TOPK (T-LAK cell-originated protein kinase) inhibitor exhibits growth suppressive effect on small cell lung cancer

Jae-Hyun Park,1 Hiroyuki Inoue,1 Taigo Kato,1 Makda Zewde,1 Takashi Miyamoto,2 Yo Matsuo,2 Ravi Salgia1,3 and Yusuke Nakamura1

1Department of Medicine, The University of Chicago, Chicago, IL, USA; 2OncoTherapy Science Inc., Kawasaki, Japan

Key words
Cancer stem cell, kinase inhibitors, molecular target, small cell lung cancer, TOPK

Correspondence
Yusuke Nakamura, Department of Medicine and Surgery, The University of Chicago, 900 E 57th Street KCBD 6130, Chicago, IL 60637, USA.
Tel: +1-773-834-1405; Fax: +1-773-702-9268; E-mail: ynakamura@bsd.uchicago.edu

Present address: Department of Medical Oncology and Therapeutics Research, City of Hope, Duarte, CA, 91010, USA

Funding Information
The University of Chicago Medicine Comprehensive Cancer Center, OncoTherapy Science Inc., Cancer Research Foundation

Received November 17, 2016; Revised December 15, 2016; Accepted December 17, 2016

Cancer Sci 108 (2017) 488–496
doi: 10.1111/cas.13160

T-lymphokine-activated killer cell–originated protein kinase (TOPK) plays critical roles in cancer cell proliferation as well as maintenance of cancer stem cells (CSC). Small cell lung cancer (SCLC) has highly aggressive phenotype, reveals early spread to distant sites, and results in dismal prognosis with little effective treatment. In this study, we demonstrate that TOPK expression was highly upregulated in both SCLC cell lines and primary tumors. Similar to siRNA-mediated TOPK knockdown effects, treatment with a potent TOPK inhibitor, OTS514, effectively suppressed growth of SCLC cell lines (IC₅₀ 0.4–42.6 nM) and led to their apoptotic cell death. TOPK inhibition caused cell morphologic changes in SCLC cells, elongation of intercellular bridges caused by cytokinesis defects or neuronal protrusions induced by neuronal differentiation in a subset of CSC-like SCLC cells. Treatment with OTS514 suppressed forkhead box protein M1 (FOXM1) activity, which was involved in stemness of CSC. Furthermore, OTS514 treatment reduced CD90-positive SCLC cells and showed higher cytotoxic effect against lung sphere-derived CSC-like SCLC cells. Collectively, our results suggest that targeting TOPK is a promising approach for SCLC therapy.

Materials and Methods

Cell lines. Three adherent SCLC cell lines (DMS114, H69AR and H446), five suspension SCLC cell lines (H69, H82, H146, H524 and H2171), and two normal fetal lung fibroblasts cell lines (IMR-90 and WI-38) were purchased from the American Type Culture Collection (ATCC) (Manassas,
VA, USA), DMS273 cell line was purchased from European Collection of Authenticated Cell Cultures (ECACC, Salisbury, UK). SBC-3 and SBC-5 cell lines were purchased from Japanese Collection of Research Biorcesources Cell Bank (JCRB) (Suitsa, Japan). IMR-90, WI-38, SBC3, and SBC5 cell lines were cultured in EMEM medium (Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimyotic solution (Sigma-Aldrich, St. Louis, MO, USA). Other SCLC cell lines were cultured in RPMI medium (Life Technologies) supplemented with 10% FBS. All cells were maintained at 37°C in humidified air with 5% CO2. Cell authentication results for SBC3, SBC5, DMS114, H446, H82 and H524 cells were previously described. 

Knockdown experiments. To knockdown expression of TOPK, SCLC cell lines were transiently transfected with 20 pmol/mL of oligo siRNA using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) reagent according to the manufacturer’s instructions. Oligo siRNAs of si-TOPK (5’-GUGUGCUUUGCCGUAUA-3’) and si-control (Mission siRNA Universal Negative Control, catalog #SIC001) were purchased from Sigma-Aldrich.

Cell viability assay. Cell viability was assessed by methyl thiazolyl tetrazolium (MTT) assay using the Cell Counting Kit-8, the 96-well plate was read at 450 nm of wavelength in the iMark microplate absorption reader (Bio-Rad, Hercules, CA, USA). All of these experiments were done in triplicate.

