Editorial

Targeting sphingosine kinase 1 localization as novel target for ovarian cancer therapy

Caterina Bernacchioni[1], Paola Turano[2], Chiara Donati[1]

[1]Department of Experimental and Clinical Biomedical Sciences, [2]CERM and Department of Chemistry, University of Florence, Florence, Italy

Correspondence to: Paola Turano. Magnetic Resonance Center CERM, University of Florence , Via Luigi Sacconi 6, 50019 Sesto Fiorentino, Florence, Italy. Email: turano@cerm.unifi.it.

Comment on: Zhu W, Jarman KE, Lokman NA, *et al*. CIB2 Negatively Regulates Oncogenic Signaling in Ovarian Cancer via Sphingosine Kinase 1. Cancer Res 2017;77:4823-34.

Submitted Sep 21, 2017. Accepted for publication Sep 27, 2017.
doi: 10.21037/tcr.2017.10.05
View this article at: http://dx.doi.org/10.21037/tcr.2017.10.05

Ovarian cancer represents the first cause of death for gynaecological malignancies in the western countries. Since specific and sensitive methods for early detection are missing, the large majority of patients are diagnosed at advanced stages (1). Aggressive chemotherapy with carboplatin and paclitaxel following surgery is initially efficacious, although most of the patients acquire chemo-resistance and the clinical outcome in advanced ovarian cancer is very poor (1). Early disease detection and novel pharmacological treatment are therefore an unmet medical need. The molecular mechanisms underlying unrestricted proliferation and resistance to apoptosis of ovarian cancer cells are, however, scarcely understood.

An emerging key player in modulating malignancy appears to be sphingosine 1-phosphate (S1P), a bioactive lipid molecule crucially involved in the regulation of fundamental processes such as proliferation, migration, survival, angiogenesis (2), all demonstrated to be essential in the development of cancer. S1P exerts most of its functions through the ligation to its specific G-protein coupled receptors (3), however recently multiple intracellular targets have been identified (3). The enzyme responsible for S1P generation is sphingosine kinase (SK) that convert sphingosine into S1P using adenosine triphosphate. Two different SK isoform exist, SK1 and SK2, which display differences in structure and cellular localization (3). SK1 and SK2 have been reported to have both overlapping and opposite functions, nonetheless, while SK1, mostly activated by extracellular stimuli affects S1P extracellular levels, SK2 seems to play more an intracellular housekeeping role. Given its crucial role in sphingolipid metabolism, SK expression and activity regulates the balance between pro-survival S1P versus pro-apoptotic sphingosine thus determining the cell fate (3).

An oncogenic role for SK1 has been well-established in a variety of cancers (4). Its therapeutic role in ovarian cancer is now starting to be clarified.

Aberrantly elevated levels of S1P have been found in ascites samples from patients with ovarian cancer (5). In human ovary cancer, plasma S1P and SK1 levels are up-regulated (6), and these increases are linked to poor prognosis in patients (7).

Functional links between SK1 and ovarian cancer cell metabolome have been highlighted; indeed, SK1 has been shown to play a crucial role in the metabolic reprogramming of human ovarian cancer cells, inducing an aerobic glycolysis switch and affecting metabolic pathways that support the synthesis of macromolecules required for enhanced cancer cell proliferation (8). Targeting SK1 might represent a comprehensive approach for antagonizing the metabolic transformation of ovarian cancer cells.

Localization of SK1 is a key element for its signaling capacity and it is important to note that translocation may involve not just general translocation to the plasma membrane but the targeting to specific membrane domains. The mechanisms that regulate agonist-induced translocation of SK1 to the plasma membrane are not fully understood. It has been shown that localization of

