

Optimization of Tumor Radiotherapy With Modulators of Cell Metabolism: Toward Clinical Applications

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Most solid tumors are characterized by unstable perfusion patterns, creating regions of hypoxia that are detrimental to radiotherapy treatment response. Because postsurgical radiotherapy, alone or in combination with other interventions, is a first-line treatment for many malignancies, strategies aimed at homogeneously increasing tumor pO₂ have been the focus of intense research over the past decades. Among other approaches of demonstrable clinical and preclinical utility, this review focuses on those directly targeting oxygen consumption to redirect oxygen from a metabolic fate to the stabilization of radiation-induced DNA damage, more particularly drugs targeting glucose and lactate metabolism, nitric oxide donors or inducers, and mitogen-activated protein kinase pathway inhibitors. Their utility as adjuvant treatments with radiotherapy has been proven preclinically, which should foster further their clinical development and evaluation.

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During the past decades, intense efforts have been made to counteract tumor hypoxia, the major cause of resistance to radiotherapy.¹⁻³ Hypoxia-dependent radioresistance has been known for more than a century⁴ and is related to the fact that oxygen enhances water radiolysis and fixes DNA damage following radiation treatment. Otherwise, DNA radicals, R●, react with sulfhydryl groups, ascorbate, or water to restore native DNA. However, the reaction of oxygen with R●

results in the formation of an organic peroxide, which is not easily repaired.⁵ In addition to direct chemical effects, oxygen has also indirect effects modulating radiosensitivity.⁶ Thus, the absence of oxygen is estimated to significantly reduce radiosensitivity at levels of oxygen below a threshold of 10 mm Hg. This is significant as pO₂ levels this low are often found in areas of human and rodent tumors, whereas normal tissue pO₂ is usually around 40 mm Hg.⁷

Hypoxia in solid tumors is the consequence of a negative balance between oxygen supply and consumption. It has many causes and can fluctuate over time. Considering chronic and acute hypoxia as 2 separate and static entities appears nowadays to be outdated. Indeed, many experimental data recently reviewed by Dewhirst et al^{8,9} and based on the direct observation of tumors often using intravital microscopy have revealed that tumor hypoxia is a cyclic phenomenon owing to the superimposition of low- and high-frequency fluctuations in pO₂ that by analogy can be compared with tides and waves. Briefly, low-frequency variations (tides) occur at a timescale of days and affect large tumor regions.^{10,11} They are due to dynamic structural changes in the tumor microvasculature.⁸ High-frequency variations (waves) occur at a timescale of a few cycles an hour and correspond to fluctuations in red blood cell (RBC) flux affecting specifically different microregions of the tumor on the whole tumor scale.^{12,13} They are more frequent in tumor areas containing vessels with a muscular coat¹⁴ and have been experimentally shown to correspond to (1) fluctuations in RBC flux that find their main origin in uneven RBC

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partitioning at vascular branches notably exacerbated by the low deformability of hypoxic RBCs,¹⁵ (2) changes in vascular diameter affecting resistance vessels,¹⁶ and (3) potentially, rapid vascular remodeling through longitudinal vascular splitting (intussusception), vascular pruning, or the connection of new vascular loops.^{8,9,17,18} Cycling hypoxia influences radial oxygen delivery from tumor blood vessels more severely when the overall oxygen vascular input in the microregion is low.^{8,9} Features at a larger scale that influence local oxygen input result from (1) the abnormal structure of the tumor vascular bed (scarcity of afferent arterioles, improper vascular orientation, low vascular density with improperly formed vessels, and arteriovenous shunts that can divert the blood away from the tumor), rheology (increased blood viscosity resulting from osmotic water leakage and decreased RBC deformability and perfusion abnormalities with some smaller vessels carrying very few to no RBCs), and cell metabolism (oxygen consumption in excess of supply with high metabolic activities at the tumor margin, which create a steep gradient of longitudinal blood deoxygenation).^{8,9,19}

