IN THE SPOTLIGHT

Not Expecting the Unexpected: Diacylglycerol Kinase Alpha as a Cancer Target

Krishna P.L. Bhat and Kenneth Aldape

Summary: In this issue of *Cancer Discovery*, Dominguez and colleagues identify diacylglycerol kinase alpha (DGKα), an enzyme that converts the membrane lipid diacylglycerol to phosphatidic acid, as a central node upstream of mTOR and other oncogenic pathways. Importantly, targeting DGKα causes apoptosis in cancer cells and tumor growth inhibition in mice with no overt toxicity, implicating DGKα as a novel cancer-specific target. *Cancer Discov; 3(7); 726–7. ©2013 AACR.*

See related article by Dominguez et al., p. 782 (8).

In addition to surgery and radiation, chemotherapy is a widely used treatment modality for patients with cancer. On the basis of the observation that soldiers who were exposed to spilled sulfur mustards had depleted bone marrow and lymph nodes, the use of nitrogen mustard as a chemotherapeutic agent has its origins in the 1940s (1). The past seven decades since have seen an unprecedented explosion of our understanding of the disease, leading to the identification of numerous cancer chemotherapeutics (1). With biologic and technological advances, personalized treatment approaches have been successful for some cancers, wherein cancer cells are specifically targeted by exploiting genetic differences that underlie these cells compared with normal cells (2). For example, targeting the BCR–ABL tyrosine kinase enzyme with imatinib is a success story for chronic myeloid leukemia treatment (3). Similar approaches have been used to treat melanoma (with the BRAF V600E inhibitor vemurafenib) and non–small cell lung cancer (with the EGFR tyrosine kinase inhibitor gefitinib; ref. 2). However, these strategies have been less successful for some cancers, such as glioblastoma. Although more than half of glioblastomas show amplification of growth factor receptors and downstream kinases, inhibitors of these pathways are essentially ineffective in clinical trials (4).

Most targeted approaches rely primarily on genetic alterations that are unique to cancer cells, but given the heterogeneity in tumor cell populations, targeting one pathway is ineffective primarily due to the evolutionary dynamics of the tumor that lead to inevitable acquisition of additional genetic changes with tumor progression. An alternative approach is to identify and target central nodes that are common to cancer cells (compared with normal cells), irrespective of the genetic makeup of the tumor. Past studies that have uncovered such mechanisms have stemmed from unexpected quarters; pathways not previously linked to cancer that either act independently or converge into oncogenic pathways. Select examples include secretory pathway Ca^{2+}-ATPase (SPCA)-Orai1 complex that links Ca^{2+} signaling to cancer (5), the protumorigenic role of oxidized low-density lipoprotein (LDL) and oxidized LDL receptor 1 (OLR1) that otherwise play a role in atherosclerosis (6), and the dependence of glioma stem cells on the vascular smooth muscle relaxant nitric oxide synthase 2 (7). In line with these unexpected discoveries, Dominguez and colleagues (8) now report the dependence of cancer cells on the lipid metabolism enzyme diacylglycerol kinase alpha (DGKα) for their proliferation and exploit DGKα inhibition as a novel anticancer-specific strategy.

DGKs are a large family of conserved membrane lipid kinases that catalyze the conversion of diacylglycerol (DAG) to phosphatidic acid (9, 10). Mammalian DGKs are categorized into five classes (types 1–5) based on functional domains and substrate specificity. DGKα is a type I DGK that has been shown to play essential functions in T-cell receptor signaling, anergy, and proliferation of T lymphocytes (9, 10). Additional known functions of DGKα include hepatocyte growth factor–induced Rac activation and membrane ruffling, cell migration, regulation of DAG sensitive isoforms of protein kinase C, and VEGF-induced angiogenesis (9, 10). The role of DGKα in cancer, however, is less clear, and its oncogenic properties remain unexplored. In this backdrop, Dominguez and colleagues (8) sought to identify targets of microRNA 297 (miR-297), which they had previously shown to be cytotoxic for glioma cells. They found that although conventional oncogenes were not predicted to be targets of miR-297, DGKα stood out as one of the top targets. Given the previous known links of DGKα to mTOR signaling and VEGF, the authors postulated that DGKα is an important cancer cell survival gene that could be a potential therapeutic target.

Using complementary siRNA and small-molecule inhibitor approaches, they found that specifically targeting DGKα caused massive apoptosis in glioma cell lines, as evidenced by Annexin V membrane staining. The authors then tested whether the converse was true. Overexpression of DGKα caused enhanced proliferation of both glioma and melanoma cell lines. In a series of follow-up experiments, the authors found that DGKα mainly targets phosphatidic

Authors' Affiliations: Department of Pathology, and Brain Tumor Center, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Corresponding Authors: Kenneth D. Aldape, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: 713-792-0634; Fax: 713-745-4049; E-mail: kaldape@mdanderson.org; and Krishna P.L. Bhat, kbhat@mdanderson.org

doi: 10.1158/2159-8290.CD-13-0244

acid and mTOR in addition to other oncogenic pathways and that apoptosis observed after silencing DGKα could be partly rescued by overexpression of mTOR or phosphatidic acid. The authors extended their analyses to The Cancer Genome Atlas dataset and found a positive correlation of *DGKA* with *MTOR* in glioblastoma. Influenced by previous findings that phosphatidic acid promotes the activity of phosphodiesterases, decreasing cyclic AMP (cAMP), and that a cAMP-dependent transcription factor could induce *MTOR* mRNA expression, the authors elegantly showed that DGKα controls *MTOR* transcription via cAMP. They showed that stable knockdown of DGKα using short hairpin RNA or treatment with a DGKα inhibitor (R59022) decreased *MTOR* transcript levels with an accompanying increase in cAMP levels. Importantly, they found, using reporter assays, that treating cells directly with cAMP caused a significant decrease in *MTOR* transcription. This identified a novel role for DGKα in the proliferation of glioblastoma by controlling *MTOR* transcription via cAMP.

