Metabolic Approaches to Treatment of Melanoma

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STATEMENT OF TRANSLATIONAL RELEVANCE

Recent information has shown that high serum lactate dehydrogenase (LDH) levels identifies melanoma patients with a poor prognosis. As a consequence many clinical trials for metastatic melanoma exclude patients with high LDH levels. The purpose of this review is to indicate new treatment approaches targeting metabolic pathways that may be effective in this subgroup of patients with rapidly progressive melanoma.
ABSTRACT

Purpose: LDH levels in blood of patients with melanoma have proven to be an accurate predictor of prognosis and response to some treatments. Exclusion of patients with high LDH levels from many trials of new treatments has created a need for treatments aimed at patients with high LDH levels. This article reviews the metabolic basis for the association of LDH with prognosis and the treatment initiatives that may be successful in this patient group.

Experimental Design: Review of current literature on the topic.

Results: A number of new treatment initiatives based on manipulation of metabolic pathways in melanoma cells are now available and await evaluation in well designed clinical trials.

Conclusions: Different cancers may require different metabolic approaches for effective treatment. In view of the high rate of glycolysis in most melanoma cells, approaches based on inhibition of acid excretion from the cells appear particularly attractive.
INTRODUCTION

The growth rates and sites of spread of metastatic melanoma vary widely between patients. In some instances the disease shows indolent growth rates and limited spread to skin, lymph nodes or lungs. In other patients the disease has rapid growth rates and early spread to multiple sites including internal organs. Several studies have identified lactate dehydrogenase (LDH) levels as the most reliable marker of the more rapidly growing highly metastatic forms of the disease \((1,2)\). LDH levels also identify melanoma that are resistant to certain forms of treatment \((3)\) and is widely used by sponsors to exclude patients with unfavourable prognosis from clinical trials of new agents. Data from the Genasense study \((3)\) suggest that approximately one-third of patients are excluded from trials if a cut off of >1.2 upper limit of normal (ULN) is taken as an entry criteria.

LDH is coded for by two genes, LDH-A (M-muscle type) and LDH-B (H-heart type) that code for two polypeptide chains that form five isoenzymes, depending on the combinations of the two chains. LDH-5 is composed of four M subunits and LDH-1 of four H subunits \((4)\). LDH-5 is the most efficient isotype in catalyzing conversion of pyruvate to lactate \((5)\). LDH-5 expression is readily detected in histological sections of melanoma and was shown to be a strong correlate of prognosis in primary melanoma. It was not an independent predictor and correlated with the other prognostic determinants of thickness and mitotic rate \((6)\) .
Increased levels of LDH within the cell are believed to be due largely to upregulation by the transcription factor “hypoxia inducible factor 1α” (HIF-1α). The latter is normally degraded by the E3 ligase von Hippel-Lindau (VHL) protein under normal oxygen levels but not under hypoxic conditions leading to post translational increases in HIF-1α (7). Hypoxia also activates the endoplasmic reticulum (ER) stress response which indirectly upregulates mRNA for HIF-1α (8) via the transcription factor XBP-1 and Akt activation (9) but this needs to be confirmed. Hypoglycemia and other factors resulting from the demands of the malignant process also contribute to ER stress and the resultant unfolded protein response (UPR) (10-12).

One of the consequences of ER stress is a change in metabolism of the cell due to activation of HIF-1α and activation of the Akt pathway. These changes are believed to result in reduced mitochondrial dependent oxidation of glucose by cancer cells referred to as the Warburg effect (13). As a consequence, melanoma cells depend largely on glycolysis for ATP production rather than oxidative phosphorylation through the Krebs cycle. This is very inefficient in that only 2 molecules of ATP are produced per glucose molecule compared to 36 ATP molecules produced in the Krebs cycle. This results in high uptake of glucose and rates of glycolysis in melanoma and generation of high lactate levels within and around the cells. Lactate is exported by the Monocarboxylate transporters 1-4 (MCT1-4) transporter family and proton pumps (7). MCT-4 appears the main transporter of lactate from the cell whereas MCT-1 is believed to transport lactate into the cell under aerobic conditions (14). Lactate was shown to be a substrate for energy production under aerobic conditions in a cervical cancer cell line (15). Cancer
cells in general appear to adapt to the acidic conditions better than surrounding cells and this is believed to give them a selective advantage (16-19).