Therefore, it seems obvious that a straightforward strategy to improve the radiosensitivity of solid tumors would consist of improving tumor oxygenation. Considering the origins of tumor hypoxia, this could logically be achieved by increasing the delivery of oxygen from the blood or by targeting the local consumption of oxygen by malignant and nonmalignant cells within the tumor, or both.²⁰

Increased oxygen delivery can be achieved by either increasing the oxygen transport capacity of the blood or increasing tumor perfusion. Historically, breathing hyperoxic or hyperbaric gases was among the first methods investigated as a modality to increase the oxygen saturation of the blood. Carbogen (95% O₂/5% CO₂) breathing is theoretically preferable to pure oxygen breathing because CO₂ counters the vascular contraction induced by oxygen.²¹ ARCON (accelerated radiotherapy combined with inhalation of hyperoxic gas and nicotinamide) was tested in the clinics, where it initially provided encouraging results (for the treatment of head and neck cancer, where study end points included local or regional control, compliance, and side effects).²² However, retrospective studies showed that the therapeutic benefit of ARCON was unclear, and also carbogen was found to be poorly tolerated.²³⁻²⁵ Limited therapeutic benefit can be at least partially attributed to the poor solubility of oxygen in the plasma, whereas RBCs are already normally fully reoxygenated in the lungs of patients breathing room air.²⁶ Another strategy tested to boost oxygen delivery to (and thus, radiosensitize) tumors was to correct the anemia often associated with cancer and cancer therapy. Unfortunately, multivariate analyses of clinical trials involving blood transfusions or erythropoietin delivery were disappointing.²⁷

When approaches aimed at increasing the oxygen transport capacity of the blood were showing their limits in clinical practice, others including our teams tested whether vasoactive drugs could be used to improve tumor perfusion and oxygenation and, therefore, the outcome of radiotherapy. This strategy is known as 'provascular'.¹⁹ Several agents and methods have provided encouraging preclinical data.^{19,28} A key for success was to achieve tumor-selective vasodilation, which has been

demonstrated for vasoactive agents activated at low pO₂ (such as S-nitrosylated hemoglobin)²⁹ or at low pH (such as nitrites),³⁰ agents targeting vasoconstriction pathways selectively activated in tumors (such as endothelin-1 receptor inhibitors),^{31,32} and, surprisingly enough, radiotherapy itself (with the first dose of X-rays sensitizing the tumor to the second dose via active vasodilation, and so on).^{33,34} When tumor-selective vasodilation was not achieved, nonselective vasodilation generally led to unpredictable responses in tumor perfusion and oxygenation because of the so-called 'steal Effect'.³⁵ Indeed, the proportion of mature, vasoactive blood vessels is consistently less in solid tumors compared with adjacent normal tissues; thus, systemic vasodilation may lead to opposing perfusion patterns between cancer and normal tissues. When vascular beds are in parallel, nonselective vasodilation would decrease the resistance to flow in normal tissues compared with the tumor, leading to a net decrease in tumor perfusion (Fig. 1). However, when vascular networks are in series, then tumor perfusion would increase. Both types of networks are inextricably interconnected in growing tumors. Therefore, the use of tumor-selective vasodilators is

