COMMENTARY

Re-programming tumour cell metabolism to treat cancer: no lone target for lonidamine

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Tumour cell metabolism is very different from normal cell metabolism; cancer cells re-programme the metabolic pathways that occur in normal cells in such a manner that it optimizes their proliferation, growth and survival. Although this metabolic re-programming obviously operates to the advantage of the tumour, it also offers unique opportunities for effective cancer therapy. Molecules that target the tumour cell-specific metabolic pathways have potential as novel anti-cancer drugs. Lonidamine belongs to this group of molecules and is already in use in some countries for cancer treatment. It has been known for a long time that lonidamine interferes with energy production in tumour cells by inhibiting hexokinase II (HKII), a glycolytic enzyme. However, subsequent studies have uncovered additional pharmacological targets for the drug, which include the electron transport chain and the mitochondrial permeability transition pore, thus expanding the pharmacological effects of the drug on tumour cell metabolism. A study by Nancolas et al. in a recent issue of the *Biochemical Journal* identifies two additional new targets for lonidamine: the pyruvate transporter in the mitochondria and the H^+-coupled monocarboxylate transporters in the plasma membrane (PM). It is thus becoming increasingly apparent that the anti-cancer effects of lonidamine do not occur through a single target; the drug works at multiple sites. Irrespective of the molecular targets, what lonidamine does in the end is to undo what the tumour cells have done in terms of re-programming cellular metabolism and mitochondrial function.

Key words: hexokinase II, lonidamine, metabolism, monocarboxylate transporters, pyruvate carrier.

Tumour cells definitely differ from normal cells in many ways; unlike normal cells, tumour cells exhibit high proliferation rate, do not senesce and resist cell death. They also re-programme normal biochemical pathways to best suit their nutritional and metabolic needs. As a result, tumour cell metabolism is quite different from normal cell metabolism. Tumour cells not only up-regulate certain metabolic pathways but also modify others for their maximal benefit. Notable examples of tumour cell-specific metabolic pathways are: (i) aerobic glycolysis that converts glucose to lactate even in the presence of sufficient oxygen supply, (ii) glutaminolysis that uses the carbon skeleton in glutamine for oxidation to generate ATP and lactate, (iii) reductive carboxylation of α-oxoglutarate to generate citrate as a carbon source for fatty acid synthesis, (iv) gain-of-function mutations in isocitrate dehydrogenase to generate the metabolite 2-hydroxyglutarate, a modifier of DNA methylation and (v) suppression of citric acid cycle at specific sites to increase the cellular levels of succinate, also a modifier of DNA methylation, and fumarate, a regulator of HIF-1α turnover [1–4]. Indeed, 2-hydroxyglutarate, succinate and fumarate are called onco-metabolites as these compounds accumulate in cancer cells at severalfold higher levels than in normal cells and also elicit profound biological effects promoting initiation and progression of cancer [5].

Aerobic glycolysis, also known as Warburg effect, is a well-recognized biochemical phenomenon in tumour cells. The relative proportion of energy derived from the cytoplasmic glycolysis goes up whereas that derived from mitochondrial oxidation goes down in tumour cells. In normal cells, oxygen suppresses glycolysis because ATP generated by oxidative metabolism of glucose-derived pyruvate in mitochondria inhibits phosphofructokinase-1, a rate-limiting step in glycolysis. This process does not occur in most tumour cells due to suppression of mitochondrial oxidation [6]. The logic behind the suppression of mitochondrial oxidation in tumour cells is not entirely clear but it is at least partly to do with the fact that mitochondrial oxidation is also a source of reactive oxygen species that could be detrimental to tumour cells. Although the decreased ATP production in mitochondria relieves ATP-dependent blockade of glycolysis, this cannot be the entire explanation for enhanced glycolysis in tumour cells. The NAD^+/NADH ratio is also an important determinant of glycolysis rate as it controls the activity of the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH). When the mitochondrial oxidation is suppressed, NADH is not oxidized to NAD^+, which would deplete NAD^+ that is needed for GAPDH. This necessitates an alternative mechanism for NAD^+ generation if glycolysis has to continue. Tumour cells hence convert pyruvate, which is generated from glycolysis but cannot be metabolized in mitochondria due to the suppressed mitochondrial oxidation, into lactate; this reaction converts NADH, which is generated at the level of GAPDH from NAD^+, back into NAD^+ to enable the GAPDH-mediated reaction to continue. The conversion of pyruvate to lactate occurs in the cytoplasm and the reaction is catalysed by lactate dehydrogenase (LDH). LDH is a tetramer consisting of two proteins encoded by two separate genes: *LDH-A* and *LDH-B*. There are five isoforms of LDH that are made up of