Several factors are believed to contribute to the Warburg effect. One factor is the induction of hexokinase II (HKII), which is bound to the outer mitochondrial membrane and catalyzes the first step in glycolysis by converting glucose to glucose-6-phosphate (20). HKII may also inhibit apoptosis by preventing interaction of Bax/Bak with the mitochondrial outer membrane (21) (see Figure 1). HIF-1α also induces pyruvate dehydrogenase kinase (PDK), which inactivates pyruvate dehydrogenase and prevents entry of pyruvate into the Krebs cycle (22,23). Abnormalities in p53 may be involved in that p53 was reported to stimulate the expression of “synthesis of cytochrome c oxidase 2” (SCO2), which is critical for oxygen use in the inner mitochondrial membrane. P53 also induced tumor protein 53 (TP53)-induced glycolysis and apoptosis regulator (TIGAR), which attenuates glycolysis (22). TP53 induced nuclear protein 1 (TP53INP1) is also involved in its antioxidant function (24). Nevertheless, p53 was not shown to play a role in xenograft models that measured glucose uptake by FDG-PET scans (25).

Another possible cause of glycolysis is a switch to the M2 (embryonic) splice form of the enzyme pyruvate kinase away from the adult M1 form. The M2 form is believed to bind to phosphotyrosine peptides, resulting in inhibition of its enzymatic activity (26,27). It is not clear why this results in higher lactate production but may involve more specific targeting of pyruvate to LDH (26).
Therapeutic Approaches Targeting Metabolic Pathways in Melanoma

Table 1 summarizes some of the possible metabolic approaches that are being considered in treatment of cancers. They are described more fully below.

1. Induction of cell death by blocking the excretion of H+ and lactate.

   All cells have proton pumps that excrete protons across cell membranes or lysosomal membranes within the cells. The most important of these are the vacuolar-H-ATPases (V-ATPases) and the H+-K+-ATPases (28). Their activity maintains a relatively neutral intracellular pH and an acidic extracellular environment. The latter is believed to be important in activation of proteases that contribute to melanoma invasion and metastasis (29,30). The activity of V-ATPases in particular appeared related to highly metastatic forms of cancer (31) and were over-expressed in many forms of cancer (30,32). Moreover, their activity was associated with resistance to chemotherapeutic agents such as Cisplatin (33), perhaps due to neutralization of weak bases such as Cisplatin. Other enzymes involved in maintenance of intracellular pH include the Carbonic Anhydrases IX and XII (34).

   Proton pump inhibitors (PPI) such as Omeprazole bind strongly but not irreversibly to SH groups on proton pumps and inhibit their activity as in treatment of peptic ulcers by blocking the gastric acid pump (Figure 2A). An attractive aspect of PPI is that they are essentially pro-drugs which are activated under acidic conditions. This
potentially gives them selectivity for tumors with acidic microenvironments. PPI were shown to induce apoptosis in human B cell tumors in vitro by a mitochondrial dependent caspase independent mechanism involving generation of oxygen reactive species and to inhibit the growth of Raji B cell tumors in severe combined immunodeficient mice (35).

A modification of proton pump inhibitors introduced by Hackett (36) was the addition of N-Acetyl-Cysteine (NAC) to Omeprazole forming the N-Acetyl Cysteine Omeprazole (NACO) disulphide complex (Figure 2B). The object was to bind the NAC SH thiol group to Omeprazole by formation of a disulphide bond. This was carried out to minimize Omeprazole binding to SH groups on proteins in the circulation system and to increase uptake into tumor cells. It was hypothesized that once within the cell the Omeprazole in the NACO complex would undergo reversible disulphide exchange, allowing it to preferentially bind to proton pumps within the cell. As reported elsewhere (37), these ideas were supported by in vitro studies in human melanoma cells showing that the combination of the two drugs was much more effective than either alone. The hypotheses were further supported by the demonstration of a drop of pH within the melanoma cells that appeared to correlate with sensitivity to apoptosis induced by NACO. Furthermore, increasing ER stress by treatment with tunicamycin resulted in increased sensitivity of the melanoma cells to NACO induced apoptosis. This evidence and results from studies with Omeprazole alone show promise that this may be an effective treatment approach in more malignant forms of melanoma.
Inhibition of Glycolysis in Melanoma Cells