An obvious question that arises is that if DGKα plays an important role in cancer, why have past studies failed to recognize an oncogenic role for this molecule, despite its being discovered nearly three decades ago? Dominguez and colleagues (8) found that although DGKα is oncogenic in cellular models, the *DGKA* locus is amplified in only 1% to 5% of cancers examined using public databases. They propose a nononcogene addiction model in which DGKα, though not significantly overexpressed in cancer cells, is nonetheless far more necessary for their survival than normal cells. To show this further, the authors examined a panel of cancer cell lines including human astrocytes and fibroblasts for basal expression of DGKα, and found them to be comparable. However, when tested in doses ranging from 5 to 100 μmol/L, the DGKα inhibitor R59022 showed powerful growth-inhibitory properties only in cancer cells, not in normal cells. Furthermore, the authors showed therapeutic benefits of DGKα inhibition in both orthotopic and subcutaneous mouse models of cancer. Convection-enhanced delivery of lentiviral particles to silence DGKα or inhibition using R59022 both caused significant reduction of tumor burden. Importantly, inhibition of DGKα caused neither apoptosis in normal cells nor toxicity in mice when tested for the period of the study. The authors also predicted the brain-penetrating capacity of R59022 using computational approaches and showed the efficacy of the drug in an intracranial model of glioma, implying that R59022 could be pursued as a therapeutic agent in future clinical trials of glioblastoma.

In conclusion, Dominguez and colleagues (8) have identified DGKα as a potential cancer-specific target with oncogenic properties in glioblastoma and melanoma. Taken together with the broad spectrum of downstream targets of DGKα shown in this study as well as others, this enzyme is implicated as a central node in cancer. The authors have used multiple approaches and convincingly shown that DGKα inhibition holds promise for cancer treatment. However, progress in this avenue is dependent on future investigations directed at studying the impact of silencing DGKα on the immune system, as genetic models of mice lacking *DGKA* and *DGKZ* have shown a role for DGKs in T-cell maturation, and a small subset of these mice develop thymic lymphoma (11). Nevertheless, in the backdrop of several failed clinical trials involving well-established oncogenic pathways in glioblastoma and other cancers, the study by Dominguez and colleagues (8) identifying a central role for DGKα in the regulation of cancer cell proliferation is welcome and holds promise as a novel therapeutic target for this disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Published online July 11, 2013.

REFERENCES

1. DeVita VT, Chu E. A history of cancer chemotherapy. Cancer Res 2008;68:8643–53.
2. Meric-Bernstam F, Mills GB. Overcoming implementation challenges of personalized cancer therapy. Nat Rev Clin Oncol 2012;9:542–8.
3. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 1996;2:561–6.
4. Wick W, Weller M, Weiler M, Batchelor T, Yung AW, Platten M, et al. Pathway inhibition: emerging molecular targets for treating glioblastoma. Neuro Oncol 2011;13:566–79.
5. Feng M, Grice DM, Faddy HM, Nguyen N, Leitch S, Wang Y, et al. Store-independent activation of Orai1 by SPCA2 in mammary tumors. Cell 2010;143:84–98.
6. Hirsch HA, Iliopoulos D, Joshi A, Zhang Y, Jaeger SA, Bulyk M, et al. A transcriptional signature and common gene networks link cancer with lipid metabolism and diverse human diseases. Cancer Cell 2010; 17:348–61.
7. Eyler CE, Wu Q, Yan K, MacSwords JM, Chandler-Militello D, Misuraca KL, et al. Glioma stem cell proliferation and tumor growth are promoted by nitric oxide synthase-2. Cell 2011;146:53–66.
8. Dominguez CL, Floyd DH, Xiao A, Mullins GR, Kefas BA, Xin W, et al. Diacylglycerol kinase α is a critical signaling node and novel therapeutic target in glioblastoma and other cancers. Cancer Discov 2013;3:782–97.
9. Mérida I, Avila-Flores A, Garcia J, Merino E, Almena M, Torres-Ayuso P, et al. Diacylglycerol kinase alpha, from negative modulation of T cell activation to control of cancer progression. Adv Enzyme Regul 2009;49:174–88.
10. Merida I, Avila-Flores A, Merino E. Diacylglycerol kinases: at the hub of cell signalling. Biochem J 2008;409:1–18.
11. Guo R, Wan CK, Carpenter JH, Mousallem T, Boustany RM, Kuan CT, et al. Synergistic control of T cell development and tumor suppression by diacylglycerol kinase alpha and zeta. Proc Natl Acad Sci U S A 2008;105:11909–14.

CANCER DISCOVERY

Not Expecting the Unexpected: Diacylglycerol Kinase Alpha as a Cancer Target

Krishna P.L. Bhat and Kenneth Aldape

Cancer Discovery 2013;3:726-727.