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## Molecular Pathways: Targeting Diacylglycerol Kinase Alpha in Cancer

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### Abstract

Lipid kinases have largely been neglected as targets in cancer, and an increasing number of reports suggest diacylglycerol kinase alpha (DGK $\alpha$ ) may be one with promising therapeutic potential. DGK $\alpha$  is one of ten DGK family members that convert diacylglycerol (DAG) to phosphatidic acid (PA), and both DAG and PA are critical lipid second messengers in the plasma membrane. A host of important oncogenic proteins and pathways affect cancer cells in part through DGK $\alpha$ , including the c-Met and VEGF receptors. Others partially mediate the effects of DGK $\alpha$  inhibition in cancer, such as mTOR and HIF-1 $\alpha$ . DGK $\alpha$  inhibition can directly impair cancer cell viability, inhibits angiogenesis, and notably may also boost T cell activation and enhance cancer immunotherapies. While two structurally similar inhibitors of DGK $\alpha$  were established decades ago, they have seen minimal *in vivo* usage and it is unlikely that either of these older DGK $\alpha$  inhibitors will have utility for cancer. An abandoned compound that also inhibits serotonin receptors may have more translational potential as a DGK $\alpha$  inhibitor, but more potent and specific DGK $\alpha$  inhibitors are sorely needed. Other DGK family members may also provide therapeutic targets in cancer, but require further investigation.

### Background

Recent evidence suggests Diacylglycerol kinase alpha (DGK $\alpha$ ) as a promising new target in the fight against cancer, with DGK $\alpha$  inhibition exhibiting multiple anti-cancer mechanisms of action. DGK $\alpha$  is one of ten DGK enzymes that convert the membrane lipid diacylglycerol (DAG) into phosphatidic acid (PA), and both DAG and PA play important roles in cellular signaling. Both DAG and PA are found in the plasma membrane, with significantly more DAG than PA present (1). However, both act as important second messengers and can bind directly to and modulate numerous proteins in cancer. DAG is known to bind directly to protein kinase C and protein kinase D family members, as well as to the Ras family and to the DGKs (2, 3). PA has been less well studied than PA, and other than mTOR most of its binding partners remain to be discovered (4). PA has been found to control activity of mTOR, Akt, and Erk, while DGK $\alpha$  has been linked to activation of NF- $\kappa$ B, HIF-1 $\alpha$ , c-met, ALK, and VEGF (Fig. 1) (5–13). Despite the association of DGK $\alpha$  and PA to a plethora of oncogenic pathways, they are little-studied in the context of cancer.

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An increasing number of reports are indicating key roles for DGK $\alpha$  in cancer. While normally DGK $\alpha$  is significantly expressed only in brain, kidney, and T cells (14), it appears to be relevant in numerous malignancies. One of the earliest studies on DGK $\alpha$  in cancer notes DGK $\alpha$  over-expression and promotion of NF- $\kappa$ B signaling in melanoma cells (13). A few reports have linked DGK $\alpha$  to cancer cell motility; one report implicates DGK $\alpha$  in cancer cell invasion through  $\alpha$ 5 $\beta$ 1 integrin recycling (RCP) (15). Dominguez and colleagues studied DGK $\alpha$  as a cancer target *in vitro* and *in vivo* (16). DGK $\alpha$  was identified as a potential cancer target through the study of tumor-suppressive microRNAs. After observing that microRNA-297 had tumor-suppressive function and was cytotoxic to glioblastoma cells, it was noted that its top predicted targets in online databases did not include established oncogenes (17). However, the kinase DGK $\alpha$  was predicted to be strongly targeted, and there were suggestions in the literature that DGK $\alpha$  and its product PA might play major roles in cancer. The possibility that DGK $\alpha$  could be a signaling hub in cancer led to testing the effects of its knockdown and inhibition in cancer cells (16). Induction of apoptosis in human glioblastoma lines was noted, including resistant glioblastoma stem cell-like lines, with both DGK $\alpha$  knockdown and with treatment with established inhibitors R59022 and R59949. Normal human cells proved insensitive to knockdown/inhibition. Importantly, these effects were specific, as glioblastoma cells were rescued by exogenous PA. Over-expression of DGK $\alpha$  increased glioblastoma cell numbers *in vitro*. Studies of downstream pathways supported the role of DGK $\alpha$  as a key signaling node in cancer, with its inhibition leading to decreased expression and/or activation of mTOR, Akt, HIF-1 $\alpha$ , c-myc, and the SREBP cholesterol synthesis pathway. Rescue experiments indicated mTOR and HIF-1 $\alpha$  suppression to be key mediators of DGK $\alpha$  inhibition in glioblastoma and melanoma cells, and a unique role of DGK $\alpha$  in regulating mTOR transcription via a novel cAMP-dependent pathway was described. The report also showed for the first time in mouse models the anti-cancer efficacy of DGK $\alpha$  knockdown and inhibition, and demonstrated potent antiangiogenic activity. *In vivo* efficacy of the small-molecule DGK $\alpha$  inhibitor R59022 was observed despite unfavorable pharmacokinetics(16).