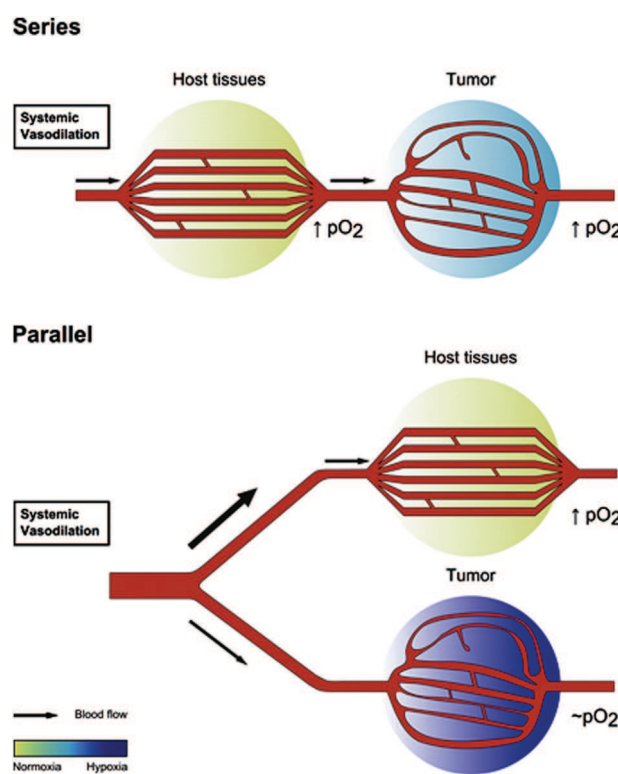


Figure 1 Steal Effect. Systemic vasodilation has unpredictable effects on tumor perfusion and oxygenation, and it is therefore not viewed as a valid treatment option with radiotherapy. Tumors contain fewer mature, vasoresponsive blood vessels than normal tissues. If vascular beds are in series, systemic vasodilation would increase tumor perfusion and oxygenation. If they are in parallel, systemic vasodilation would decrease tumor perfusion and oxygenation, because blood would ooze out of the tumor to the normal tissues that offer a lower resistance to flow (steal effect). Because vascular networks in series and in parallel coexist in vivo, it is difficult to predict the overall oxygen and perfusion effects of systemic vasodilation in tumors. (Color version of the figure is available online.)

the only viable option to increase consistently tumor perfusion and radiosensitivity. Details on the vasoactive treatments validated preclinically are available in recent reviews.^{3,19,36}

In the same line of investigation, several treatments have been tested for their ability to induce structural modifications to increase tumor perfusion. For example, nicotinamide improves the deformability of RBCs³⁷ and is part of the ARCON protocol. However, the rationale behind its radiosensitizing properties could be primarily owing to the conversion of NADH to NAD⁺, which would lead to the stimulation of many metabolic processes, including oxidative metabolism and glycolysis, with shifts in the oxygen consumption rate and acid-base balance.³⁸ Calcium antagonists, especially flunarizine, can also increase RBC deformability and, thereby, decrease blood viscosity.¹⁵ Although these treatments are aimed at targeting RBCs, other compounds target immature tumor blood vessels. Chemotherapy used in a metronomic mode and antiangiogenic drugs in their initial response phase or used at low dose have been shown to increase tumor perfusion through the removal of inappropriately located endothelial cells from blood vessels. This results in the restoration of the endothelial continuum and enhanced tumor perfusion, a process that has been widely explored and is known as 'vascular normalization'.^{39,40} The clinical significance of this strategy has been the topic of a recent review.⁴¹

This review focuses on a different determinant of the oxygen balance in tumors, cellular metabolism. By comparison with strategies intended to improve oxygen delivery to tumors, mathematical models⁴²⁻⁴⁴ suggest that targeting cell metabolism is a more efficient mean to radiosensitize tumors. In principle, any amount of oxygen spared from metabolism could be used to stabilize DNA damage at the time of radiotherapy. Based on several pieces of experimental data, it is hypothesized that even a modest decrease in cell respiration could abolish tumor hypoxia, whereas large increases in tumor perfusion or arterial pO₂ would be needed to achieve the same effect.⁴²⁻⁴⁴ Targeting oxygen consumption also offers the theoretical advantage of reducing oxygen heterogeneities within the tumor tissue.⁴²

