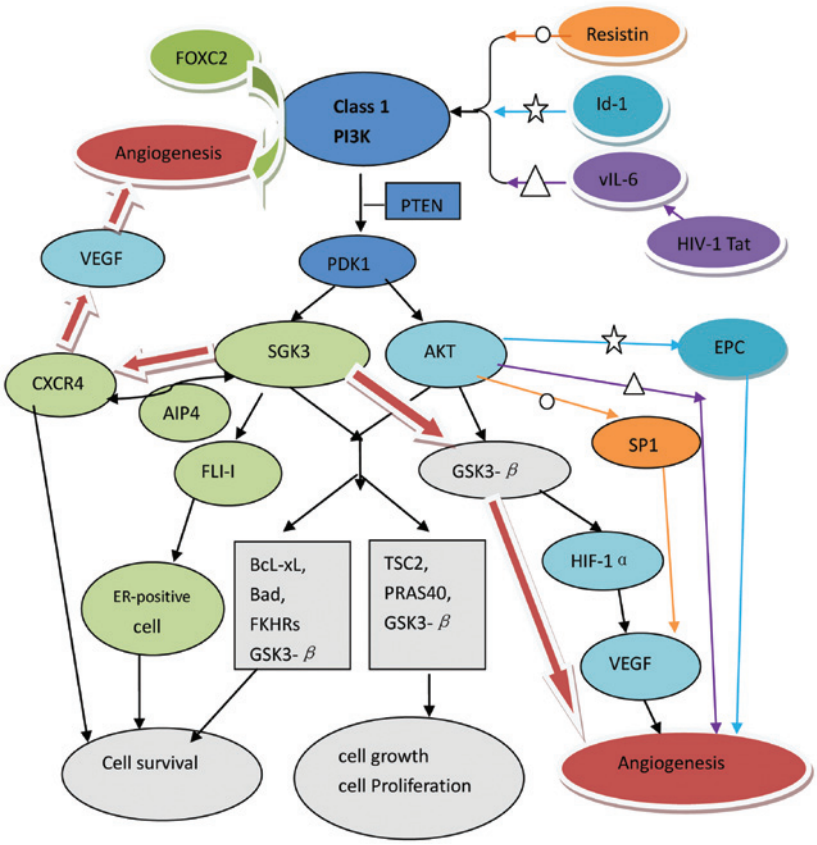

Figure 1. Signaling of SGK3 and AKT. Activation of PI3K leads to phosphorylation of PDK1, subsequently leading to phosphorylation and activation of AKT and SGK3. Cell survival and cell growth/proliferation: By regulating the downstream targets, SGK3 and AKT show a parallel function in cell survival (Bcl-xL, BAD, FKHRs, GSK3-β) and cell growth/proliferation (GSK3-β, TSC2, PRAS40). SGK3 also induces cell survival by downregulating CXCR4 through the interaction with ubiquitin ligase AIP4. SGK3 mediates ER-positive cancer cells survival by phosphorylating its co-activator, FLI-I. Angiogenesis: PI3K signaling pathways involve in the Foxc2-mediated angiogenesis process. Inhibition of GSK3-β by PI3K/AKT up-regulates the expression of HIF-1α, which induces VEGF transcriptional activation to promote angiogenesis. °Resistin activates PI3K/AKT to increase the interaction with Sp1, resulting in upregulation of VEGF expression to promote angiogenesis. *PI3K/AKT is mediated by Id1 to enhance EPC angiogenesis in ovarian cancer. $^{\Delta}$By regulating PI3K/PTEN/AKT/GSK-3β pathway, HIV-1 Tat in KSHV vIL-6-induced angiogenesis. SDF-1/CXCR4 receptor ligand system up-regulates VEGF expression to promote angiogenesis. Hypothesis: The red arrow pathways are our hypothesis: SGK3 may involve in tumor angiogenesis by regulating CXCR4 or/and GSK-3β. PI3K, phosphoinositide 3-kinase; PDK1, 3-phosphoinositide-dependent kinase 1; AKT, v-akt murine thymoma viral oncogene homolog; SGK3, serum- and glucocorticoid-inducible protein kinase 3; Bcl-xL, B-cell lymphoma-extra large; FKHRs, forkhead family of transcription factors; BAD, Bcl-2-associated death promoter; FLI-I, flightless-I; GSK3-β, glycogen synthase kinase-β; TSC2, tuberous sclerosis factor 2; PTEN, phosphatase and tensin homolog; PRAS40, proline-rich AKT substrate of 40 kDa; SDF-1, stromal cell-derived factor 1; CXCR4, C-X-C chemokine receptor type 4; HIF1α, hypoxia-inducible factor-1α; VEGF, vascular endothelial growth factor; Id1, inhibitor of DNA binding/differentiation 1; EPC, endothelial progenitor cell; KSHV vIL-6, Kaposi's sarcoma-associated herpesvirus viral interleukin-6; HIV-1, human immunodeficiency virus type 1; Tat, transactivator of transcription; ER, estrogen receptor; AIP4, atrophin-1-interacting protein 4; FOXC2, forkhead box protein C2; Sp1, specificity protein 1.

2. Hypothesis

An abundance of experiments have been performed to investigate the function of the PI3K/AKT pathway in regulating tumor angiogenesis, and so the mechanism of angiogenesis regulated by the PI3K/AKT pathway is, to a certain extent, clear. However, the association between angiogenesis and SGK3 remains unclear. As they are downstream mediators of the PI3K/PDK1 signaling pathway, AKT and SGK3 have certain similar substrates and share certain targeting molecules. Thus, we hypothesize that a strong signaling connection may exist between angiogenesis and SGK3, contributing to tumor malignant transformation.

