Cell Chemical Biology

CellPress

Preview

The Right Tool for the Job: A Chemical and Genetic Toolkit for Interrogating DCLK1 Function

Linglan Fang[1] **and Dustin J. Maly**[1,2,*]
[1]Department of Chemistry, University of Washington, Seattle, WA 98195, USA
[2]Department of Biochemistry, University of Washington, Seattle, WA 98195, USA
*Correspondence: djmaly@uw.edu
https://doi.org/10.1016/j.chembiol.2020.09.007

Cell permeable, small molecule inhibitors are powerful tools for interrogating kinase function and validating drug targets. In this issue of *Cell Chemical Biology*, Liu and colleagues (2020) describe the development of a toolkit containing a highly selective DCLK1 inhibitor and complementary DCLK1 mutants for interrogating DCLK1-dependent cellular processes.

Small molecule chemical inhibition offers numerous advantages over pre-translational perturbation methods for studying intracellular protein function (Weiss et al., 2007). Small molecule inhibitors confer high temporal control over protein function, allowing primary biological effects to be discriminated from indirect secondary events. In addition, small molecule inhibitors provide more graded and reversible control over protein function than pre-translational perturbation methods. Importantly, demonstration that pharmacological inhibition of a protein provides a desired phenotype is an important component of the drug discovery process because most clinically approved therapeutics that target intracellular proteins are small molecules. Despite these advantages, the true potential of small molecule inhibitors as reagents for interrogating biology is often limited because it can be difficult to determine whether an observed phenotype is due to on-target rather than off-target effects. This limitation can be especially acute for small molecules that target protein kinases because most potent inhibitors of this enzyme family are ATP-competitive and discriminating between the >500 ATP-binding sites in the human kinome can be challenging. In this issue of *Cell Chemical Biology*, Liu et al. (2020) address this issue for the intriguing anticancer target Doublecortin-like kinase 1 (DCLK1) by developing a suite of chemical and genetic tools that can be used to interrogate the on-target effects of DCLK1 inhibition.

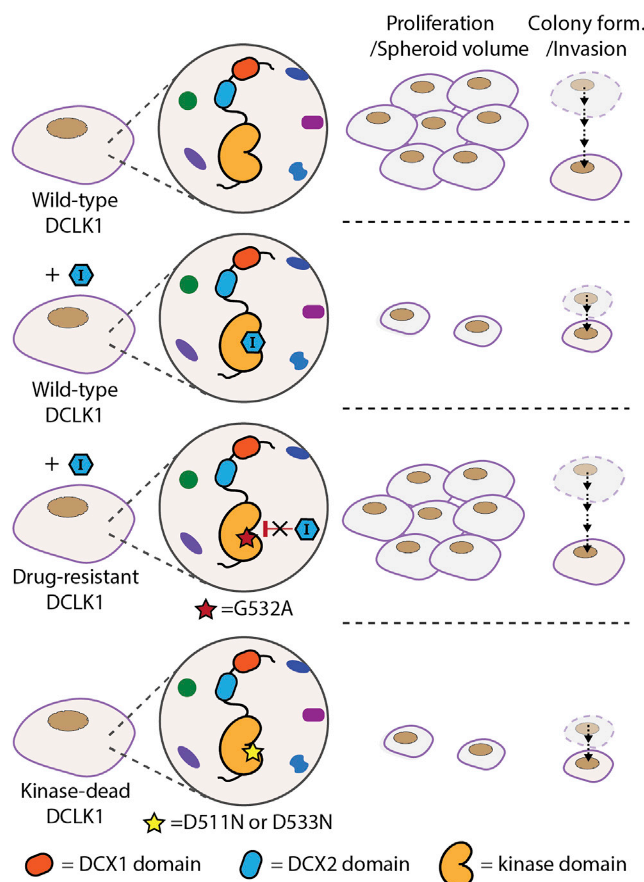

Figure 1. An Overview of the Molecular Toolkit for Interrogating DCLK1 Function
Liu et al. (2020) generated a panel of DCLK1 mutants (two kinase-dead mutants and a drug-resistant variant) that were used in combination with a highly selective small molecule inhibitor (DCLK1-IN-1) for studying DCLK1's function in DLD-1 cells.

DCLK1 is a multi-domain serine/threonine kinase that contains tandem microtubule-binding doublecortin domains at its N terminus that are connected to a C-terminal kinase domain through a linker rich in proline, glutamic acid, serine and threonine residues. Although DCLK1 was first characterized as a mediator of microtubule polymerization in migrating neurons, recent studies suggest that DCLK1 may play a prominent role in certain cancers (Cancer Genome Atlas Research Network, 2014; Weygant et al., 2015). DCLK1 is overexpressed in several cancers, and high DCLK1 expression levels are associated with poor clinical outcomes in colon and gastric cancer patients (Chandrakesan et al., 2017; Wu et al., 2020).