Knockdown experiments. To knockdown expression of TOPK, SCLC cell lines were transiently transfected with 20 pmol/mL of oligo siRNA using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) reagent according to the manufacturer’s instructions. Oligo siRNAs of si-TOPK (5’-GUGUGCUUUGCCGUAUA-3’) and si-control (Mission siRNA Universal Negative Control, catalog #SIC001) were purchased from Sigma-Aldrich.

Cell viability assay. Cell viability was assessed by methyl thiazolyl tetrazolium (MTT) assay using the Cell Counting Kit-8, the 96-well plate was read at 450 nm of wavelength in the iMark microplate absorption reader (Bio-Rad, Hercules, CA, USA). All of these experiments were done in triplicate.

Knockdown experiments. To knockdown expression of TOPK, SCLC cell lines were transiently transfected with 20 pmol/mL of oligo siRNA using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) reagent according to the manufacturer’s instructions. Oligo siRNAs of si-TOPK (5’-GUGUGCUUUGCCGUAUA-3’) and si-control (Mission siRNA Universal Negative Control, catalog #SIC001) were purchased from Sigma-Aldrich.

Cell viability assay. Cell viability was assessed by methyl thiazolyl tetrazolium (MTT) assay using the Cell Counting Kit-8, the 96-well plate was read at 450 nm of wavelength in the iMark microplate absorption reader (Bio-Rad, Hercules, CA, USA). All of these experiments were done in triplicate.

Knockdown experiments. To knockdown expression of TOPK, SCLC cell lines were transiently transfected with 20 pmol/mL of oligo siRNA using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) reagent according to the manufacturer’s instructions. Oligo siRNAs of si-TOPK (5’-GUGUGCUUUGCCGUAUA-3’) and si-control (Mission siRNA Universal Negative Control, catalog #SIC001) were purchased from Sigma-Aldrich.

Cell viability assay. Cell viability was assessed by methyl thiazolyl tetrazolium (MTT) assay using the Cell Counting Kit-8, the 96-well plate was read at 450 nm of wavelength in the iMark microplate absorption reader (Bio-Rad, Hercules, CA, USA). All of these experiments were done in triplicate.

Knockdown experiments. To knockdown expression of TOPK, SCLC cell lines were transiently transfected with 20 pmol/mL of oligo siRNA using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) reagent according to the manufacturer’s instructions. Oligo siRNAs of si-TOPK (5’-GUGUGCUUUGCCGUAUA-3’) and si-control (Mission siRNA Universal Negative Control, catalog #SIC001) were purchased from Sigma-Aldrich.

Cell viability assay. Cell viability was assessed by methyl thiazolyl tetrazolium (MTT) assay using the Cell Counting Kit-8, the 96-well plate was read at 450 nm of wavelength in the iMark microplate absorption reader (Bio-Rad, Hercules, CA, USA). All of these experiments were done in triplicate.

Knockdown experiments. To knockdown expression of TOPK, SCLC cell lines were transiently transfected with 20 pmol/mL of oligo siRNA using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) reagent according to the manufacturer’s instructions. Oligo siRNAs of si-TOPK (5’-GUGUGCUUUGCCGUAUA-3’) and si-control (Mission siRNA Universal Negative Control, catalog #SIC001) were purchased from Sigma-Aldrich.

Cell viability assay. Cell viability was assessed by methyl thiazolyl tetrazolium (MTT) assay using the Cell Counting Kit-8, the 96-well plate was read at 450 nm of wavelength in the iMark microplate absorption reader (Bio-Rad, Hercules, CA, USA). All of these experiments were done in triplicate.

Knockdown experiments. To knockdown expression of TOPK, SCLC cell lines were transiently transfected with 20 pmol/mL of oligo siRNA using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) reagent according to the manufacturer’s instructions. Oligo siRNAs of si-TOPK (5’-GUGUGCUUUGCCGUAUA-3’) and si-control (Mission siRNA Universal Negative Control, catalog #SIC001) were purchased from Sigma-Aldrich.