Although aerobic glycolysis, known as the 'Warburg effect', after decades, has eventually been recognized as a hallmark of solid tumors and gained renewed attention,⁴⁵ it is of importance to realize that Warburg-phenotype tumor cells probably only represent a small population of cells that coexists with (1) cells that are glycolytic only because they are hypoxic and (2) cells that primarily perform oxidative metabolism. We have shown that glycolytic tumor cells cooperate with oxidative tumor cells to survive,⁴⁶⁻⁴⁸ stressing the importance of both metabolic populations in tumor development. In fact, recently obtained *in vivo* data suggest that mitochondria generally retain full oxidative capacities in solid tumors⁴⁹ although some exceptions exist that are associated with mutations in genes of the tricarboxylic acid (TCA) cycle.⁵⁰ Therefore, targeting mitochondrial metabolism to enhance tumor oxygenation appears to be a suitable approach to radiosensitize tumors. This argument is strengthened by the use of drugs affecting tumor metabolism with no or negative effects on the tumor blood flow, which nevertheless have been shown to radiosensitize tumors owing to an oxygen effect (see details later).⁵¹⁻⁵⁵

In this review, we focus on drugs targeting glucose and lactate metabolism, nitric oxide (NO) donors or inducers, and mitogen-activated protein kinase (MAPK) pathway inhibitors. These treatments have been shown preclinically to enhance the radiosensitivity of solid tumors. Where relevant, we have mentioned what we believe are the next steps for a successful translation of these approaches to clinical applications. Other strategies, hyperthermia⁴⁷ and the use of antiinflammatory drugs,⁵⁶ have been reviewed elsewhere.

Targeting Glucose Metabolism

A salient feature of solid tumors is their avidity for glucose, supported by a large body of data, including clinical data obtained using the glucose tracer [18F]-fluorodeoxyglucose (FDG) in positron emission tomography.⁵⁷ Although positron emission tomography positivity with FDG is most often interpreted to reflect a high glycolytic rate, there is also convincing evidence that OXPHOS is upregulated in some cancer types.^{49,58}

Two radiosensitizing strategies are possible that target glucose metabolism. The first one aims at inhibiting the general use of glucose and has been explored with the glucose competitor 2-deoxyglucose (2DG). Results from *in vitro* experiments revealed that 2DG is an efficient radiosensitizer acting through (1) blocking glutathione recycling (which largely depends on the glucose-fueled pentose phosphate pathway) thereby preventing reactive oxygen species detoxification^{59,60} and (2) inhibition of ATP-dependent DNA repair.⁶¹⁻⁶³ However, high variability was reported in the response of human tumor cells,⁶³ which may be linked to their p53 status. For example, 2DG radiosensitized p53-positive lung tumor cells, whereas it conferred dose-dependent radioresistance to cells lacking p53, which was confirmed by re-expressing p53 in deficient cells and silencing p53 in wild types.⁶⁴ In MIA PaCa-2 (p53-mutated pancreatic cancer cell line) xenografts, the combination of 2DG (0.5 g/kg) with radiotherapy (1 × 15 Gy) induced a tumor growth retardation that was significant compared with 2DG alone but not with radiotherapy alone.⁶⁰ Similarly, p53-expressing non-small cell lung tumors in mice were only marginally sensitized to radiotherapy (10 × 3 Gy) when treated chronically with 2DG (3 g/kg).⁶⁴ Despite variations in 2DG effects, a first single-arm clinical trial combining phases I and II was performed with 20 patients with glioma treated with whole-brain γ -ray irradiation (4 × 5 Gy/week) and 2DG (200 mg/kg).⁶⁵ The study reported limited toxicity and an improved quality of life. In a second clinical trial,⁶⁶ 12 patients with glioblastoma received higher irradiation (7 × 5 Gy/week) and 2DG (250 or 300 mg/kg) doses, and 2DG was administered orally ~30 minutes before each irradiation. Dose escalation with 2DG revealed that symptoms similar to hypoglycemia were dose limiting, and the report suggested a clinical benefit in quality of life and survival. Finally, a single-arm multicentric phase II clinical trial with 70 patients with glioblastoma was conducted using the same irradiation protocol as for the dose escalation trial in combination with 2DG (250 mg/kg). Clinical details

have not been published yet; however, a review from 2009 claims clinical benefits compared with standard of care temozolomide treatment, as well as preservation of normal brain tissue.⁶⁷ These trials collectively indicate that combining γ -ray radiotherapy with 2DG to treat brain tumors is feasible, safe, and clinically relevant. Of note, combining 2DG with the pentose phosphate pathway inhibitor 6-aminonicotinamide (6-AN) has also been investigated *in vitro* as a way to decrease the dose of 2DG; as expected, the combined targeting of related metabolic pathways allowed for the retention of radiosensitizing effects, even when low doses of the inhibitors were used (2DG, 5 mM; 6-AN, 5 μ M).⁶⁸