The potential promise of DCLK1 as an anti-cancer target has motivated the development of small molecule inhibitors that target DCLK1's ATP-binding site. Recently, the authors of this study used available crystal structures of DCLK1's kinase domain to develop the highly selective ATP-competitive inhibitor DCLK1-IN-1 and the structurally similar negative control DCLK1-NEG (Ferguson et al., 2020; Patel et al., 2016) DCLK1-IN-1 was demonstrated to be highly selective for DCLK1/2 over other protein kinases, capable of potently engaging DCLK1's ATP-binding site *in situ*, and to possess suitable pharmacokinetics for *in vivo* studies. Furthermore, DCLK1-IN-1, but not DCLK1-NEG, was found to block the proliferation of a subset of patient-derived pancreatic ductal adenocarcinoma (PDAC) organoids. The antiproliferative effects of DCLK1-IN-1 in patient-derived PDAC organoids correlated with DCLK1 expression and the downregulation of proteins associated with cell motility, suggesting a potential role for DCLK1 in modulating this process.

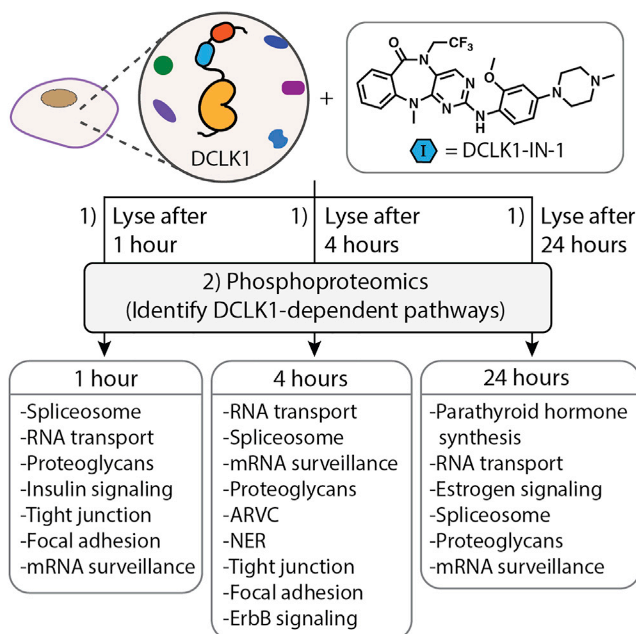

Figure 2. Strategy for Elucidating DCLK1-Dependent Processes with DCLK1-IN-1

DLD-1 cells overexpressing wild-type DCLK1 were treated for various amounts of time with DCLK1-IN-1 and comparative phosphoproteomic analyses were perfomred.

Liu et al. (2020) generated a small panel of DCLK1 mutants—two kinase-dead DCLK1 mutants and a drug-resistant DCLK1 variant—to complement DCLK1-IN-1 and DCLK1-NEG as tools for investigating the role of this kinase in diverse biological contexts (Figure 1). While the use of a drug-resistant protein variant is considered to be the "gold standard" for validating the on-target cellular effects of a small molecule inhibitor (Kapoor and Miller, 2017), identifying mutations that confer resistance without altering protein function can often be difficult. By analyzing co-crystal structures of kinases that are insensitive to DCLK1-IN-1 but capable of binding to structurally similar analogs, the authors of this study were able to identify a DCLK1 mutant—G532A—that was highly resistant to DCLK1-IN-1 but had similar phosphotransferase activity and effects on cell proliferationwhen overexpressed as the wild-type variant.

With this chemical genetic toolset in hand, Liu et al. (2020) first investigated cellular properties mediated by DCLK1's phosphotransferase activity in the colorectal adenocarcinoma DLD-1 cell line. Specifically, they focused on characterizing DCLK1's modulation of phenotypes associated with cellular malignancy. Overexpression of kinase-dead DCLK1 mutants, but not the wild-type variant, was found to reduce cell proliferation, colony formation, invasion, and spheroid formation in DLD-1s. Moreover, treatment of wild-type DLK1-overexpressing DLD-1 cells with DCLK1-IN-1 led to a similar attenuation of all four phenotypes, which was rescued by overexpression of the drug-resistant G532A DCLK1 mutant. Together, these results provide confidence that cell proliferation, colony formation, invasion, and spheroid formation are dependent on DCLK1's phosphotransferase activity in DLD-1 cells and that DCLK1-IN-1's ability to attenuate all four phenotypes is through inhibition of DCLK1.