Cell viability assay. Cell viability was assessed by methyl thiazolyl tetrazolium (MTT) assay using the Cell Counting Kit-8, the 96-well plate was read at 450 nm of wavelength in the iMark microplate absorption reader (Bio-Rad, Hercules, CA, USA). All of these experiments were done in triplicate.

Knockdown experiments. To knockdown expression of TOPK, SCLC cell lines were transiently transfected with 20 pmol/mL of oligo siRNA using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) reagent according to the manufacturer’s instructions. Oligo siRNAs of si-TOPK (5’-GUGUGCUUUGCCGUAUA-3’) and si-control (Mission siRNA Universal Negative Control, catalog #SIC001) were purchased from Sigma-Aldrich.

Cell viability assay. Cell viability was assessed by methyl thiazolyl tetrazolium (MTT) assay using the Cell Counting Kit-8, the 96-well plate was read at 450 nm of wavelength in the iMark microplate absorption reader (Bio-Rad, Hercules, CA, USA). All of these experiments were done in triplicate.

Knockdown experiments. To knockdown expression of TOPK, SCLC cell lines were transiently transfected with 20 pmol/mL of oligo siRNA using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) reagent according to the manufacturer’s instructions. Oligo siRNAs of si-TOPK (5’-GUGUGCUUUGCCGUAUA-3’) and si-control (Mission siRNA Universal Negative Control, catalog #SIC001) were purchased from Sigma-Aldrich.

Cell viability assay. Cell viability was assessed by methyl thiazolyl tetrazolium (MTT) assay using the Cell Counting Kit-8, the 96-well plate was read at 450 nm of wavelength in the iMark microplate absorption reader (Bio-Rad, Hercules, CA, USA). All of these experiments were done in triplicate.

Knockdown experiments. To knockdown expression of TOPK, SCLC cell lines were transiently transfected with 20 pmol/mL of oligo siRNA using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) reagent according to the manufacturer’s instructions. Oligo siRNAs of si-TOPK (5’-GUGUGCUUUGCCGUAUA-3’) and si-control (Mission siRNA Universal Negative Control, catalog #SIC001) were purchased from Sigma-Aldrich.

Cell viability assay. Cell viability was assessed by methyl thiazolyl tetrazolium (MTT) assay using the Cell Counting Kit-8, the 96-well plate was read at 450 nm of wavelength in the iMark microplate absorption reader (Bio-Rad, Hercules, CA, USA). All of these experiments were done in triplicate.

Knockdown experiments. To knockdown expression of TOPK, SCLC cell lines were transiently transfected with 20 pmol/mL of oligo siRNA using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) reagent according to the manufacturer’s instructions. Oligo siRNAs of si-TOPK (5’-GUGUGCUUUGCCGUAUA-3’) and si-control (Mission siRNA Universal Negative Control, catalog #SIC001) were purchased from Sigma-Aldrich.

Cell viability assay. Cell viability was assessed by methyl thiazolyl tetrazolium (MTT) assay using the Cell Counting Kit-8, the 96-well plate was read at 450 nm of wavelength in the iMark microplate absorption reader (Bio-Rad, Hercules, CA, USA). All of these experiments were done in triplicate.

Knockdown experiments. To knockdown expression of TOPK, SCLC cell lines were transiently transfected with 20 pmol/mL of oligo siRNA using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) reagent according to the manufacturer’s instructions. Oligo siRNAs of si-TOPK (5’-GUGUGCUUUGCCGUAUA-3’) and si-control (Mission siRNA Universal Negative Control, catalog #SIC001) were purchased from Sigma-Aldrich.

Cell viability assay. Cell viability was assessed by methyl thiazolyl tetrazolium (MTT) assay using the Cell Counting Kit-8, the 96-well plate was read at 450 nm of wavelength in the iMark microplate absorption reader (Bio-Rad, Hercules, CA, USA). All of these experiments were done in triplicate.
conjugated anti-human CD56 antibody (eBioscience) for 20 min at room temperature. To measure expression levels of CD90 surface protein, cells were stained with APC-conjugated anti-human CD90 antibody (5E10) (BD Pharmingen) for 15 min at room temperature. After washing with PBS, samples were subjected to flow cytometry instruments (FACS Calibur or FACS LSRII; Becton Dickinson, San Jose, CA, USA) and analyzed using Flow Jo software (Treestar, Ashland, OR, USA).