Abbreviations: ANT, adenine nucleotide translocase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HK, hexokinase; HKII, hexokinase II; IMM, inner mitochondrial membrane; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; MPC, mitochondrial pyruvate carrier; OAA, oxaloacetate; OMM, outer mitochondrial membrane; PM, plasma membrane; SDH, succinate dehydrogenase; SLC, solute-linked carrier; SMCT, sodium-coupled monocarboxylate transporter; VDAC, voltage-dependent anion channel.

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the two subunits in different combinations: A_4, A_3B_1, A_2B_2, A_1B_3 and B_4. The kinetic features of the two subunits for the reaction catalysis are such that A_4 is more tuned to convert pyruvate to lactate whereas B_4 is more tuned to convert lactate to pyruvate. Accordingly, tumour cells silence the expression of LDH-B, thus making the conversion of pyruvate to lactate more efficient than the reverse reaction [7]. The end result is that glycolysis is accelerated even with the suppression of mitochondrial function and the proportion of ATP generated from glycolysis (substrate-level phosphorylation) is increased compared with ATP generated from mitochondria (oxidative phosphorylation). Even though the amount of ATP generated in the cytoplasm is increased in tumour cells, the inhibition of phosphofructokinase-1 by this excess ATP is relieved because tumour cells also up-regulate the bifunctional enzyme phosphofructokinase-2/fructose-2,6-bisphosphatase-3, thus increasing the cellular levels of fructose-2,6-bisphosphate, a potent activator of phosphofructokinase-1.

Interestingly, there is a parallelism between normal cells compared with tumour cells and naïve T-cells compared with activated T-cells in terms of glucose and glutamine metabolism [8,9]. Similar to normal cells, naïve T-cells exhibit a low proliferation rate and utilize glycolysis and oxidative metabolism for ATP generation. In contrast, when these naïve T-cells are activated by antigen presentation, their proliferation rate goes up and the cells adopt a metabolic profile that is similar to that of tumour cells. Activated T-cells rely on aerobic glycolysis as well as glutaminolysis with suppressed mitochondrial oxidation for their energy production and proliferation. Both in tumour cells and activated T-cells, the hypoxia-inducible factor-1α and the oncogene c-Myc mediate the metabolic re-programming to facilitate aerobic glycolysis and glutaminolysis.

Although tumour cells have solved the problem of how to keep glycolysis going while suppressing mitochondrial oxidation at the same time by converting pyruvate to lactate, the cells now face the issue of how to deal with lactate. Lactic acid, unless effectively removed from the cell, will cause intracellular acidification, thereby compromising cell survival. To solve this problem, tumour cells up-regulate the H^+-coupled monocarboxylate transporters MCT1 and MCT4, which mediate the efflux of lactic acid from the cells [10]. The resulting acidification and increase in lactate levels in the extracellular milieu provide additional advantages for the tumour by facilitating acid pH-induced necrosis of the surrounding cells to make room for tumour growth and by lactate-induced autocrine signalling via the cell-surface G-protein-coupled lactate receptor GPR81 whose expression is up-regulated in tumour cells [11–13].