Downstream effects of DGK $\alpha$  in cancer may be due largely to modulation of total PA, or specific PA molecules, or PA in specific cellular locations. There are numerous PA (and DAG) species that differ in their two hydrocarbon side chains, but whether different PA molecules functionally diverge has yet to be determined. Modulating PA levels likely mediates DGK $\alpha$  effects through direct binding of PA to oncogenes, as has been demonstrated for mTOR (4). Effects of DGK $\alpha$  on oncogenes can also be indirect, with one example being the regulation of HIF-1 $\alpha$  via modulating the interaction of the degradative von Hippel Lindau (vHL) protein with HIF-1 $\alpha$ ; the role of PA in this interaction is not established (12, 18). DGK $\alpha$  effects in cancer might also stem from affecting DAG levels (19)—though this seems less likely given the high concentration of DAG in the membrane, the numerous DGK family members, and the existence of other DAG-modulating pathways; DAG can be generated by lipase action on triacylglycerols, phospholipase action on phospholipids, phosphatase action on PA, and acyltransferase action on monoacylglycerols (20).

It is unknown whether there is functional redundancy of DGK family members, and whether other DGK family members or PA-synthesizing enzymes can compensate for DGK $\alpha$  knockdown or inhibition. In addition to the DGKs, the lysophosphatidic acid acyltransferases (LPAATs) and phospholipase D (PLD) enzymes also generate PA. LPAAT and PLD enzymes have also been linked to cancer, further supporting roles for PA in malignant cells (21–24). Though all DGKs convert DAG to PA, they may still play unique roles in cancer given their different cellular localizations. For example, DGK $\alpha$  and a few other DGKs are generally thought to translocate from the nucleus to the plasma membrane when active (Fig. 1) (25–27), while DGK $\beta$  is localized on actin fibers (28). Different DGKs may also have varying specificity against different DAG species (29), potentially contributing to diverse roles for the DGK family members in cancer. Notably, a recent report indicates that DGK $\zeta$  may also play a prominent role in cancer (30). DGK $\delta$  has been shown to regulate expression of the epidermal growth factor receptor (EGFR) (31), and DGK $\eta$  may be a downstream mediator of EGFR signaling in cancer (32). DGK $\iota$  increases activity of Ras/Rap signaling in cancer (33).

While roles for most of the DGKs in cancer have not yet been explored, TCGA data (accessed through the cBioPortal) show frequent mutations and amplifications of some of the DGKs in a number of cancers (34)—suggesting that they may have oncogenic functions in certain settings. Over 40% of melanomas have at least one mutation in a DGK family member, with cases of clustered and repetitive mutations suggesting oncogenic function (34). Further studies need to be performed to dissect the roles of other DGK family members in cancer and determine any overlap; it is very possible that other DGKs may be cancer targets in their own right.

Some insight has been gained into upstream activators of DGK $\alpha$ . The oncogenic Src kinase has been found to phosphorylate DGK $\alpha$  to promote its activity (6, 35), but whether Src inhibitors such as dasatinib have a significant effect on DGK $\alpha$  activity remains to be tested. Src may also lie downstream of DGK $\alpha$ , with Src and DGK $\alpha$  comprising a positive feedback loop (36). The Abl oncogene product also modulates DGK $\alpha$ , through regulation of its export from the nucleus (37). It is possible that DGK $\alpha$  mediates a number of oncogenic stimuli. DGK $\alpha$  is activated by estrogen signaling in endometrial CA (38), and it promotes cell invasiveness downstream of SDF-1 $\alpha$  and HGF (39). Calcium plays a well-established and important role in activation of DGK $\alpha$ . DGK $\alpha$ , DGK $\beta$ , and DGK $\gamma$  are all Type I DGKs that contain a calcium-binding region important for activation, which is not the case for the other seven DGK family members (40). Intracellular calcium and DGK $\alpha$  might in fact act in a positive feedback loop, as DGK $\alpha$  inhibitors have been found to reduce intracellular calcium levels (Fig. 1) (41, 42).