Alternative methods of modifying glucose metabolism and oxygen consumption for therapeutic benefit also exist. For example, in 2002 we reported that insulin can be used to decrease the consumption rate of oxygen by tumor cells *in vivo*.⁵¹ The underlying molecular mechanism involves NO biology. In particular, inhibition of NO synthase fully prevented insulin-induced tumor reoxygenation and abolished the radiosensitizing effect (Fig. 2). The current hypothesis is that insulin triggers the production of NO by the endothelial NO synthase isoform, which in turn reversibly inhibits cytochrome *c* oxidase in complex IV of the mitochondrial electron transport chain.³ Whether a decrease in glucose consumption participates in the therapeutic benefit remains to be tested. In a follow-up study,⁶⁹ we showed that the systemic hypoglycemia induced by insulin in tumor-bearing mice can be corrected by concomitant glucose delivery without affecting the gain in radiosensitivity, thus opening a path and

offering a rationale toward testing this strategy in future clinical trials.

A second strategy targeting glucose metabolism exploits the Crabtree effect, ie, inhibition of cellular respiration by glucose.⁵⁸ Here, the idea is that a moderate increase in glucose delivery to tumors could improve the radiotherapeutic response.⁷⁰ Accordingly, Snyder et al⁷¹ showed that hyperglycemia (achieved by infusing glucose *i.v.* at 1 g/kg) combined with hyperbaric oxygen breathing improved the oxygenation of rat mammary carcinoma tumors better than either individual treatment. In this study, the median pO₂ increase for the combination treatment amounted to 27 mm Hg compared with 3–5 mm Hg for individual treatments. Another study⁷² combined hyperglycemia (300 mg glucose per 100 ml blood, corresponding to approximately 75% of what was achieved in the Snyder study) with mild hyperthermia and radiotherapy for the treatment of rhabdomyosarcoma-bearing rats. A dose-modifying benefit of glucose on thermoradiotherapy (used at TCD₅₀) was reported, with a glucose enhancement ratio of 1.2. Despite these promising results, using glucose as a radiosensitizer did not reach clinical practice, probably because of the constraints associated with continuous blood glucose monitoring and the fear of life-threatening side effects. In one of the few clinical trials reported to date (78 patients, 2 arms), Nagata et al⁷³ concluded that hyperglycemia can enhance the effectiveness of thermoradiotherapy for the treatment of superficial tumors. However, additional studies are needed to confirm these data.