As tumour cells re-programme metabolic pathways to support their growth, these tumour cell-specific pathways offer novel drug targets for cancer therapy. The logic is simple: counter the re-programmed metabolic pathways to negate their tumour-promoting advantages. In theory, this can be done by blocking glycolysis, inhibiting LDH or preventing lactic acid efflux via MCTs. Indeed, all of these avenues are actively being pursued as potential targets for cancer therapy in the design of new anti-cancer drugs. Lonidamine [1-(2,4-dichlorobenzyl)-1*H*-indazole-3-carboxylic acid] represents one such drug; it is approved in many European countries for cancer treatment and has been shown to improve the efficacy of other chemotherapy agents when used in combination [14,15]. In the United States, the drug was evaluated in a phase 3 clinical trial for the treatment of benign prostatic hyperplasia, but the Food and Drug Administration has suspended the trial due to development of liver toxicity in some patients on the drug. However, other clinical trials to determine the efficacy of lonidamine for different solid tumours are still ongoing. Investigations into the molecular mechanisms of the anti-cancer effects of lonidamine have revealed that the drug is an inhibitor of the glycolytic enzyme hexokinase II (HKII) [16]. There are four isoforms of hexokinase, and HKII is selectively up-regulated in tumours [17]. In addition, this isoform is bound to the outer membrane of the mitochondrion where it is physically associated with the mitochondrial permeability transition pore, which consists of the adenine nucleotide translocase (ANT) in the inner mitochondrial membrane (IMM) and the voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane (OMM) [18,19]. The interaction of the enzyme with the permeability pore occurs via VDAC. The physical proximity of HKII to the permeability pore is important to tumour cells because it allows the enzyme ready access to mitochondrial ATP via ANT. The end result of lonidamine treatment is therefore the blockade of glycolysis, thereby interfering with ATP production in tumour cells. Subsequent to the original discovery of HKII as a target, two additional targets have been identified for lonidamine: VDAC and Complex II. VDAC as a component of the mitochondrial permeability transition pore plays a critical role in the control of mitochondria-initiated apoptosis [20]. The anti-apoptotic proteins such as Bcl-2 maintain the permeability pore in a closed state, preventing the cytoplasmic release of pro-apoptotic proteins such as cytochrome *c* and apoptosis-inducing factor, which are normally located in the inter-membrane space of mitochondria. Lonidamine disrupts the barrier function of this permeability pore, thus triggering apoptosis [21]. Inhibition of Complex II in the electron transport chain, also known as succinate dehydrogenase (SDH), by lonidamine interferes with the citric acid cycle and also with the electron transport chain [22]. Later studies have revealed the complexity of the interaction between lonidamine and Complex II [23]; the drug blocks the transfer of electrons from succinate to Coenzyme Q but does not interfere with the conversion of succinate to fumarate.

In a recent issue of the *Biochemical Journal*, Nancolas et al. [24] have identified two new targets for lonidamine: the mitochondrial pyruvate carrier (MPC) and the H^+-coupled monocarboxylate transporters MCT1 and MCT4. Using purified mitochondria, the authors have shown that lonidamine blocks the mitochondrial import of pyruvate via the pyruvate transporter located in the IMM. This transporter is known as the MPC, which has been recently characterized at the molecular level [25], and its transport function is energized by the H^+ gradient that exists across the IMM. The inhibition is potent, with the inhibition constant of $\sim$2.5 μM. The authors suggest that the drug-induced blockade of pyruvate entry into mitochondria with consequent interference with pyruvate oxidation and ATP production within the mitochondria is at least partly responsible for the anti-cancer effects of the drug. The drug also blocks the function of the lactic acid efflux transporters MCT1 and MCT4, though with comparatively less potency; the inhibition constant is 35–40 μM. Various clinical trials in humans have shown that the peak plasma levels of lonidamine are in the range of 15–100 μM at a daily dose of 150 mg or 180–520 mg/m^2 [26,27], clearly demonstrating that significant inhibition of the MPC and the monocarboxylate transporters is achievable in clinics with lonidamine. These latest targets of lonidamine along with the already known ones, their cellular locations and their biological functions are depicted in Figure 1. With the study by Nancolas et al., the total number of pharmacological targets for lonidamine has reached five. It seems that there is no lone target for lonidamine to fight cancer.

The apparent lack of specificity for the interaction of lonidamine with its targets is intriguing. HKII catalyses the phosphorylation of glucose, VDAC is an anion channel, Complex II converts succinate to fumarate and transfers electrons to Coenzyme Q, MPC transports pyruvate but not lactate and MCT1

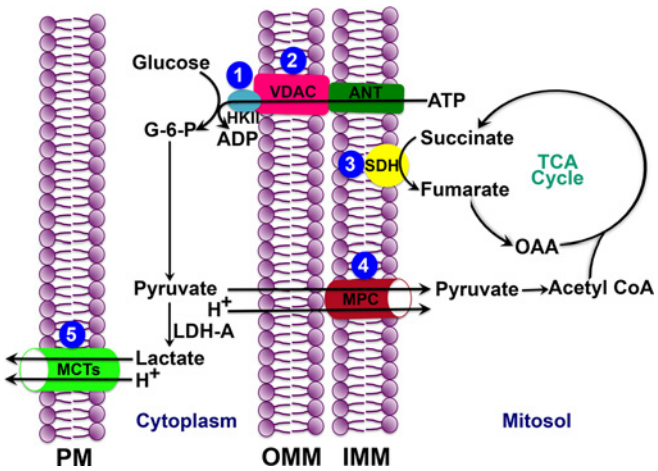

Figure 1 Molecular targets of the anti-cancer drug lonidamine in tumour cells

To date, five targets have been identified for the drug, which are indicated by numbers 1–5. OAA, oxaloacetate.

