



Perspective on targeting salt-inducible kinase 2 (SIK2) in ovarian cancer metastasis

Nathaniel Jensen^{1,2,3}, Hiu Wing Cheung^{1,2,3}

¹Department of Pathology and Laboratory Medicine, ²Hollings Cancer Center, ³Center for Genomic Medicine, Medical University of South Carolina, Charleston, SC, USA

Correspondence to: Hiu Wing Cheung, Ph.D. Department of Pathology and Laboratory Medicine, Medical University of South Carolina, 68 President Street, Room BE413, MSC 908, Charleston, SC 29425, USA. Email: cheungh@musc.edu.

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Abstract: Ovarian cancer has a predilection for metastasis to the omentum in abdominal cavity. Omental adipocytes have been shown to secrete adipokines to attract implantation of ovarian cancer cells and provide free fatty acids to promote rapid growth of omental metastases. However, how ovarian cancer cells take advantage of the adipocyte-rich metastatic niche is not understood. In a recent report by Miranda and colleagues published in *Cancer Cell*, they show that salt-inducible kinase 2 (SIK2) is overexpressed in omental metastases compared with primary tumors. Omental adipocytes activate SIK2 in co-cultured ovarian cancer cells, leading to increased fatty acid oxidation and proliferation of ovarian cancer cells. Inhibition of SIK2 significantly reduces growth of metastases in omentum and other adipocyte-rich tissues. These findings identify SIK2 as a highly promising target in suppressing ovarian cancer metastasis. This perspective will describe the current understanding of the molecular mechanisms by which SIK2 promotes ovarian cancer metastasis and discuss the promise of targeting SIK2 in improving the therapeutic efficacy of chemotherapy.

Keywords: Ovarian cancer; salt-inducible kinase 2 (SIK2); metastasis

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Introduction

Ovarian cancer is the seventh leading cause of cancer deaths among women worldwide, with 225,000 new cases and 140,000 deaths estimated to occur every year. More than 70% of deaths are of patients presenting with advanced stage, high-grade serous ovarian cancer (HGSOC) that has widely disseminated into the peritoneal cavity by the time of diagnosis (1). Current standard therapy involves debulking surgery to remove bulky tumors optimally followed by intraperitoneal carboplatin-paclitaxel based chemotherapy to kill residual/microscopic tumors. Despite experiencing initial objective response, most patients will have recurrence with progressively chemo-resistant tumors within 12 to 18 months. Current treatment options available for recurrent HGSOCs are of palliative nature, aiming to delay progression or alleviate patient symptoms (2).

The major cause of death remains carcinomatosis within peritoneal cavity which leads to bowel obstruction and malnourishment in many cases. Hence, the important goals of ovarian cancer research are to identify approaches that can effectively treat peritoneal metastases and potentially improve the efficacy of the current first-line and second-line therapies.

HGSOC primarily disseminates via the transcoelomic route into the peritoneal cavity due to lack of an anatomic barrier (3). In addition to direct extension into adjacent pelvic tissues, HGSOC disseminates by shedding into peritoneal fluid, either as single cells or multicellular spheroids, before implanting intraperitoneally. Studies of the distribution pattern indicate that peritoneal metastasis is not random. Other than the contralateral ovary, the most common sites for metastasis are the omentum, the right diaphragm, the small bowel mesentery, and the lower

portions of pelvis (4). Approximately 80% of women with HGSOCS present with omental metastases. The omentum is a large fat pad (20 cm × 12 cm × 3 cm) that extends from the stomach and covers the intestines and the lower abdomen (4). Understanding the molecular mechanisms underlying this predilection of HGSOCS metastasis to the omentum will provide avenues for therapeutic invention.

A seminal work by Nieman and colleagues identified omental adipocytes as major promoters of ovarian cancer metastasis to the omentum (5). Using co-culture of primary omental adipocytes and ovarian cancer cells, the authors found that adipocytes not only secrete adipokines such as interleukin-6 (IL-6), IL-8, monocyte chemoattractant protein-1 (MCP-1), and tissue inhibitor of metalloproteinase-1 (TIMP1) to promote homing, migration and invasion of cancer cells to the omentum, but also provide fatty acids to fuel rapid metastatic tumor growth. Upregulation of fatty-acid binding protein 4 (FABP4), a lipid transporter, is found in omental metastases compared to primary tumors. Metastatic tumor growth is dramatically suppressed in Fabp4-deficient mice compared to wildtype mice in an orthotopic model of ovarian cancer. This study not only highlights the important contribution of tumor microenvironment to ovarian cancer metabolism and metastatic growth, but also indicates that therapeutic strategies targeting fatty acid metabolism or trafficking is effective in suppressing peritoneal metastasis of ovarian cancer in adipocyte-rich tissues.