**Sphere formation assays.** 1 × 10⁴ of adherent SCLC cells (SBC3, H446, and H69AR) were initially seeded onto the ultra-low attachment 96-well plate (Corning, Lowell, MA, USA) and cultured for 8 days to examine lung sphere (LS) formation under treatment with TOPK inhibitor OTS514. Subsequently, through gentle pipetting, the detached SCLC cells were transferred into another ultra-low attachment 96-well plate for additional 7-day culture. Then LS formation was examined by an inverted microscope Axio Vert.A1 TL (Carl Zeiss Microscopy, Thornwood, NY, USA).

**Statistical analyses.** Data were expressed as mean ± one standard deviation. Differences between two groups were examined for the statistical significance by the student’s t-test, using GraphPad Prism 6 software (GraphPad, San Diego, CA, USA). Differences at P < 0.05 were considered to be significant.

**Results**

**High levels of TOPK expression in SCLC cell lines and primary SCLC tissues.** To examine TOPK expression levels in SCLC, we performed immunoblot analyses using 11 human SCLC cell lines (six adherent cells and five suspension cells) and two normal fetal lung fibroblast (NFLF) cell lines. Our results showed that TOPK protein was highly expressed in the majority of the SCLC cell lines examined, whereas it was hardly detectable in two NFLF normal cell lines (Fig. 1a,b). In addition, the Oncomine database(22) revealed that TOPK expression in primary SCLC tissues was significantly higher than in normal lung tissues (P = 0.0008) (Fig. 1c).

**Decrease of cell viability by siRNA-mediated knockdown of TOPK.** To examine the growth promoting effect of TOPK in SCLC cells, we performed a loss of function approach with specific siRNA (si-TOPK) using six adherent SCLC cell lines. Our results from quantitative RT-PCR showed that knockdown of TOPK by si-TOPK significantly decreased the TOPK expression in all of six adherent SCLC cell lines, compared with si-control (**P < 0.01, ***P < 0.001) (Fig. 2a). Immunoblot analyses confirmed reduction of TOPK protein levels in the SCLC cell lines tested at 48 h after si-TOPK transfection (Fig. 2b). MTT assay concordantly revealed significant decrease of the cell viability in the SCLC cell lines transfected with si-TOPK, compared with those transfected with si-control (*P < 0.05, **P < 0.01 or ***P < 0.001) (Fig. 2c). These results suggested that TOPK is likely to play critical roles in the proliferation and/or survival of SCLC cells.

**Growth-suppressive activity of TOPK inhibitor in SCLC cell lines.** We then assessed growth-suppressive effects of a potent TOPK inhibitor, OTS514.(14) Since TOPK is known to be auto-phosphorylated,(1) we first examined effect of OTS514 on TOPK protein itself in the SCLC cells. We treated four SCLC cell lines with 10 or 20 nM of OTS514 for 48 h, and found that OTS514 treatment reduced the TOPK protein level in a dose-dependent manner (Fig. 3a), indicating that the autophosphorylation might be required to maintain the stability of TOPK protein. Similar to the TOPK knockdown effect, OTS514 treatment exhibited strong growth-suppressive effects on all of the six adherent SCLC cell lines with the half-maximum inhibitory concentration (IC₅₀) of 1.3–8.4 nM (Fig. 3b).

We also examined five suspension SCLC cell lines and found that OTS514 treatment revealed stronger growth-suppressive effects on three cell lines (H69, H82, and H524 with IC₅₀ of 0.4–7.2 nM) that have very high levels of TOPK expression, but revealed relatively weaker growth-suppressive effects on two cell lines (H146 and H2171) with IC₅₀ of 39.3 nM and 42.6 nM, respectively), which showed relatively lower TOPK expression levels (Fig. 3c). It is notable that H446 cells bearing high CSC properties,(23) and H69AR cells which are resistant to multi-cytotoxic agents, were also very sensitive to this compound with IC₅₀ values of 8.4 nM and 7.3 nM, respectively. Microscopic observation apparently exhibited cytotoxic effects of OTS514 in adherent SCLC cells in a dose-
Fig. 3. Treatment with TOPK inhibitor shows marked growth-suppressive activity against SCLC cells. (a) Western blot analyses were performed to measure TOPK protein levels in adherent and suspension SCLC cells, 48 h after treatment with OTS514 (0, 10, and 20 nM). MTT assay was performed in six adherent SCLC cells (b) or in five suspension SCLC cells (c). All cells were measured their viability after treatment with OTS514 at different concentrations (0–50 nM) for 72 h. Graphs indicate relative cell viability at each OTS514 concentration, compared to a negative control (untreated). (d) The cellular effects induced by OTS514 treatment (10 nM or 20 nM) in three adherent SCLC cells and two NFLF cells were evaluated at 72 h after treatment by microscopic observation (×200 magnification). Scale bar indicates 50 μm.
dependent manner, while two NFLF cells remained undamaged with the treatment at the same concentration (Fig. 3d).