SK1 is crucial for eliciting its oncogenic signaling. It has been reported that SK1 has intrinsic catalytic activity (9) but further activation induced by extracellular stimuli is required for its oncogenic role. Activation of SK1 is directly mediated by the ERK1/2-dependent phosphorylation on Ser225 residue and more importantly, this modification is responsible for translocation of the enzyme from cytosol to plasma membrane (10). It has been demonstrated that SK1 associates with phosphatidylserine in a phosphorylation-dependent manner, facilitating its retention at the plasma membrane (11). Moreover, generation of highly localized phosphatidic acid by phospholipase D at plasma membrane induces translocation and membrane retention of SK1 (12). In literature, several SK1 interacting protein have been reported (13); overexpression of RPK118 (14), a phox homology domain containing protein, seems to have effects on localization of SK1, suggesting its possible involvement in the retention of SK1 in precise subcellular fractions before agonist-induced translocation to plasma membrane. Future investigations, however, are required to establish the exact roles of the interaction of this protein with SK1. Recently Jarman *et al*. (15) have shown that CIB1, a Ca^{2+}-myristoyl switch protein, is essential for the translocation of SK1 from the cytoplasm to the plasma membrane. When Ca^{2+} is not present, the myristoyl group of CIB1 is sequestered into a hydrophobic pocket of the protein, while in the presence of Ca^{2+}, a conformational change occurs so that the myristoyl group is extruded allowing the interaction with partner proteins. CIB1, which has been found to be up-regulated in a large number of human cancers (4), has been demonstrated to enhance SK1 localization to the plasma membrane and represents an important effector of Ras oncogenic signaling (16).

Very recently, the paper by Zhu *et al*. (17) has added another piece of information to the SK1 complex signaling axis in cancer. They reported that another member of the CIB family, CIB2, is a novel tumor suppressor that negatively regulates the translocation and the subsequent activation of SK1. CIB2 is down-regulated in ovarian cancer patients, suggesting a role as potential prognostic marker for ovary tumors.

The authors found that CIB2 interacts with SK1 in the cytoplasm independently from the kinase phosphorylation on Ser225. Moreover, they identified the calmodulin binding site of SK1 being the CIB2 binding region, the same of CIB1 association (15,18). Zhu *et al*. (17) demonstrated that, differently from CIB1, despite being myristoylated, CIB2 lacks Ca^{2+}-myristoyl switch behavior. Moreover, the interaction between CIB2 and SK1 is not dependent on the presence of Ca^{2+} or Mg^{2+}. Therefore, unlike CIB1, CIB2 does not mediate the translocation of SK1 to the plasma membrane. Interestingly, the authors demonstrated that CIB2, associating with SK1 at the same site of CIB1, acts as an endogenous inhibitor of SK1 translocation. Phorbol myristate acetate (PMA)-induced SK1 translocation to the plasma membrane is indeed abolished in cells overexpressing CIB2. Consistent with a reduced access of SK1 to the plasma membrane where its substrate sphingosine is located, Zhu *et al*. (17) showed that S1P generation is significantly inhibited by CIB2 overexpression even if SK1 total activity is unaffected. The inhibition of SK1 translocation by CIB2 is likely mediated by direct binding, being the PMA-induced ERK1/2 phosphorylation and consequent SK1 activation unaffected by CIB2 overexpression.

The subcellular localization of SK1 is crucial for its role in cellular signaling (19). Hence, as a consequence of the inhibition of SK1 plasma membrane localization, CIB2 blocks the biological effects downstream to SK1 activation. In particular, Zhu *et al*. (17) demonstrated that CIB2 expression significantly inhibits the pro-survival action of TNFα and the neoplastic transformation induced by Ras, being these oncogenic signaling mediated by SK1 activation and plasma membrane translocation (15,16,20).

Given the well-known role of SK1 signaling in cancer (4), the authors analyzed CIB2 expression in different human tumors compared to the normal tissues and found that the expression of this protein is markedly reduced in ovarian cancer tissues compared to normal ovary, thus suggesting a tumor suppressor role for CIB2.

Zhu *et al*. (17) evaluated the protective role of CIB2 against ovarian cancer using two human ovarian cancer cell lines, SKOV3 and OV90, that show a significant reduction in CIB2 expression compared to normal ovarian tissues. The authors demonstrated that the stable re-expression of CIB2 blocks SK1 plasma membrane localization and suppresses the neoplastic growth of ovarian cancer *in vitro* and tumor growth in mice. Moreover, CIB2 re-expression inhibits migration and invasion of ovarian cancer cells both *in vivo* and *in vitro*, in accordance with the described role of SK1 in promoting cell migration and invasion in ovarian cancer (21).

