Drug Resistant Melanoma May Be Vulnerable to Inhibitors of Serine Synthesis

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NRAS-driven melanomas frequently develop resistance to MAPK/extracellular signal—regulated kinase inhibitors (MEKis), which limits their therapeutic utility. Nguyen et al. (2020) show that MEKi-resistant cells upregulate phosphoglycerate dehydrogenase (PHGDH), the rate-limiting enzyme in serine synthesis. Suppression of PHGDH rendered cells sensitive to MEKis, suggesting that PHGDH may be a therapeutic target for melanoma.

To increase the likelihood of identifying physiologically relevant resistance mechanisms, Nguyen et al. (2020) established MEKi-resistant human melanoma lines from subcutaneous tumors that developed in nude mice. Using a high-throughput antibody-based reverse-phase protein array and western blotting, the authors determined that cells isolated from resistant tumors expressed higher levels of MEK/ERK signaling compared with control cells, suggesting that upregulation of downstream MAPK pathways may play a role in the resistance. However, the authors posit that reactivation of the MEK pathway is not solely responsible for the resistance because both control and MEKi-resistant cells remained equally sensitive to ERK1 and/or ERK2 inhibition—a hypothesis that would require additional functional studies to validate. The authors also used RNA sequencing to explore transcriptomic changes in MEKi-resistant cells and noted that serine metabolic pathway genes, including PHGDH, were among the most highly upregulated genes.

PHGDH is the rate-limiting enzyme in the serine synthesis pathway (SSP) and is commonly overexpressed in many cancers, including breast, cervical, pancreatic, and colorectal cancer (Locasale et al., 2011; Possemato et al., 2011). After validating the upregulation of PHGDH protein in resistant cells, the authors genetically depleted PHGDH. This markedly inhibited vitro proliferation in both control and MEKi-resistant cells and also seemed to restore MEKi sensitivity to the previously resistant lines.

Finally, the authors probe the mechanism responsible for PHGDH upregulation in resistant cells and show that it does not likely result from RAS downstream factors. Future work to define PHGDH regulators might identify other therapeutic targets.

Tumors undergo major metabolic reprogramming to support continuous cell proliferation and, as Nguyen et al. (2020) suggest, to acquire therapeutic resistance. These adaptations often include increased glucose uptake and utilization through glycolysis. The glucose transporters GLUT1 and/or GLUT3 are expressed in 85% of melanomas, but they are notably absent from benign nevi. Furthermore, GLUT1 and/or GLUT3 expression is associated with significantly decreased survival in melanoma (Ruby et al., 2019). This is relevant to this study because increased cytoplasmic glucose not only increases glycolysis but also several side branches of the glycolytic pathway, including the SSP.

The SSP begins when PHGDH converts 3-phosphoglycerate, a glycolytic intermediate, into 3-phosphohydroxypyruvate in a nicotinamide adenine dinucleotide (NAD+)—dependent fashion. Phosphoserine aminotransferase 1 then converts 3-phosphoserine (3PS) through the donation of one-carbon units to the folate and methionine cycles, which can be used for nucleotide synthesis, NAD phosphate generation for antioxidant defense, and methyltransfer reactions. Serine can further be converted to glycine through serine hydroxymethyltransferase, and this...
reaction provides a major source of methyl groups required for DNA methylation. In addition, glycine is a component of the peptide glutathione (GSH), which serves several functions in the cell, including detoxification reactions, scavenging free radicals, and modulating DNA synthesis. Although GSH is known to play a protective role in normal cells against harmful reactive oxygen species (ROS), cancer cells are able to hijack GSH's antioxidant effects to confer a pro-proliferative and chemoresistant advantage. In melanoma, GSH status is directly correlated with increased cell growth and metastatic potential (Carretero et al., 1999). Further mechanistic studies have shown that high intracellular levels of GSH protect B16 melanoma cells from oxidative stress found in the hepatic microvasculature and that the depletion of GSH sensitizes melanoma to combination therapy (Anasagasti et al., 1998; Mena et al., 2007). Nguyen et al. (2020) find similar results in the well-defined melanoma cell line WM1366, suggesting that PHGDH upregulation and the subsequent potentiation of the GSH antioxidant system specifically benefits MEKi-resistant cells by neutralizing additional stress caused by treatment with the targeted inhibitor. However, the cellular effects of ROS are complex and vary with cell type and tissue context. In some melanoma models, ROS seems to prevent metastasis (Piskounova et al., 2015), suggesting that targeting ROS itself as an anticancer strategy may be especially difficult.