Whereas the above approach depends on accumulation of protons and lactate within the cell, the second approach is to try and inhibit production of lactate and restore ATP production from the Krebs cycle. One such approach is the use of dichloroacetate (DCA). As outlined in Figure 3, one of the explanations for increased lactate levels is the induction by HIF-1α of pyruvate dehydrogenase kinase (PDH), the enzyme which converts pyruvate to acetyl CoA. DCA is an orally available small molecule which can inhibit PDK and which has been used for treatment of lactic acidosis. Bonet et al (38) reported that DCA could induce antitumor effects against xenografts of A549 human non-small cell lung cancer in immunodeficient rats and could reverse mitochondrial changes. DCA was also shown to induce apoptosis in endometrial (39) and prostate cancer cells (40). DCA has been in clinical use since 1969 for treatment of lactic acidosis in doses ranging from 12.5 to 100mg/kg/day (41). Peripheral neuropathy appeared to be a prominent side effect in studies on adult patients with a rare mitochondrial disorder but not in children with congenital lactic acidosis. Well conducted clinical trials with DCA have not been reported but internet searches suggest that it has been used in a non-controlled fashion in patients with a range of different cancers.

A second approach to reduction of lactate is based on inhibition of the M2 splice isoform of pyruvate kinase. This embryonic form of the kinase was reported to be overexpressed in cancer cells such as colon cancer and to be associated with glycolysis and lactate production from pyruvate rather than metabolism of pyruvate through
mitochondrial pathways. The mechanism involved is unclear (26). Nevertheless, a cyclic heptapeptide referred to as TLN-232 or CAP-232 which targets M2PK has been developed and a small phase II study conducted on 13 patients with metastatic melanoma by Thallion (www.thallion.com). One patient had a partial response and three had stabilization of their disease.

Topoisomerase 1 inhibitors such as Topotecan were shown to inhibit translation of HIF-1α (42,43) and transcription of its target genes such as vascular endothelial growth factor (44). Studies on the metabolic consequences of treatment with this class of drugs requires further study.

**Starvation of Cancer Cells**

A third metabolic approach may best be described as starvation of cancer cells. 3-Bromopyruvate (3-Br-PA) was reported to deplete cancer cells of ATP and to have antitumor effects against hepatocellular carcinoma in rats (45) without damage to normal tissues. Similar reports described activity against breast carcinoma in rats (46). 3-Br-PA is an alkylating agent that has a structural similarity to lactate (45). Although described as an inhibitor of HK II, it may also have activity against proton pumps in cancer cells (47). A combination of 3-Br-PA and a heat shock protein 90 inhibitor was reported to have synergistic activity against pancreatic xenografts (48). Other inhibitors of HK include Lonidamine (49).
Depletion of glucose in cancer cells can be achieved by the use of 2-deoxyglucose which is taken up into cancer cells and phosphorylated by hexokinases but is not further metabolized. As a single agent it was shown to improve results of radiotherapy in 20 patients with glioma (50) at doses of 200mg/kg. It was also shown to have single agent activity in vitro against a range of different cancers (51). Killing of human colon or breast carcinoma cells appeared to be associated with generation of reactive oxygen species (52). It was reported (53) that 2DG could induce ER stress and give rise to the UPR, which may explain the protective effect of 2DG at some concentrations either by activation of Akt (54) or MAPKinase (55). 2DG was reported to bypass Bcl-2 and Akt resistance in a rat model (56).

Although glucose is believed to be the major energy source for most cancer cells, fatty acid oxidation may be equally important in certain cancers like prostate carcinoma (57). Hernlund et al (58) investigated whether a combination of 2DG and Etomoxir may potentiate cancer chemotherapy drugs in vitro. Etomoxir is believed to inhibit transport of fatty acids into mitochondria. Both drugs were shown to reduce ATP levels in colon carcinoma cells and potentiated Cisplatin induced killing.