Overexpression of SK1 is involved in conferring resistance to chemotherapeutics, promoting the survival of lung, breast, colon, prostate and leukemia cancer cells (4). In human ovarian carcinoma cells resistant to the synthetic

retinoid N-(4-hydroxyphenyl)retinamide (HPR), the level and activity of SK1 were found elevated. Interestingly, pharmacological blockade of SK1 increased sensitivity to HPR, while overexpression of SK1 was sufficient to induce resistance to HPR in A2780 human ovarian cancer cells (22). Of note, Zhu *et al*. (17) confirmed the reported role of SK1 in ovarian cancer chemoresistance (7,22,23), demonstrating that higher SK1 expression correlates with poorer survival of ovarian cancer patients treated with standard chemotherapy. Moreover, they provided the first evidence of a correlation between low levels of CIB2 expression and poor survival of ovarian cancer patients treated with platinum and taxane-based chemotherapy. These findings are very important because suggest a tumor suppressor role for CIB2 in human ovarian cancer. This concept is strengthened by the demonstration that re-expression of CIB2 sensitized ovarian cancer cells to carboplatin.

Zhu *et al*. (17) have made a very significant advance in the understanding of SK1 involvement in ovarian cancer development and resistance to chemotherapeutics, identifying CIB2 as a novel endogenous suppressor of SK1 signaling and potential prognostic biomarker.

Taken together, these findings provide the evidence of a new mechanism regulating SK1 signaling depending on Ca^{2+} intracellular content. Under basal conditions characterized by low cytoplasmic Ca^{2+} levels, SK1 mainly interacts with CIB2 in the cytoplasm: the formation of the complex hinders SK1 translocation to the plasma membrane. However, increased intracellular Ca^{2+} content induced by cell stimulation with different cues enhances CIB1 interaction with SK1 thus inducing CIB1-mediated translocation of the enzyme to the plasma membrane, independently from its phosphorylation state on Ser225. Thus, both CIB1 and CIB2 regulate SK1 signaling through the modulation of its plasma membrane localization. The disruption of the mechanism that regulates SK1 location will lead to aberrant SK1 signaling, critical for cancer development. Indeed, the up-regulation of CIB1, recently described in multiple cancers, including ovarian cancer (16), or the down-regulation of CIB2 reported by Zhu *et al*. (17) in ovarian cancer, lead to increased SK1 plasma membrane translocation and resultant oncogenic signaling.

Altogether, these studies indicate that dysregulation of human SK1 localization might be a crucial aspect in the acquisition of malignant phenotype, enhancing cell proliferation and protecting ovarian cancer cells from apoptosis. Targeting the mechanisms that regulate SK1 membrane translocation will provide innovative hints for future therapies against ovarian cancer.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Zheng Li (Department of Gynecologic Oncology, The Third Affiliated Hospital of Kunming Medical University, Kunming, China).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2017.10.05). The authors have no conflicts of interest to declare.

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.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013;63:11-30.
2. Maceyka M, Harikumar KB, Milstien S, et al. Sphingosine-1-phosphate signaling and its role in disease. Trends Cell Biol 2012;22:50-60.
3. Newton J, Lima S, Maceyka M, et al. Revisiting the sphingolipid rheostat: Evolving concepts in cancer therapy. Exp Cell Res 2015;333:195-200.
4. Heffernan-Stroud LA, Obeid LM. Sphingosine kinase 1 in cancer. Adv Cancer Res 2013;117:201-35.
5. Wang D, Zhao Z, Caperell-Grant A, et al. S1P differentially regulates migration of human ovarian cancer and human ovarian surface epithelial cells. Mol Cancer