Intriguingly, DGK $\alpha$  and DGK $\zeta$  limit T cell activation, and a steadily increasing number of reports are showing that DGK inhibition enhances the T-cell anti-tumor response and immunotherapies such as chimeric antigen receptor-modified T (CART) cells (43–48). DGK $\alpha$  and DGK $\zeta$  may be more important than other DGK family members in T cells due to higher expression. Several years ago, initial reports appeared indicating that DGK $\alpha$  played a role in T cell anergy, and that its inhibition or knockdown could rescue T cell activation (46, 47). *DGKA*-knockout mice have hyperactive T cells resistant to anergy (47). A few

mechanisms have been posited to explain this. In T cells, DGK $\alpha$  inhibition elevates Ras/Erk pathway activity, which is well-known to promote T cell activity (49). T-cell receptor engagement drives movement of the Ras partner Sos to the cell membrane (50), fostering Ras activation, and Ras activation drives IL-2 receptor expression and T cell proliferation (51). Ras activation by DGK $\alpha$  inhibition in T cells stands in contradistinction to reports of Ras pathway suppression by DGK $\alpha$  inhibition in cancer (52), and suggests that DGK $\alpha$  inhibition may have context-dependent effects that vary across different cells and tissues. Other mechanisms for the effect on T cells have been suggested as well, including regulation of the immunologic synapse (53). DGK $\alpha$  inhibition has also been reported to boost the activity of NK cells, providing another potential immunologic anti-cancer benefit (54). From a speculative viewpoint, DGK $\alpha$  inhibition might have another mechanism for boosting the anti-tumor immune response *in vivo* through its antiangiogenic activity; this should generate areas of necrosis within tumors, and necrotic cell death is more immunogenic than structured cell death via apoptosis. A few reports have suggested that DGK $\zeta$  may be stronger than DGK $\alpha$  in modulating T cells. However, DGK $\zeta$  may be a less appealing target than DGK $\alpha$  for increasing the anti-tumor immune response, as one report indicates that DGK $\zeta$  may be especially critical in restraining immunosuppressive Treg cells (55). DGK $\zeta$  has also been found to suppress the oncogenic NF- $\kappa$ B pathway, providing another potential drawback to targeting it for cancer therapy (56).

## Clinical-Translational Advances

The prospect that DGK $\alpha$  inhibition may directly attack cancer cells, suppress angiogenesis, and boost immunotherapies makes it an attractive target in cancer. The ability of DGK $\alpha$  inhibition to affect multiple cancer pathways, and in particular mTOR and HIF-1 $\alpha$ , broadens its potential applicability. However, the lack of adequate small-molecule inhibitors is a clear barrier to its clinical translation. The only known DGK $\alpha$  inhibitors are R59022 and R59949, which share highly similar structures. They have modest inhibitory potency against DGK $\alpha$  and have only been tested *in vivo* against cancer in one report (16). A limited intraperitoneal course of R59022 increased median mouse survival in an orthotopic xenograft GBM model by approximately 20% (p=0.01). However, the half-life in mice of R59022 appears to be very short, on the order of one to two hours (16), while R59949 has not been tested *in vivo*. It would take years and substantial resources to optimize these compounds for potential clinical usage, with the need for SAR (structure-activity relationship) studies to better understand their interaction with DGK $\alpha$ . It may be possible to attack cancer with *DGKA* knockdown by siRNA or shRNA, as shown in one report (16), but this of course presents the tremendous challenge of efficient delivery to cancers in patients.

Recently it has been noted that the known DGK $\alpha$  inhibitor R59022 differs structurally only by a single fluorine from the established serotonin receptor inhibitor ritanserin (57). Ritanserin has been tested for applications including schizophrenia, alcoholism, and insomnia. While it was bypassed by other schizophrenia medications and never put forward for FDA approval, it was shown to be safe in human trials. Ritanserin is orally bioavailable, has a 40-hour half-life in humans, and has some degree of blood-brain barrier penetrability. Our group has now found that ritanserin inhibits DGK $\alpha$  activity more potently than R59022, and we are further testing its potential as a DGK $\alpha$  inhibitor that may be translated to the

clinic with relative rapidity. Early experiments with single-agent ritanserin in intracranial glioblastoma and melanoma xenograft models suggest increases in median mouse survival of up to 30% ( $p < 0.05$ ; B. Purow; unpublished data). However, ritanserin only has potency in the low micromolar range and is not very specific, with much more potent inhibition of serotonin 5HT<sub>2A</sub> and 5HT<sub>2B</sub> receptors and possibly with effects on dopamine signaling as well. That being said, some of the non-DGK $\alpha$ -mediated effects of ritanserin may be beneficial in cancer patients, such as having a stimulating effect when awake but also improving sleep. Ultimately there is a clear need for new small-molecule inhibitors of DGK $\alpha$  with greater potency and specificity, and efforts are ongoing to identify candidate compounds.