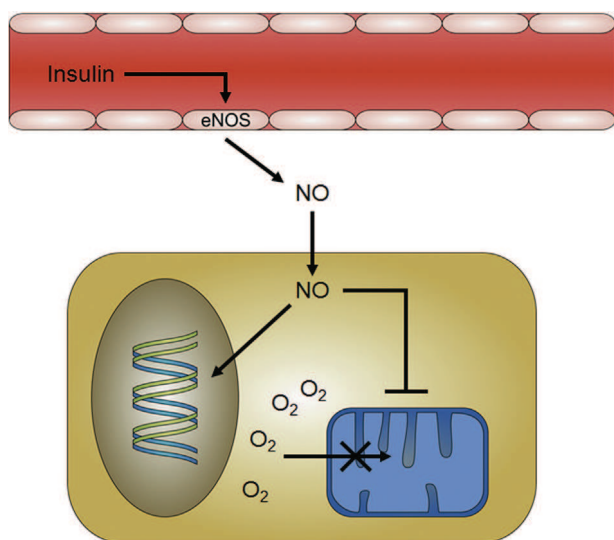


Figure 2 Insulin as a radiosensitizer. Following systemic delivery, insulin triggers endogenous NO production in tumors through the stimulation of endothelial NO synthase (eNOS) activity. eNOS can be expressed in vascular endothelial cells and in tumor cells (not represented here). Once produced, NO freely diffuses. In tumors, insulin has 2 documented actions through NO: inhibition of mitochondrial oxygen consumption and improved stabilization of radiation-induced DNA damage.^{51,102} This makes insulin an attractive treatment modality to radiosensitize tumors.⁶⁹ (Color version of the figure is available online.)

Targeting Lactate Metabolism

During hypoxia, OXPHOS becomes uncoupled from the TCA cycle, making anaerobic glycolysis the main source of cellular ATP. If hypoxia persists and after cells have consumed all internal resources (autophagy), cell death as a short-term outcome is the only alternative possibility. Although anaerobic glycolysis produces ATP less efficiently than aerobic metabolism, it does so at a faster rate, which would give a proliferative advantage to cells engaged in glycolysis. Therefore, hypoxia is believed to further pressure cells to adopt constitutive glycolysis (Warburg-phenotype).⁷⁴ But tumors also comprise oxidative tumor cells located in oxygenated compartments close to perfused blood vessels. Interestingly, there is increasing evidence that these distinct cell populations not only coexist but also cooperate and exploit differences in metabolic patterns between tumor and stromal cells to sustain and drive tumor progression.⁴⁸

To cope with the inefficiency of glycolysis to produce ATP, cells must dramatically increase the flux of glycolytic metabolites. This is achieved through the activation of key transcription factors, notably HIF-1 (which controls the expression of enzymes and transporters necessary for glycolysis)⁷⁵ and c-Myc (which controls the activity of pyruvate kinase and glutamine metabolism).^{76–78} Activation of the appropriate transcription factors and cell machinery allows for metabolic adaptation and sustained cell survival as long as enough glucose and glutamine are available. When fueled, glycolysis

produces up to 2 molecules of lactate per molecule of glucose. The lactate pool is further increased by glutamine metabolism. Because of poor blood and lymphatic drainage, these metabolic patterns tend to create a gradient of high lactate concentrations in tumors.^{48,79}

In contrast to these metabolic patterns, cells located within 100–200 μm from perfused blood vessels receive oxygen and glucose. These conditions may favor oxidative metabolism or, when they are proliferating, cells may switch to Warburg glycolysis. In what we believe was an important contribution to this field, we found that oxidative tumor cells preferentially use lactate vs glucose to fulfill their oxidative activities, which could create a metabolic symbiosis among different metabolic subpopulations of cancer cells (Fig. 3).⁴⁶ This metabolic preference

for lactate over glucose can be explained by (1) a competition between the oxidation of lactate to pyruvate (the lactate dehydrogenase-1 reaction) and the oxidative phosphorylation of glyceraldehyde-3-phosphate to glycerate-1,3-bisphosphate (the GAPDH reaction of glycolysis) for NAD^+ ,⁸⁰ (2) allosteric inhibition of glycolysis by lactate,⁸¹ and (3) the fact that the production of pyruvate from lactate produces energy (in the form of NADH), whereas glycolysis requires the maintenance of many enzymes and an initial input of energy (in the form of ATP).⁴⁶ Consequently, glucose spared in the oxygenated tumor compartment is made available for glycolytic tumor cells, in this metabolic symbiosis (Fig. 3).⁴⁶ Data from other studies suggest a converging paradigm and support the importance of metabolic symbiosis in the tumor