and MCT4 transport pyruvate as well as lactate. There is no obvious commonality among these different lonidamine targets either in terms of their biological function or in terms of their substrates. It is interesting however to note that MPC and MCTs accept monocarboxylates as substrates and that lonidamine does possess a monocarboxylate group. But we do not know yet whether lonidamine is a transportable substrate for either of these transporters. The study by Nancolas et al. [24] has only shown that lonidamine inhibits pyruvate transport mediated by MPC and lactate transport mediated by MCTs, but these findings do not address the issue of whether or not lonidamine is a transportable substrate for these transporters. The observed inhibition could be either due to competition between the monocarboxylate substrates and lonidamine for the transport process or due to the blockade of the transport process by lonidamine without itself being transported across the membrane by the transporters. Studies from our laboratory have shown that α-methyltryptophan blocks the function of the amino acid transporter SLC6A14 but is not a transportable substrate [28]. Additional studies are therefore needed to determine the mechanism of action of the drug on MPC and MCTs. About a year ago, we investigated whether lonidamine is transported via SLC5A8 (also known as SMCT1), a Na^+-coupled monocarboxylate transporter. We did not find any evidence of lonidamine transport via the transporter, but we also did not examine if the drug works as a blocker of the transporter (E. Babu and V. Ganapathy, unpublished work).

Another interesting issue related to the findings of Nancolas et al. [24] is the relevance of the observed robust inhibition of MPC by lonidamine to the anti-cancer efficacy of the drug. Most tumour cells re-programme their metabolic pathways that result in defective mitochondrial metabolism of pyruvate. The major aspect of this re-programme is the tumour cell-specific up-regulation of the enzyme pyruvate dehydrogenase kinase (PDK), which phosphorylates the α-subunit of the pyruvate dehydrogenase complex and inhibits the activity of the complex [29]. Forcing the tumour cells to metabolize pyruvate by mitochondrial oxidation is effective in killing these cells. This seems to be the principal mechanism underlying the anti-cancer effects of dichloroacetate, an inhibitor of PDH kinase [30]. The ability of dichloroacetate to promote pyruvate oxidation is evident from the fact that this compound is used as a drug to treat lactic acidosis. Facilitation of pyruvate metabolism by the drug alters the equilibrium between lactate and pyruvate by promoting the LDH-mediated conversion of lactate to pyruvate, thus reducing lactate levels. The well-established anti-cancer effects of dichloroacetate and the facilitation of mitochondrial oxidation of pyruvate as the underlying mechanism raise questions as to the contribution of lonidamine-mediated inhibition of MPC to the anti-cancer efficacy of the drug. On the surface, the ability of both dichloroacetate and lonidamine to function as anti-cancer drugs seems paradoxical given their opposite effects on mitochondrial pyruvate metabolism. A possible explanation for the paradox is the heterogeneity of tumour cells within a tumour. Tumour cells within a given tumour are not biochemically homogeneous; some reside in a relatively hypoxic environment whereas some have adequate supply of oxygen. Therefore, suppression of mitochondrial oxidative function is not likely to be a universal phenomenon among the tumour cells within a tumour; it is thus plausible that dichloroacetate and lonidamine actually target different groups of tumour cells to elicit their anti-cancer effects. The ability of lonidamine to inhibit SDH does not raise similar issues. Loss-of-function mutations in SDH do cause cancer (e.g. paraganglioma, gastrointestinal stromal tumours) [31], but lonidamine inhibits the ability of the enzyme to transfer electrons to Coenzyme Q without blocking the conversion of succinate to fumarate [23]. Inactivation of SDH causes cancer most likely through cellular accumulation of the onco-metabolite succinate; lonidamine may not impact on cellular levels of this metabolite.

With the lack of a rational structural or molecular basis for the interaction of lonidamine with so many seemingly different proteins with diverse biological functions (enzymes, channels and transporters), one wonders whether we have seen the last of the lonidamine pharmacological targets or if more are yet to come.

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