Cerulenin is a small molecule inhibitor of fatty acid synthase (FAS) which was shown in vitro to induce killing of human multiple myeloma cells perhaps by induction of ER stress (59). Cerulenin was also shown to induce apoptosis in vitro in A375 melanoma cells (60).
In conclusion, many drugs targeting metabolic pathways in cancer cells have been described (61). It is likely that different agents will be used in different cancers depending on the major metabolic pathways in the cancer cells. Targeting products downstream of different metabolic pathways such as lactate production has particular appeal, especially in highly glycolytic cancers such as melanoma. Well controlled clinical trials are now needed to test these hypotheses with currently available drugs.
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Table 1. Targeting Metabolic Pathways in Melanoma

<table>
<thead>
<tr>
<th>1. Block excretion of acid from cell (cell suicide approach)</th>
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<tbody>
<tr>
<td>Proton pump inhibitors (with or without n-acetyl cysteine).</td>
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<tr>
<td>Inhibition of monocarboxylic transporters MCT1-4</td>
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<td>Inhibition of carbonic anhydrase in cell membranes (IX &amp; XII) by aromatic sulfonamides</td>
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<th>2. Reduce glycolysis and lactate production</th>
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<tr>
<td>Dichloroacetate (DCA), an inhibitor of PDK that blocks conversion of pyruvate to acetyl CoA</td>
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<td>TLN232, a cyclic heptapeptide inhibitor of the embryonic M2 isoform of pyruvate kinase</td>
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<th>3. Depletion of energy sources for the tumor</th>
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<td>3-Bromopyruvate - depletes cells of ATP</td>
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<tr>
<td>2-Deoxyglucose – inhibits hexokinases</td>
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<tr>
<td>Etomoxir – inhibitor of fatty acid oxidation</td>
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<tr>
<td>Cerulenin – small m.w. inhibitor of fatty acid synthase</td>
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LEGENDS TO FIGURES

Figure 1
Dual role of Hexose Kinase II (HK). HKII phosphorylates glucose as it enters the cell which allows for the metabolism of glucose. HKII also binds to VDAC on the outer mitochondrial membrane which inhibits BAX-mediated opening of the mitochondrial pores, preventing the release of Cytochrome c and ultimately apoptosis.

Figure 2
A. Omeprazole binding to sulfydryl groups on the proton pumps.
B. Role of N-Acetyl Cysteine in acting as a transporter molecule for Omeprazole to proton pumps in cancer cells.

Figure 3
An abbreviated schematic of Glycolysis showing the two end points of lactate production and the TCA cycle. Various metabolic inhibitors, 2-deoxyglucose, 3-bromopyruvate, TLN232, dichloroacetate, Etoxomir and Cerulenin, and their sites of inhibition are indicated in the diagram.
Figure 2

A) OMP-S=O → H⁺ or {Enzymes} → OMP-S → S-[K⁺H⁺ ATPase]  
SH-[K⁺H⁺ ATPase]↑  

B) OMP-S=O → H⁺ or {Enzymes} → OMP-S → S-NAC  
SH-NAC ↑(Transporter) ↓  
SH-[K⁺H⁺ ATPase] → ↓← {Cancer Protease Enzymes}  
(Facilitate Di-Sulphide Interchange) ↓  
SH-NAC ↑(Transporter) + OMP-S → S-[K⁺H⁺ ATPase]  

Where OMP-S=O is Omeprazole, SH-NAC is N-Acetyl Cysteine and  
SH-[K⁺H⁺ ATPase] is the Proton Pump
Figure 3

Glucose → Glucose-6-P → Glyceraldehyde-3-P → 1,3-Diphosphoglycerate → Phosphoenolpyruvate (M2-Pyruvate Kinase) → Pyruvate

- Hexose Kinase
- 3-BrPA
- 2-DG
- M2-Pyruvate Kinase

Pyruvate → Lactate (LDH) → Acetyl-CoA → TCA Cycle

- LDH
- DCA → PDK

Alternative energy sources:
- Cerulenin

Amino Acids → Fatty Acids
- Etomoxir
- Cerulenin

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