PHGDH activity promotes numerous downstream pathways that may promote tumor growth and is commonly overexpressed in many cancers, including 40% of melanomas (Locasale et al., 2011). Melanoma cell lines with amplified PHGDH expression are especially sensitive to PHGDH depletion, which agrees with the results presented in Nguyen et al. (2020) (Locasale et al., 2011). Similar PHGDH dependency is observed in breast cancer models (Pacold et al., 2016; Possemato et al., 2011). In spontaneous human pancreatic, cervical, gastric, and colorectal cancer, increased PHGDH expression correlates with poor prognosis (Locasale et al., 2011; Possemato et al., 2011).

In diverse tumor settings, PHGDH contributes to therapy resistance. In triple-negative breast cancer treated with doxorubicin, PHGDH upregulation antagonizes doxorubicin formation of ROS, and upon PHGDH repression, cells become sensitive to chemotherapy (Zhao et al., 2020). In addition, PHGDH knockdown in cervical adenocarcinoma renders cells sensitive to cisplatin chemotherapy (Zhao et al., 2020). PHGDH elevation also contributes to targeted therapy resistance, as shown by Nguyen et al., in response to MEK inhibition in melanoma. Further studies in melanoma have shown that PHGDH is upregulated in response to vemurafenib (BRAF inhibition), which ultimately supports cell proliferation through increased flux through the folate cycle (Zhao et al., 2020). Similar phenomena have been reported in lung adenocarcinoma in response to EGFR inhibitor treatment (Zhao et al., 2020). Importantly, in all of these studies, PHGDH repression through either genetic or pharmacologic approaches increased cell sensitivity to therapy.

PHGDH inhibitors appear especially attractive as it seems that cancer cells that overexpress PHGDH are especially sensitive to inhibition. Several groups have identified allosteric inhibitors of PHGDH, including CBR-5884 (Mullarky et al., 2016) and NCT-503 (Ruby et al., 2019), which were identified through unbiased screening of compound libraries in a PHGDH assay. CBR-5884 modestly inhibited breast cancer and melanoma cell growth in vitro, with growth inhibition ranging from 35% to 60% at 30 μM (Mullarky et al., 2016). Unfortunately, CBR-5884 is unstable in mouse plasma and therefore is not a viable PHGDH inhibitor candidate (Mullarky et al., 2016). NCT-503 exhibits a lower half maximal inhibitory concentration than CBR-5884 in breast cancer cells and was further shown to inhibit tumor growth in vivo (Pacold et al., 2016). In addition to reducing total serine and glycine in the cell, NCT-503 significantly reduced adenosine monophosphate and deoxythymidine monophosphate levels, suggesting that PHGDH inhibition reduces nucleosides used for cell proliferation. In addition to breast cancer, NCT-503 inhibits therapy-resistant renal cell carcinoma and multiple myeloma (Zhao et al., 2020). Other groups have identified competitive inhibitors of PHGDH, including indole amide compounds and a prodrug of NAD+/NAD hydrogen, which bind the NAD+ pocket of PHGDH and inhibit activity at low nanomolar affinity (Zhao et al., 2020). However, these compounds have not yet been thoroughly tested in cancer cells. These small molecule PHGDH inhibitors, combined with PHGDH genetic antagonism, could be used in preclinical studies to establish the potential utility of targeting PHGDH for melanoma and to determine whether any antimelanoma activity results from PHGDH inhibition in tumor cells versus host immune and/or stromal cells.

Although many studies have shown that PHGDH is commonly overexpressed in melanomas, the selective pressures and mechanisms driving the upregulation are unknown. UVR and specifically UVA can cause indirect DNA damage through the formation of ROS. UVR also activates facultative pigmentation in melanocytes, which forms either black and/or brown eumelanin or red and/or light brown pheomelanin. Because pheomelanin production requires cysteine, which is also needed for GSH production, UVR might result in less GSH production and a corresponding reduction in antioxidant potential. Together, this might lead to increased ROS within melanocytes and potentially increased genomic instability. Perhaps,
melanocytes upregulate PHGDH to increase flux through the SSP and, subsequently, GSH levels in an effort to reduce the oxidative stress they experience from UVR, which then also serves to protect them from oncogenic stress upon transformation to melanoma.

Furthermore, as Nguyen et al. (2020) and others suggest, PHGDH may be a common resistance mechanism that is relevant to both targeted and chemotherapy. Upon examining publicly available data from treated versus naive melanoma, Nguyen et al. (2020) found that there is a heterogeneous expression of PHGDH after treatment. It would be interesting if certain genetic mutations or perhaps other predictive biomarkers exist that would indicate which populations may be expected to experience PHGDH upregulation after treatment and thereby identify those patients most appropriate for combination therapy with a PHGDH inhibitor. Although PHGDH inhibition seems to be an attractive therapeutic strategy, it has yet to be proven efficacious in humans. The next most appropriate step seems to be to establish whether PHGDH inhibitors such as NCT-503 extend survival in preclinical murine melanoma models with or without targeted and/or immune therapy.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

REFERENCES