Liu et al. (2020) next used DCLK1-IN-1 to identify potential DCLK1 substrates and elucidate DCLK1-dependent biological processes. To do this, comparative phosphoproteomic studies with wild-type DCLK1-overexpressing DLD-1s treated with DCLK1-IN-1 were performed (Figure 2). How DCLK1-IN-1 treatment influenced three parameters—significantly downregulated phosphopeptides, alteration of biologically related phosphopeptide levels, and changes in inferred kinase activity—was monitored over time. The authors found that DCLK1-IN-1 treatment most substantially reduced the phosphorylation of three proteins—the cyclin-dependent kinase CDK11, the DNA/RNA-binding protein MATR3, and DNA topoisomerase 2-beta (TOP2B)—that play roles in nucleic acid processing at the earliest time point monitored, suggesting that these proteins are direct substrates of DCLK1. A role for DCLK1 in modulating RNA processing/trafficking was previously unknown, but this study shows that DCLK1-IN-1 treatment leads to a persistent perturbation of RNA processing-related processes in DLD-1 cells. Coordinated alterations in phosphorylation events associated with biological pathways related to cell growth and migration were also observed in DCLK1-IN-1-treated DLD-1 cells. A GSEA analysis with a customized database that contains known kinase substrates was performed, and the inferred activities of several kinases (including ERK2 and CDK1/2) involved in signaling pathways that influence cell growth and migration appeared to be altered by DCLK1-IN-1 treatment, providing pathway-specific insight into how DCLK1 inhibition may influence these processes. These results are consistent with a previous report that suggests that DCLK1 indirectly promotes ERK activation and cyclin D1 upregulation through its interaction with the tyrosine kinase SRC (Ikezono et al., 2017). Together, these results provide additional insight into DCLK1's role in cell signaling and suggest new avenues for future exploration.

Beyond providing fundamental insight into DCLK1's function, this study (Liu et al., 2020) suggests that selective, small molecule inhibition of DCLK1 is a strategy worthy of further exploration for targeting certain cancers. The chemical and genetic DCLK1 toolkit described in this work will facilitate additional investigations into DCLK1's role in different cancer contexts. Furthermore, these efforts could result in the discovery of biomarkers that predict a cancer's response to selective DCLK1 inhibition. Liu et al. (2020) clearly demonstrate the advantages of combining genetic methods with chemical inhibition to study DCLK1 function. Hopefully, this work will motivate the development of similar toolsets for additional protein kinases. The availability of these types of reagents for numerous kinases would allow fundamental new insight into cell signaling to be obtained and greatly accelerate the drug target validation process.

ACKNOWLEDGMENTS

D.J.M. acknowledges support from grant R01GM086858 from the National Institutes of Health.

REFERENCES

Cancer Genome Atlas Research Network (2014). Comprehensive molecular characterization of gastric adenocarcinoma. Nature *513*, 202–209.

Chandrakesan, P., Yao, J., Qu, D., May, R., Weygant, N., Ge, Y., Ali, N., Sureban, S.M., Gude, M., Vega, K., et al. (2017). Dclk1, a tumor stem cell marker, regulates pro-survival signaling and self-renewal of intestinal tumor cells. Mol. Cancer *16*, 30.

Ferguson, F.M., Nabet, B., Raghavan, S., Liu, Y., Leggett, A.L., Kuljanin, M., Kalekar, R.L., Yang, A., He, S., Wang, J., et al. (2020). Discovery of a selective inhibitor of doublecortin like kinase 1. Nat. Chem. Biol. *16*, 635–643.

Ikezono, Y., Koga, H., Akiba, J., Abe, M., Yoshida, T., Wada, F., Nakamura, T., Iwamoto, H., Masuda, A., Sakaue, T., et al. (2017). Pancreatic Neuroendocrine Tumors and EMT Behavior Are Driven by the CSC Marker DCLK1. Mol. Cancer Res. *15*, 744–752.

Kapoor, T.M., and Miller, R.M. (2017). Leveraging Chemotype-Specific Resistance for Drug Target Identification and Chemical Biology. Trends Pharmacol. Sci. *38*, 1100–1109.

Liu, Y., Ferguson, F.M., Li, L., Kuljanin, M., Mills, C.E., Subramanian, K., Harshbarger, W., Gondi, S., Wang, J., Sorger, P.K., et al. (2020). Chemical Biology Toolkit for DCLK1 Reveals Connection to RNA Processing. Cell Chem. Biol., this issue, 1229–1240.

Patel, O., Dai, W., Mentzel, M., Griffin, M.D., Serindoux, J., Gay, Y., Fischer, S., Sterle, S., Kropp, A., Burns, C.J., et al. (2016). Biochemical and structural insights into doublecortin-like kinase domain 1. Structure *24*, 1550–1561.

Weiss, W.A., Taylor, S.S., and Shokat, K.M. (2007). Recognizing and exploiting differences between RNAi and small-molecule inhibitors. Nat. Chem. Biol. *3*, 739–744, https://doi.org/10.1038/nchembio1207-739.

Weygant, N., Qu, D., May, R., Tierney, R.M., Berry, W.L., Zhao, L., Agarwal, S., Chandrakesan, P., Chinthalapally, H.R., Murphy, N.T., et al. (2015). DCLK1 is a broadly dysregulated target against epithelial-mesenchymal transition, focal adhesion, and stemness in clear cell renal carcinoma. Oncotarget *6*, 2193–2205.

Wu, X., Qu, D., Weygant, N., Peng, J., and Houchen, C.W. (2020). Cancer stem cell marker DCLK1 correlates with tumorigenic immune infiltrates in the colon and gastric adenocarcinoma microenvironments. Cancers (Basel) *12*, 274.