PDK1: a new therapeutic target for glioblastoma?

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Glioblastoma multiforme (GBM) remains the most lethal and difficult-to-treat cancer of the CNS despite aggressive surgical resection and adjuvant chemo-radiation approaches. Marginal impact has been made with most recent survival characteristics varying between 14 and 20 months with maximal intervention [1]. While targeted therapeutics are increasingly being investigated based upon molecular data, simultaneously an alternative renaissance in the oncological perspective has been suggested with dysregulated bioenergetic metabolism as the driver of oncogenesis. Termed the Warburg effect, this observed phenomena initially described in 1956 is hypothesized to divert necessary precursors to purine and fatty acid synthesis in rapidly dividing tumor [2]. This is at the expense of glucose being shunted away from the tri-carboxylic acid cycle (Krebs cycle) with diminished Acetyl-CoA production. Aerobic glycolysis predominates with lactate production despite the adequate presence of oxygen and other precursors necessary for normal cellular bioenergetics.

There is no mutual exclusivity of metabolic oncogenesis, however, as initial evidence indicate ubiquitous cell surface signaling and subsequent tyrosine kinase

modulation of metabolic pathways through mitochondrial docking via Src family kinases. Thus, with further identification of the key regulatory mechanisms of the Warburg effect, potential antagonism at this juncture of mitochondrial metabolism and cell surface mechanisms may serve as a rationale to reverse the Warburg effect. Because of the current dearth of existing targets in mitochondrial-mediated cancer metabolism in glioblastoma, novel agents designed to interfere with regulating such activity have increasingly gained attention [3]. More recently, we along with other groups reported PDK1 - a Ser/Thr kinase and a gatekeeper of glucose oxidation that represses the flux of pyruvate into acetyl-CoA to promote glioblastoma formation and progression [4, 5].

The mechanisms that regulate glucose metabolism present an interesting phenomenon, since glucose consumption and lactate production were correlated with significant increase of several glycolytic enzymes in variety of cancers. Since PDK1 is required for the conversion of pyruvate into lactate, targeting PDK1 might present itself as a therapeutic target that can be combined with conventional targets. Very few studies have evaluated the correlation

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of PDK1 in cancer prognosis. For example, Hur *et al.* [6] reported that the overexpression of PDK-1 correlated with HIF-1 α expression and poor prognosis in gastric cancers. In melanoma cells, silencing of PDK1 by its respective shRNA after doxycycline administration demonstrated considerable tumor regression indicating the multiple roles of PDK1 are not only essential for tumor initiation, but also for tumor maintenance and progression, suggestive of a beneficial target for therapeutic intervention [7]. In addition, the findings from a study conducted by analyzing the mRNA expression from a set of 140 patients with head and neck carcinoma, demonstrated up-regulated expression of PDK1 when compared with the normal tissues. This corroborates with the outcomes in another study conducted in patients with non-small-cell lung cancer, where 73% of patients demonstrating PDK-1 expression experienced poor clinical outcome, illustrating PDK-1 as a novel target for tumor therapy [8, 9]. In another study, Wigfield *et al.* (2008), using a cDNA microarray in SCC-25 in HNSCC cells, confirmed that PDK-1 indeed modulates pyruvate and lactate metabolism and likewise is associated with a significantly poor prognosis in patients with high levels of expression [10]. In another study tested in human head and neck cancer cells, PDK-1 inhibition showed a dramatic reduction in lactate levels in 22B cells following knockdown of PDK-1 with shPDK-1 cells showed significantly higher cell death and a profound reduction in their ability to form colonies in soft agar *in vitro*, while xenograft studies using PDK-1 knockdown showed suppressed tumor growth [11]. Furthermore, the data obtained from a study on induced pluripotent stem cells revealed elevated PDK1 protein expression. Administration of PDK1 inhibitors lead to reduced human embryonic stem cell-like colony formation. PDK1 thus may represent itself as an early marker of metabolic reprogramming involved in the Warburg-like restructuring associated with the conversion to pluripotency [12].

Earlier, we have demonstrated that PDK-1 is highly expressed in human glioblastoma multi-forme surgical specimens when compared with the normal brain using qualitative and semi quantitative analysis [5]. We were also able to demonstrate that silencing of PDK1 exhibited apoptotic and anti-proliferative effects on U251 and 5310 cells. As literature on this topic is

sparse, our study is the first report that represents first-described preliminary results elucidating the role of PDK1 in regulating glucose and energy metabolism in glioblastoma and suggest that PDK1 in glioblastoma may serve as a therapeutic target affecting cancer cell metabolism and inducing tumor regression. Several research groups including ours have demonstrated that pharmacologic inhibition of PDK-1 reverses the Warburg effect [5]. One such pharmacologic inhibitor is the small molecule dichloroacetate (DCA), a well-known inhibitor of PDK that has been used clinically for the treatment for lactic acidosis [13]. DCA inhibits PDK-1 activity and preferentially diverts glucose metabolism from glycolysis towards oxidative phosphorylation. Some initial evidence indicates that DCA attenuates glioblastoma cell proliferation and tumor growth by targeting PDK1 in a clinical trial involving small number of patients [14] and is consistent with previous observations; for example, in A549 lung cancer cells [15]. However, these findings in glioblastoma are limited and our results await more definitive validation in targeting PDK1.

As it currently stands, targeting the mitochondria is attractive as initial evidence is supportive. As a downstream element in the regulation of cancer growth, perhaps modulated by cell surface mechanisms, the convergence may serve as a more efficacious means to control neoplasia. This situation may particularly lend itself to the recurrent setting when cancer has altered its *de novo* signaling pathways from the cell surface typically encountered in the initial response to chemotherapy. It will be of great interest to investigate the relevance of the PDK-1 pathway and determine the points of enhanced lactate production by suppressing deregulated mitochondrial function. Moreover, further studies investigating the impact of PDK-1 inhibitors, such as DCA, alone or with other adjuvants hold promise in suppressing glioblastoma growth.

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