Salt-inducible kinase 2 (SIK2) drives growth of HGSOCS metastases in adipocyte-rich tissues

A recent study by Miranda and colleagues published in *Cancer Cell* has identified an important role of SIK2 in metastatic growth of HGSOCS in adipocyte-rich tissues (6). SIK2 is an AMP-activated protein kinase (AMPK)-related protein kinase that has been shown to regulate metabolism. When they analyzed expression of SIK2 by immunohistochemistry in paired samples from primary HGSOCS and omental metastases, they observed that SIK2 was expressed at higher levels in omental metastases with the highest SIK2 level being at the interface between omental adipocytes and tumors. Subsequently, they performed both gain- and loss-of-function studies to investigate whether SIK2 overexpression promoted peritoneal metastasis in two orthotopic xenograft tumor models using SKOV3 and OVCA432 serous ovarian cancer cell lines. They found that ectopic overexpression of wildtype SIK2, but not the kinase-dead SIK2 K49M mutant, in both cell lines promoted their metastatic growth in the omentum and

mesentery of immunodeficient mice after implantation of these cells at the ovarian bursa, as evidenced by increased tumor weight and the number of metastatic nodules. They further showed that silencing of SIK2 by siRNAs delivered by dioleoylphosphatidylcholine (DOPC) nanoliposomes in both cancer cell lines resulted in more than 70% reduction of the tumor burden in immunodeficient mice compared to control siRNA. Of note, siRNA treatment was initiated 7 days post implantation of cancer cells, and no significant difference in tumor burden was observed between SIK2-targeting siRNA and control siRNA groups in the first 21 days. These findings indicate that SIK2 is not required for invasion or metastatic colonization of ovarian cancer cells into omentum or other adipose tissues but promotes growth of metastases in those tissues.

Miranda and colleagues continued the investigation into the mechanisms by which SIK2 promotes ovarian cancer metastasis. They first examined the effect of freshly isolated omental adipocytes on proliferation of co-cultured ovarian cancer cells and SIK2 activity. They showed that co-culturing with adipocytes increased proliferation of SKOV3 cells or SIK2-overexpressing SKOV3 cell lines that was accompanied by increased phosphorylation (at serine 358 site) and activity of SIK2. They found that free fatty acids, such as oleic and linoleic acids, provided by omental adipocytes increased intracellular calcium levels, and calcium chelation by the cell permeable chelator BAPTA-AM blocked adipocyte-induced SIK2 activation in co-cultured cancer cells. Increased calcium levels activate Ca^{2+} -calmodulin-dependent protein kinases that are known to activate AMPK. These results indicate that adipocytes transfer fatty acids to induce calcium-dependent activation of SIK2. Suppression of SIK2 by siRNAs or a small molecule inhibitor HG-9-91-01 prevented the adipocyte-mediated increase in cancer cell proliferation, indicating that SIK2 is required for adipocyte-induced proliferation. Previous studies have shown that SIK2 indirectly upregulates transcription of key genes involved in fatty acid metabolism to promote fatty acid oxidation, a catabolic process by which fatty acid molecules are broken down in mitochondria to generate acetyl-co-enzyme A (CoA). The authors showed that co-culture of adipocytes with SKOV3 cells increased expression of carnitine palmitoyltransferase (CPT1), which is an enzyme responsible for acyl-CoA transfer to mitochondria for initiation of fatty acid oxidation. Importantly, they obtained novel evidence showing that SIK2 directly phosphorylated (at serine 79 residue) and inactivated acetyl-CoA carboxylase (ACC), which is a critical enzyme required for irreversible carboxylation of acetyl-CoA to malonyl-CoA to promote the biosynthesis of

fatty acid. SIK2-induced ACC phosphorylation is additive to AMPK-induced phosphorylation. They showed that inhibition of SIK2 in SKOV3 and OVCA432 cells decreased adipocyte-induced ACC phosphorylation, CPT1 expression, and extracellular oxygen consumption (EOC). Reduced EOC indicated decreased fatty acid oxidation. These findings identify SIK2 as a major mediator of fatty acid oxidation in ovarian cancer cells following increased fatty acid availability provided by adipocytes.