Treatment of TOPK inhibitor induces apoptosis in SCLC cells. To address the molecular mechanism of cytotoxic effects by OTS514, we evaluated its effects on apoptosis during the treatment. Flow cytometry analyses after 48 h of OTS514 treatment revealed increased proportion of apoptotic cells in a dose-dependent manner (Fig. 4a). We then explored the use of

Fig. 4. Treatment with TOPK inhibitor causes apoptosis in SCLC cells. (a) Three adherent SCLC cell lines were treated with 10 or 20 nM of OTS514. At 48 h of the treatment, Annexin-V and PI staining assay was performed to detect apoptosis. The numbers represent the percentage of cells in each quarter. (b) At 72 h of treatment with 10 or 20 nM of OTS514, flow cytometry analysis was conducted to comparatively quantify levels of cleaved caspase-3 activity in SCLC cells. The numbers depict the percentage of cells in each gate.
an antibody against an active (cleaved) form of caspase-3 for the detection of the execution phase of apoptotic events. As shown in Fig. 4b, OTS514 treatment induced activation of caspase-3 in a dose-dependent manner in SCLC cells.

Cell morphological changes by TOPK inhibition. To further understand the mechanism of action of OTS514, we examined possible TOPK-signaling pathways in SCLC cells. Since forkhead box protein M1 (FOXM1) was reported to function as an oncogenic transcriptional factor and a master regulator of mitosis and stemness in CSC, we investigated FOXM1 activity at protein level in the OTS514-treated SCLCs. We found that an active form of FOXM1, phosphorylated FOXM1 protein, was reduced (although the amounts of total FOXM1 protein were different in different cell lines) in adherent SCLC cells treated with OTS514 (Fig. 5c). Accordingly, OTS514 treatment reduced protein level of MELK, which is a downstream of FOXM1 and involved in the cancer stemness as shown in Fig. 5a. It was also interesting that OTS514 treatment downregulated FOXM1 transcriptional level in two out of three SCLC cell lines (Fig. S1b), likely as we previously observed in kidney cancer cells after TOPK knockdown. Collectively, these results suggested that OTS514 treatment suppressed FOXM1 and MELK activity that play important roles in the proliferation/stemness of CSC.

TOPK inhibitor preferentially suppresses the lung sphere formation. To further evaluate the therapeutic potential of OTS514 on CSC subpopulation, we examined the protein expression level of CD90, one of the putative SCLC CSC markers, in OTS514-treated and -untreated SCLC cells. Flow cytometry analysis showed that OTS514 treatment clearly decreased proportion of CD90-positive cells (Fig. 6a) as well as the intensity of CD90 (Fig. 6b) in all SCLC cells examined. We also conducted lung sphere (LS) formation assay because adherent SCLC cells can grow as spheres that are enriched with CSC subpopulation harboring higher in vitro clonogenic and in vivo tumorigenic potentials. The LS formation was developed through serial passage of cancer cells under low attachment culture condition as described previously. After microscopic confirmation of LS development after 15 days of culture, we mechanistically dissociated LS into single cell suspension and treated these LS-derived SCLC cells with or without OTS514. Subsequently, we compared the sensitivity to OTS514 treatment between the LS-derived SCLC cells and parental adherent SCLC cells by MTT assay, and found that OTS514 treatment more significantly suppressed the cell viability of LS-derived SCLC cells than that of parental adherent SCLC cells in a dose-dependent manner (Fig. 6c), indicating a possibility that OTS514 treatment might more effectively suppress the CSC subpopulation of SCLC cells.