3. Evidence and discussion

The deteriorative progress of tumor growth includes several alterations that collectively dictate malignant transformation, including insensitivity to growth-inhibitory signals, evasion of cell apoptosis, limitless cell proliferation, sustained angiogenesis, and tissue invasion and metastasis (8). SGK3, a downstream effector of PI3K, induces several pro-malignant pathways through the PI3K/PDK1/SGK3 route. Functionally parallel with AKT (Fig. 1) (3,4), SGK3 participates in cell growth, cell survival and cell migration (1,2,9,10). In contrast to the AKT pleckstrin homology domain, SGK3 contains a phox homology domain, through which SGK3 binds to phosphatidylinositol 3-phosphate-rich endosomal and vesicular compartments to remain active. Overexpression of SGK3 increases cell cycle progression through G_1 by inactivating glycogen synthase kinase-β (GSK3-β) and stabilizing CCND1, as previously observed in hepatocellular carcinoma (3,7,9). Similar to AKT, SGK3 is involved in cell growth signaling by the increase of phosphorylated tuberous sclerosis factor 2, ribosomal protein S6, proline-rich AKT substrate of 40 kDa and eIF4E-binding protein 1 in normal

cell physiology and malignant transformation (9,11). Induced by interleukin (IL)-3 (4), SGK3 increases the level of B-cell lymphoma-extra large (Bcl-xL), and inhibits the pro-apoptotic proteins Bcl-2-associated death promoter (BAD) and forkhead family of transcription factors (FKHRs) (3,4,9,10), thus promoting cell survival. Consistent with the fact that SGK3 and AKT are functionally parallel, SGK3 and AKT have synergetic responses to the cell survival pathways through Bcl-xL, BAD and FKHRs. SGK3 mediates estrogen receptor-positive cancer cell survival by phosphorylating its co-activator, flightless-I (2,6). Observations by Slagsvold *et al* (12) support the potential role of SGK3 as a cell survival effector by the downregulation of C-X-C chemokine receptor type 4 (CXCR4) through the interaction with ubiquitin ligase atrophin-1-interacting protein 4. CXCR4 is strongly associated with promoting cell invasion, migration and adhesion during the process of metastasis in breast cancer and liver tumor cells (13,14), showing the potentially significant role of SGK3 in cell migration. Thereby, further studies are required to more specifically characterize the role of SGK3 in these processes.

Tumor angiogenesis is recognized as an essential step for tumor growth, invasion and metastasis, and has become an intriguing target of tumor investigators worldwide for the development of anticancer drugs. A number of studies have shown that vascular endothelial growth factor (VEGF)/VEGF receptor are essential in the tumor angiogenesis process (15,16).

Study has also been focused on the role of the PI3K/AKT pathway in angiogenesis (Fig. 1) (17). Inhibition of GSK3-β by PI3K/AKT upregulates the expression of hypoxia-inducible factor-1α, inducing VEGF transcriptional activation to promote angiogenesis (17). In early 2013, Su *et al* (18) reported that PI3K/AKT is mediated by inhibitor of DNA binding/differentiation 1 to enhance endothelial progenitor cell angiogenesis in ovarian cancer. In a study by You *et al* (19), extracellular signal-regulated kinases and PI3K signaling pathways showed strong involvement in the forkhead box protein C2-mediated angiogenesis process. Zhou *et al* (20) showed the promotion of human immunodeficiency virus type 1 transactivator of transcription in Kaposi's sarcoma-associated herpesvirus viral IL-6-induced angiogenesis by regulating the PI3K/phosphatase and tensin homolog/AKT/GSK-3β pathway *in vivo*. A recent study revealed that PI3K/AKT is activated by resistin and increases the interaction with specificity protein 1, resulting in the upregulation of VEGF expression to promote angiogenesis (21).

The stromal cell-derived factor 1/CXCR4 receptor ligand system has also been reported to play a potential role in cancer metastases via the upregulation of VEGF expression to promote angiogenesis (22-24).

Bevacizumab, a monoclonal antibody targeted against VEGF, and a number of other anti-angiogenesis molecules have been used in numerous anti-angiogenesis strategies (25). The use of anti-angiogenesis strategies may present a new epoch in tumor research, however, the exact pathway of the angiogenesis mechanism remains unknown.

Since PI3K/AKT plays an important role in tumor angiogenesis, we hypothesize that SGK3, as a downstream target of PI3K and functionally parallel to AKT, may also be involved in the malignant transformation of tumors by promoting angiogenesis. It has also been reported that CXCR4 and GSK3-β, both downstream of SGK3, may also have potential capacity in angiogenesis (3,17). These facts demonstrated the potential role of SGK3 in promoting angiogenesis and is evidence that confirms our hypothesis (Fig. 1).

SGK3 has been studied in depth with regard to tumor malignant transformation, but the exact association between angiogenesis and SGK3 is rarely reported. Possible reasons for this include the fact that SGK3 does not significantly correlate with angiogenesis, or that the amplification and overexpression of SGK3 may be an early stage event in tumor growth (3).

In conclusion, the mechanism of SGK3 in oncogenesis is, to a certain extent, clear. However, its role in malignant transformation, particularly in angiogenesis, remains to be elucidated. Based on the present datum, detailed characterization of any role of SGK3 in the promotion of angiogenesis via CXCR4 and GSK3-β, the association between SGK3 and VEGF, and the exact mechanisms behind this are required.

4. Clinical implications

These data generates an overall impression of SGK3 as an important oncogenic signaling mediator, and stresses the vital nature of further research on the elucidation of the signaling mechanisms associated with SGK3 in tumor angiogenesis. Determining the role of SGK3 in tumor angiogenesis will surely present a novel perspective on tumor malignant transformation, as well as a target for tumor therapy.

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