Ther 2008;7:1993-2002.
6. Sutphen R, Xu Y, Wilbanks GD, et al. Lysophospholipids are potential biomarkers of ovarian cancer. Cancer Epidemiol Biomarkers Prev 2004;13:1185-91.
7. Beach JA, Aspuria PJ, Cheon DJ, et al. Sphingosine kinase 1 is required for TGF-beta mediated fibroblastto-myofibroblast differentiation in ovarian cancer. Oncotarget 2016;7:4167-82.
8. Bernacchioni C, Ghini V, Cencetti F, et al. NMR metabolomics highlights sphingosine kinase-1 as a new molecular switch in the orchestration of aberrant metabolic phenotype in cancer cells. Mol Oncol 2017;11:517-33.
9. Pitson SM, D'Andrea R J, Vandeleur L, et al. Human sphingosine kinase: purification, molecular cloning and characterization of the native and recombinant enzymes. Biochem J 2000;350 Pt 2:429-41.
10. Pitson SM, Xia P, Leclercq TM, et al. Phosphorylation-dependent translocation of sphingosine kinase to the plasma membrane drives its oncogenic signalling. J Exp Med 2005;201:49-54.
11. Stahelin RV, Hwang JH, Kim JH, et al. The mechanism of membrane targeting of human sphingosine kinase 1. J Biol Chem 2005;280:43030-8.
12. Delon C, Manifava M, Wood E, et al. Sphingosine kinase 1 is an intracellular effector of phosphatidic acid. J Biol Chem 2004;279:44763-74.
13. Wattenberg BW, Pitson SM, Raben DM. The sphingosine and diacylglycerol kinase superfamily of signaling kinases: localization as a key to signaling function. J Lipid Res 2006;47:1128-39.
14. Hayashi S, Okada T, Igarashi N, et al. Identification and characterization of RPK118, a novel sphingosine kinase-1-binding protein. J Biol Chem 2002;277:33319-24.
15. Jarman KE, Moretti PA, Zebol JR, et al. Translocation of sphingosine kinase 1 to the plasma membrane is mediated by calcium- and integrin-binding protein 1. J Biol Chem 2010;285:483-92.
16. Zhu W, Gliddon BL, Jarman KE, et al. CIB1 contributes to oncogenic signalling by Ras via modulating the subcellular localisation of sphingosine kinase 1. Oncogene 2017;36:2619-27.
17. Zhu W, Jarman KE, Lokman NA, et al. CIB2 Negatively Regulates Oncogenic Signaling in Ovarian Cancer via Sphingosine Kinase 1. Cancer Res 2017;77:4823-34.
18. Sutherland CM, Moretti PA, Hewitt NM, et al. The calmodulin-binding site of sphingosine kinase and its role in agonist-dependent translocation of sphingosine kinase 1 to the plasma membrane. J Biol Chem 2006;281:11693-701.
19. Wattenberg BW. Role of sphingosine kinase localization in sphingolipid signaling. World J Biol Chem 2010;1:362-8.
20. Xia P, Wang L, Moretti PA, et al. Sphingosine kinase interacts with TRAF2 and dissects tumor necrosis factor-alpha signaling. J Biol Chem 2002;277:7996-8003.
21. Dai L, Xia P, Di W. Sphingosine 1-phosphate: a potential molecular target for ovarian cancer therapy? Cancer Invest 2014;32:71-80.
22. Illuzzi G, Bernacchioni C, Aureli M, et al. Sphingosine kinase mediates resistance to the synthetic retinoid N-(4-hydroxyphenyl)retinamide in human ovarian cancer cells. J Biol Chem 2010;285:18594-602.
23. Zhang H, Wang Q, Zhao Q, et al. MiR-124 inhibits the migration and invasion of ovarian cancer cells by targeting SphK1. J Ovarian Res 2013;6:84.

Cite this article as: Bernacchioni C, Turano P, Donati C. Targeting sphingosine kinase 1 localization as novel target for ovarian cancer therapy. Transl Cancer Res 2017;6(Suppl 7):S1277-S1280. doi: 10.21037/tcr.2017.10.05