Discussion

T-LAK cell-originated protein kinase has been shown to be highly upregulated in various types of human cancer and its overexpression is correlated with poor prognosis of cancer patients, but its biological or clinicopathological roles in SCLC, an aggressive phenotype of tumor enriched with CSC...
subpopulation, have not been addressed. Since it was suggested as one of CSC consensus markers, TOPK may play pivotal roles in development/progression of SCLC. Indeed, this study demonstrated that TOPK was highly expressed in the great majority of SCLC cell lines as well as primary SCLC tumors, compared with normal control cells or normal lung tissues.

We attempted to address the biological role of TOPK in growth of SCLC cells by two loss-of-function approaches, depletion of TOPK expression by siRNA and inhibition of TOPK kinase activity by a small molecular compound OTS514. Both approaches led to strong growth-suppressive effects on the majority of SCLC cell lines with rapid induction...
of apoptosis, clearly indicating that TOPK is indispensable for the proliferation and/or survival of SCLC cells. Interestingly, OTS514 treatment could induce apoptosis even in the CSC-like H446 cells, suggesting that OTS514 is a promising treatment modality for refractory SCLC where CSCs are enriched and anti-apoptotic proteins are overexpressed. (36) Except two SCLC cell lines (H146 and H2171) having low levels of TOPK expression, nine SCLC cell lines with high TOPK expression were very sensitive to OTS514 treatment with IC50 TOPK expression, nine SCLC cell lines with high TOPK expression. (21) In addition, TOPK inhibition induced morphological changes in SCLC cells, such as intercellular bridges and neuronal protrusions. Considering TOPK’s roles in cancer cell mitosis and stemness, it was likely that most proliferating cancer cells might undergo cytokinetic failure that resulted in cell death, meanwhile a subpopulation of CSC was provoked to differentiate into neuron-like cell morphology. Thus, TOPK inhibitor might also have the function as a differentiation inducer, which aims to force some subpopulation of SCLC cells to resume the neuronal differentiation from progenitor phenotype. Related with a possible TOPK pathway, our analyses indicated that OTS514 treatment decreased the activity of FOXM1, which was accompanied by reduction of MELK protein. MELK is a downstream of FOXM1 and also plays important roles in SCLC CSCs. (21) It is likely that autophosphorylation of TOPK is critical for maintenance of TOPK protein stability, thus TOPK inhibition by OTS514 treatment is a very effective way to suppress TOPK’s downstream molecules, FOXM1 and MELK. Therefore, targeting these correlative molecules may provide a new modality to attack the vulnerabilities of CSC, as we previously showed preferential effects of MELK inhibitor for cancer treatment, as we reported in xenograft models of human cancer. (14) In addition, TOPK inhibition induced transcriptional changes in SCLC cells, such as intercellular bridges and neuronal protrusions. Considering TOPK’s roles in cancer cell mitosis and stemness, it was likely that most proliferating cancer cells might undergo cytokinetic failure that resulted in cell death, meanwhile a subpopulation of CSC was provoked to differentiate into neuron-like cell morphology. Thus, TOPK inhibitor might also have the function as a differentiation inducer, which aims to force some subpopulation of SCLC cells to resume the neuronal differentiation from progenitor phenotype. Related with a possible TOPK pathway, our analyses indicated that OTS514 treatment decreased the activity of FOXM1, which was accompanied by reduction of MELK protein. MELK is a downstream of FOXM1 and also plays important roles in SCLC CSCs. (21) It is likely that autophosphorylation of TOPK is critical for maintenance of TOPK protein stability, thus TOPK inhibition by OTS514 treatment is a very effective way to suppress TOPK’s downstream molecules, FOXM1 and MELK. Therefore, targeting these correlative molecules may provide a new modality to attack the vulnerabilities of CSC, as we previously showed preferential effects of MELK inhibitor on SCLC CSCs. (21) There is a possibility of complex cross-talk mechanism between TOPK and FOXM1. For example, our results showed that OTS514 treatment could downregulate transcriptional level of the FOXM1 gene in SBC5 and DMS273 cells. Indeed, our recent work using kidney cancer cell lines showed significant reduction of FOXM1 transcriptional level after knockdown of TOPK. (7) It was still unclear why total FOXM1 protein level was drastically reduced in the OTS514-treated SBC5 cells, but considering higher FOXM1 protein level and more CD90+ cell population in SBC5 cells than those in SBC3 and DMS273 cells, the TOPK-mediated upregulation of FOXM1 might be critical in the SBC5 cells which harbored more CSC-like subpopulation.