It is vital to consider potential side effects of DGK $\alpha$  inhibition as it is developed further at the preclinical stage and hopefully moved on to the clinic. Early mouse studies have not revealed any major side effects of DGK $\alpha$  inhibition, in keeping with the findings in DGK $\alpha$  knockout mice mentioned above. The effects on T cells may result in increased autoimmunity with DGK $\alpha$  inhibition, but this is speculative. Drugs such as ritanserin with effects on serotonin receptors might affect serotonin-related processes such as coagulation, but this has not been shown previously. Mice with other DGK family members knocked out have displayed significant pathologic phenotypes—including glucose intolerance in DGK $\delta$  knockout mice and neurologic disorders in DGK $\beta$  knockout mice (58, 59)—but these concerns do not seem to cross over to DGK $\alpha$  inhibition, perhaps due to tissue-specific expression. It might be expected that downstream targets observed in cancer cells such as the Ras and mTOR pathways would result in substantial side effects of DGK $\alpha$  inhibition *in vivo*, but these may not arise because of context-dependent differential effects on various cell types.

It is clear that much further study is needed on the effects of DGK $\alpha$  on downstream oncogenic pathways, immunity, angiogenesis, and combinatorial effects with other anti-cancer agents. Ongoing work is investigating the combination of DGK $\alpha$  inhibition with standard therapies such as radiation and chemotherapy, as well as with other targeted therapies and immunotherapies. With the recent dramatic successes of cancer immunotherapies, the potential for DGK $\alpha$  inhibition to boost them is especially intriguing. DGK $\alpha$  inhibition may be most useful in enhancing T cell or NK cell immunotherapies, though this requires further exploration. It is also tempting to speculate that a more complete attack on PA synthesis might have even greater efficacy against cancer, through combining DGK $\alpha$  inhibition with blockade of other DGKs or other PA-synthesizing enzymes LPAATs or PLDs. At present there are no known inhibitors of other DGKs—besides possible non-specific and limited effects by the known DGK $\alpha$  inhibitors—but inhibitors of LPAAT and PLD already exist. Numerous therapeutic opportunities such as this remain to be explored. While study of the DGKs remains a field in its infancy, it appears increasingly likely to yield important biological and therapeutic advances.

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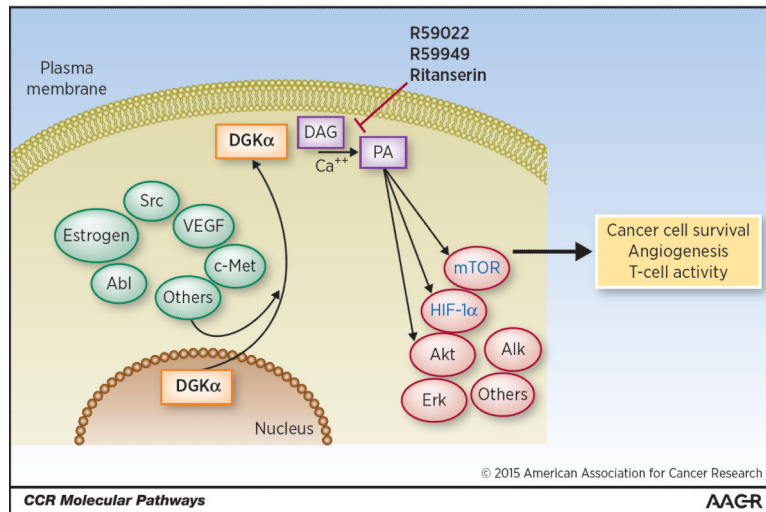
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**Figure 1. DGKα regulation and activity**

DGKα is located in the nucleus till activated by regulators such as Src, at which point it translocates to the inner leaflet of the plasma membrane. There it converts diacylglycerol to phosphatidic acid, acting as a regulator or mediator of numerous oncogenic pathways.