Targeting Oxidative Phosphorylation: Why, When, and How

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In this issue of *Cancer Cell*, Vazquez and colleagues report reduced glycolysis and increased oxidative phosphorylation in certain melanomas, revealing metabolic plasticity rather than stable Warburg pathophysiology. Furthermore, Haq and colleagues (also in this issue of *Cancer Cell*) show situations where increased oxidative phosphorylation is required for melanomas to survive inhibition of B-RAF, suggesting investigation of therapeutic combinations of B-RAF inhibitors with biguanides.

Increased glycolysis has been recognized to be a characteristic of neoplastic cells since the classic observations of Otto Warburg. It is now appreciated that the "Warburg effect" does not usually involve compensation for defects in mitochondrial ATP production as originally proposed. Rather, it is a consequence of genetic lesions that, among other sequellae, alter control systems that regulate cellular metabolism. While increased glycolytic flux may provide some benefit to cancer cells by increasing ATP production, recent studies suggest that it is advantageous mainly because it generates chemical "building blocks" required for the anabolic processes that must occur prior to cell division.

The conspicuous glycolysis of neoplastic cells has long provided a rationale for investigating inhibitors of glycolysis such as 2-deoxyglucose, but this direction of research has not yet led to clinical advances in cancer treatment. Observations reported in this issue of *Cancer Cell* by Haq et al. (2013) and Vazquez et al. (2013) provide evidence that there are circumstances in which neoplastic cells are critically dependent on oxidative phosphorylation rather than glycolysis, at least in the context of melanoma. This may represent a vulnerability that could be therapeutically exploited.

Both reports show that the lineage-specific transcription factor MITF upregulates PGC1α in a subset of melanomas, and, expectedly, this results in increased oxidative phosphorylation. Haq et al. (2013) show that mutational B-RAF activation, a common derangement in melanoma, leads to declines in MITF, PGC1α, and oxidative phosphorylation, yielding a glycolytic phenotype.

B-RAF inhibitors such as vemurafenib are used in the clinic but commonly provide only short-term benefit, followed by the rapid development of drug resistance. Interestingly, when cells with activated B-RAF are exposed to a B-RAF inhibitor, they upregulate PGC1α and demonstrate, at least for a critical period of time, increased dependency on oxidative phosphorylation. This phenomenon suggests that the efficacy of B-RAF inhibitors may be, at least in part, attributable to drug-induced reprogramming of cellular metabolism, leading to a reduction of glycolysis and an increased requirement for oxidative phosphorylation. While under this stress, cells are under selective pressure to revert to a glycolytic phenotype by activating another oncogene to substitute for the pharmacologically inhibited B-RAF (Nazarian et al., 2010). However, it is possible that before this reversion takes place, there is an opportunity for synthetic lethality by combining the kinase inhibitor with inhibition of oxidative phosphorylation.

The studies by (Haq et al. (2013) and Vazquez et al. (2013) are specific to melanoma, so it will be of interest to investigate the generality of the key findings. Many cancers with prominent glycolytic phenotypes are treated with kinase inhibitors, which reduce the oncogenic signaling that promotes glycolysis, among other consequences. Most kinase inhibitors provide a period of benefit followed by drug resistance. It is possible that one of several consequences of exposing sensitive cells to a kinase inhibitor is reduction of high glycolytic flux. Apart from the melanoma data, an example is provided by glycolytic leukemic cells driven by BCR-ABL that demonstrate upregulated oxidative phosphorylation upon exposure to imatinib (Gottschalk et al., 2004; the kinase inhibitor that targets BCR-ABL).

Consistently, Vazquez et al. (2013) show that melanomas can be classified into two groups: a conventional glycolytic group and a group characterized by high PGC1α, high rates of oxidative phosphorylation, resistance to oxidative stress, and aggressive clinical behavior. The latter group also appears to demonstrate sensitivity to the targeted inhibition of oxidative phosphorylation. Experimentally, this point is convincingly made by knocking down proteins required for oxidative phosphorylation. However, this approach is not one that is applicable to clinical testing.