Finally, we investigated therapeutic potential of OTS514 on CSC and found that OTS514 treatment was effective to eliminate CD90+ CSC subpopulation in all seven SCLC cell lines examined. We also used a LS formation approach and demonstrated stronger growth-suppressive effects of OTS514 on the CSC-like SCLC cells. Our results suggested that OTS514 could target CSC and thus might be applied to a subset of human cancers where CSCs play dominant roles, such as glioblastoma, neuroblastoma, and malignant peritoneal mesothelioma.

In summary, we have clarified that TOPK plays pivotal roles in cancer progression and/or stem cell maintenance in SCLC cells. Our findings collectively suggest that the TOPK inhibitor OTS514 may be a promising molecular-targeted therapy to treat patients with SCLC.

Acknowledgments

This work was supported in part by a Team Science Award of UCCC (The University of Chicago Medicine Comprehensive Cancer Center), that from Cancer Research Foundation, and by a grant from OncoTherapy Science Inc. We appreciate Mr. Masao Mizuno for his gift to support this work.

Disclosure Statement

Y.N. is a stock holder and a scientific advisor of OncoTherapy Science, Inc. J.P. is a scientific advisor of OncoTherapy Science, Inc. T.M. and Y.M. are employees of OncoTherapy Science, Inc. The other authors declare no competing interests.

References

1. Park JH, Lin ML, Nishidate T, Nakamura Y, Katagiri T. PDZ-binding kinase/T-LAK cell-originate protein kinase, a putative cancer/testis antigen with an oncogenic activity in breast cancer. Cancer Res 2006; 66: 9186–95.
2. Komatsu M, Yoshimaru T, Matsuo T et al. Molecular features of triple negative breast cancer cells by genome-wide gene expression profiling analysis. Int J Oncol 2013; 42: 478–506.
3. Park JH, Nishidate T, Nakamura Y, Katagiri T. Critical roles of T-LAK cell-originated protein kinase in cytokinesis. Cancer Sci 2010; 101: 403–11.
4. Zhu F, Zyкова TA, Kang BS et al. Bidirectional signals transduced by TOPK-ERK interaction increase tumorigenesis of HCT116 colorectal cancer cells. Gastroenterology 2007; 133: 219–31.
5. Park JH, Nishidate T, Nakamura Y, Katagiri T. Critical roles of T-LAK cell-originated protein kinase in cytokinesis. Cancer Sci 2010; 101: 403–11.
6. Zhu F, Zyкова TA, Kang BS et al. Bidirectional signals transduced by TOPK-ERK interaction increase tumorigenesis of HCT116 colorectal cancer cells. Gastroenterology 2007; 133: 219–31.
7. Herrera-Martin D, Osuna D, Odone JL et al. Stable interference of EWS-FLI1 in an Ewing sarcoma cell line impairs IGF-1/IGF-1R signalling and reveals TOPK as a new target. Oncotarget 2015; 6: 33410–25.
8. Kato T, Inoue H,imoto S et al. Oncogenic roles of TOPK and MELK, and effective growth suppression by small molecular inhibitors in kidney cancer cells. Oncotarget 2016; 7: 17652–64.
9. Fujibuchi T, Abe Y, Takeuchi T et al. Expression and phosphorylation of TOPK during spermatogenesis. Dev Growth Differ 2005; 47: 637–44.
Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Effects of OTS514 treatment in SCLC cells. (a) Western blot analyses were performed to measure protein levels of phosphorylated FOXM1, total FOXM1, and MELK in 3 adherent SCLC cells untreated or treated with 10 nM of OTS514 for 48 h. (b) Real-time RT-PCR showed relative expression levels of FOXM1 in three adherent SCLC cells untreated or treated with 10 nM of OTS514 for 48 h.