Interestingly, a previous report by Ahmed and colleagues has identified SIK2 as an important activator of AKT pathway in multiple ovarian cancer cell lines (7), but the underlying mechanisms are not understood. In this study, inhibition of PI3K could prevent adipocyte-mediated increase in cancer cell proliferation but only had a minor effect on fatty acid oxidation, suggesting that the PI3K pathway is a separate signaling arm utilized by SIK2 in promoting ovarian cancer cell proliferation. To clarify the mechanism, the authors first performed an elegant stable isotope labeling with amino acid (SILAC) experiment in which a T96G gatekeeper mutant form of SIK2 or wildtype SIK2 was expressed in SKOV3 cells which were cultured in heavy or control SILAC media for a quantitative comparison. The T96G mutant, but not wildtype form, was capable of utilizing the “bulky” N6-benzyl- and N6-phenylethyl-ATP- γ S analogs, thus enabling identification of phosphorylation substrates of SIK2 T96G. They identified a phosphorylation site at S154 on p85 α , the regulatory subunit of the PI3K complex. Detailed phosphopeptide mapping of p85 α following incubation with recombinant SIK2 proteins further revealed that p85 α was phosphorylated at the S154 in the BH domain and S541 in the iSH2 domain by SIK2. They obtained evidence showing that p85 α mutant with mutated S154A site indeed exhibited reduced ability to activate the PI3K-AKT pathway. These results indicate that SIK2 directly phosphorylates p85 α S514 to activate the PI3K-AKT pathway and promotes ovarian cancer cell proliferation.

Discussion

Overall this study shows that overexpression of SIK2 is a key promoter of metastatic growth of HGSOc in omentum and adipocyte-rich tissues by promoting ovarian cancer cell proliferation, oxidation of fatty acids, and activation of the PI3K-AKT pathway by interaction with adipocytes at the metastatic niche. Mechanistic studies demonstrate that SIK2 is activated in ovarian cancer cells by the PLC/intracellular Ca²⁺ pathway in response to increased availability of free fatty acids provided by co-cultured adipocytes, and that upon activation,

SIK2 directly phosphorylates p85 α and ACC to promote the PI3K-AKT pathway and fatty acid oxidation, respectively. Previous studies have shown that higher expression of SIK2 in primary HGSOcs significantly correlated with poor survival in patients (7). Therefore, this work provides a plausible mechanism by which overexpression of SIK2 in HGSOcs promotes aggressive growth of metastases in adipocyte-rich tissues including omentum.

Another important aspect of SIK2 function is its role as a centrosome kinase during mitosis (7). In a previous study, Ahmed and colleagues performed a high content siRNA kinome screen and identified SIK2 as an important cell cycle regulator. Silencing of SIK2 by siRNAs in ovarian cancer cells results in prolonged mitotic progression and delayed G1/S transition. SIK2 has been shown to directly phosphorylate C-Nap1, a centrosome linker protein, and regulates its subcellular localization and function during centrosome separation in mitosis. Silencing of SIK2 synergizes with paclitaxel to inhibit proliferation, survival, and xenograft tumor growth of multiple ovarian cancer cell lines. More recently, a novel small molecular SIK2 inhibitor, ARN-3236, has been developed by the Robert Bast group (8). Combinatorial treatment with ARN-3236 and paclitaxel shows greater inhibitory effects on proliferation and survival in 8 out of 10 ovarian cancer cell lines tested compared to monotherapy, with a synergistic effect being observed in three cell lines. Combination therapies targeting different hallmarks of cancer may achieve greater anti-tumor efficacy, as exemplified by recently approved bevacizumab or lapatinib in combination with platinum-based chemotherapy for treatment of recurrent ovarian cancer. Inhibitors against SIK2 hold great promise in enhancing the efficacy of chemotherapy that is currently used in the first-line and second-line therapies.

To realize the promise of developing targeted therapy against SIK2, it is important to understand the long-term benefits and potential tumor resistance mechanisms of SIK2 inhibition alone and in combination with chemotherapy to suppress tumor recurrence. In this study, the *in vivo* experiments with siRNAs were designed to end by 6 weeks after inoculation with cancer cells in immunodeficient mice (6). However, it remains unclear whether the observed 70% reduction in tumor burden in mice treated with SIK2 siRNA/DOPC will be sufficient to extend the survival of these mice (6). In addition, although many molecularly targeted therapies, such as erlotinib targeting mutated EGFR in lung adenocarcinoma and trastuzumab targeting HER2-positive breast cancer, provide clinical benefits, their efficacies are limited by development of drug resistant tumors (9-11). In this study, the tumor burden in mice

appears to increase by 5 weeks after treatment with SIK2 siRNA/DOPC. Given that SIK2 and AMPK phosphorylate ACC in an additive manner, will long-term inhibition of SIK2 lead to adaptive AMPK activation to become the dominant mediator of ACC phosphorylation and allow cancer cells to bypass the requirement of SIK2?

In summary, the study by Miranda and colleagues identifies SIK2 as a highly promising target for preventing or suppressing abdominal metastasis of HGSOCS. SIK2 plays a key role in ovarian cancer cells following interaction with adipocytes to promote the PI3K-AKT pathway and fatty acid metabolism. Inhibition of SIK2 will have the potential to block the metastatic growth of HGSOCS at the adipocyte-rich microenvironment.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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