Are there practical ways to target oxidative phosphorylation in the clinic? An initial impression is that this is impossible, as classic inhibitors of cellular respiration such as cyanide are well-known poisons without a suitable therapeutic index. However, there are oxidative phosphorylation inhibitors that are in common clinical use, including the antidiabetic biguanide metformin and the antimalarial agent atovaquone. Incidentally, the inhibition of cytochrome b by atovaquone is effective against *Plasmodium falciparum*, not because it inhibits mitochondrial ATP production (which is not required by the parasites), but rather because of a requirement for oxidative phosphorylation

to regenerate ubiquinone (Painter et al., 2007). (In *Plasmodium falciparum*, ubiquinone is required as the electron acceptor for dihydroorotate dehydrogenase, an essential enzyme for pyrimidine biosynthesis.) Nevertheless, the safety of atovaquone relates to the fact that it is a better inhibitor of *Plasmodium* than human cytochrome c, so it may not be a strong candidate for oncology applications.

Metformin, on the other hand, is widely used for the treatment of type II diabetes, has a favorable toxicity profile, and is already under investigation for possible oncology applications (Pollak, 2012). There are several hypotheses to explain the activity of metformin as an antineoplastic agent in experimental models. One involves systemic effects such as the reduction of insulin levels (which may be relevant for a subset of patients where the drug induces significant declines in insulin, provided their cancers are insulin responsive). A second hypothesis involves a direct action on neoplastic cells, characterized by decreased ATP levels secondary to reduced oxidative phosphorylation. This can lead to predominately cytostatic or cytotoxic effects, depending on the specific characteristics of targeted cells. In cells with intact control systems for dealing with energy stress, ATP deficit leads to the activation of AMPK, inhibition of mTOR, reduced energy consumption and growth inhibition. In cells with loss of function of these control systems (such as inactivation of LKB1), the ongoing high level of ATP consumption, despite reduced oxidative phosphorylation, leads to an energetic crisis.

Could metformin or other biguanides be practical inhibitors of oxidative phosphorylation in the context of the studies by Haq et al. (2013) and Vazquez et al. (2013)? This might not require complete inhibition of oxidative phosphorylation, but rather blocking the increase in oxidative phosphorylation following PGC1α upregulation.

If further preclinical work provides sufficient evidence that metformin can delay the development of resistance to vemurafenib or other kinase inhibitors, the safety profile of biguanides suggests that clinical trials would be feasible.

However, there are important gaps in knowledge to consider. First, with clear credentials as oxidative phosphorylation inhibitors, why are the biguanides so different from cyanide in terms of therapeutic index? Part of the reason may relate to whole organism, cellular, and subcellular pharmacokinetics. Following oral administration, biguanides accumulate in a nonhomogeneous fashion in different organs, and cellular uptake of metformin is greatly influenced by the level of anion transporter OCT1. Furthermore, mitochondrial uptake of biguanides is facilitated by the membrane potential, so as biguanide concentration rises at complex I, the membrane potential falls, reducing further import and limiting toxicity. Thus, in vivo, it is not trivial to determine the extent to which a biguanide is inhibiting oxidative phosphorylation in a particular tissue. Thus, it is unclear if the dose of metformin used in diabetes patients would be optimal for partial inhibition of oxidative phosphorylation in melanoma patients or if phenformin or other lipophilic biguanides would be more effective.

There are relevant clues and cautions in the literature. In tissue culture, biguanides clearly inhibit oxidative phosphorylation in transformed cells, often leading to compensatory increases in glycolysis (Pollak, 2012). Also, an early report provided in vitro evidence for synergy between vemurafenib and metformin (Niehr et al., 2011). On the other hand, metformin has been reported in certain contexts to upregulate VEGF expression, which is an adaptive response by the transformed cell to the inhibition of oxidative phosphorylation. This response obviously has the potential for undesired consequences, although this research revealed synergy between metformin and VEGF inhibitors (Martin et al., 2012).

Whereas many clinical trials of biguanides for cancer treatment are ongoing, most were designed with the rationale that the reduction of systemic hyperinsulinemia, if achievable, would be beneficial. The ongoing trials address neither hypotheses related to specific strategic therapeutic combinations of biguanides with kinase inhibitors nor the possibility of activity in specific tumor subsets defined by tumor characteristics such as PGC1α status. Future studies aimed at rigorously examining these possibilities will need to establish, as a first step, the extent to which clinically achievable biguanide exposure inhibits oxidative phosphorylation in neoplastic tissue in vivo.

Despite the considerable challenges, the clues concerning the sensitivity of a subset of cancers to the inhibition of oxidative phosphorylation, particularly in the context of resistance to kinase inhibitors, are tantalizing. Further work will reveal if there are practical opportunities for synthetic lethality by pharmacologically limiting the adaptive ability of transformed cells to upregulate oxidative phosphorylation when facing stresses such as oncogenic kinase inhibition.

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