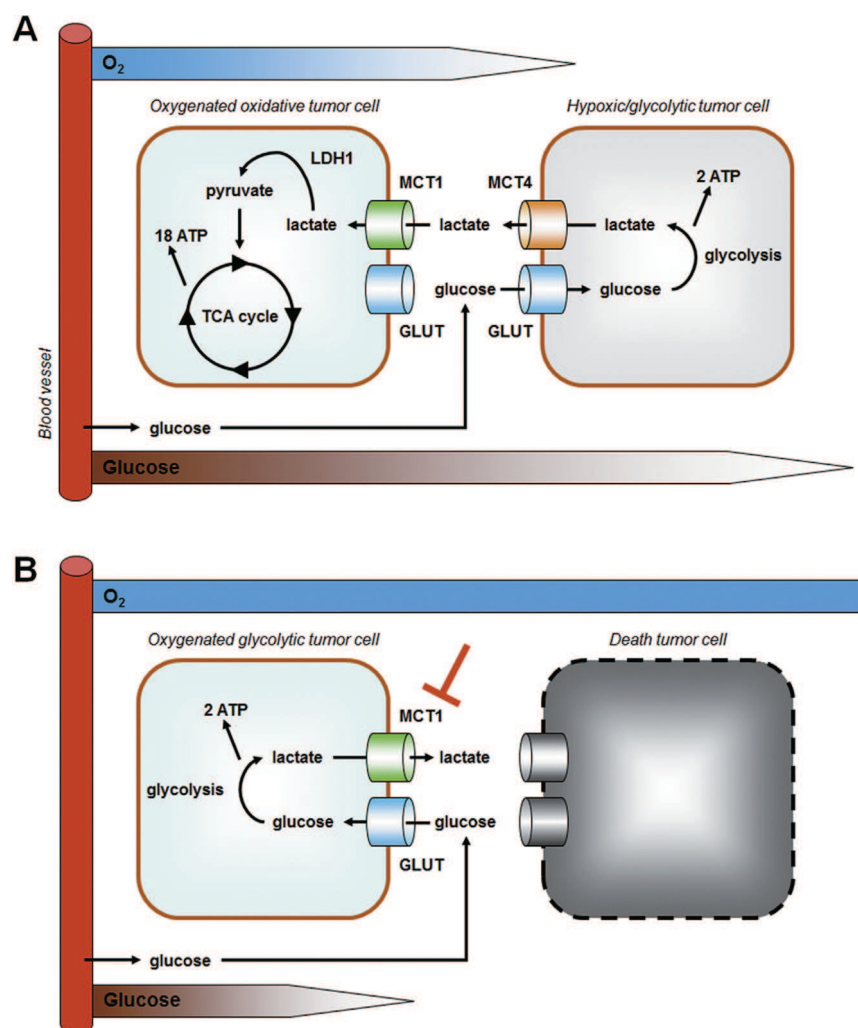


Figure 3 Simplified representation of intercellular lactate shuttling and its inhibition in cancer. (A) Tumors comprise oxygenated tumor cells close to perfused blood vessels and hypoxic cells at remote locations. Hypoxic cells use accelerated glycolysis to produce ATP; they are therefore addicted to glucose and release lactate through hypoxia-induced MCT4.^{85,86} Oxygenated cells receive glucose from the blood and lactate from glycolytic cells. They preferentially use lactate to fuel their oxidative metabolism via MCT1,⁴⁶ thus increasing the availability of glucose for glycolytic cells. The oxidative use of lactate requires lactate oxidation to pyruvate by LDH1. (B) MCT1 inhibition induces a switch from lactate-fueled respiration to glucose-fueled glycolysis in oxidative tumor cells.⁴⁶ Their survival now depends on glycolysis at high rate, which indirectly causes the death of hypoxic cells by virtue of glucose starvation. This metabolic rearrangement abolishes tumor hypoxia, offering the opportunity to eradicate the remaining tumor cells with radiation therapy. GLUT, glucose transporter; LDH, lactate dehydrogenase. (Adapted with permission from Porporato et al⁴⁷) (Color version of the figure is available online.)

microenvironment. For example, Lisanti et al^{82,83} showed that oxidative tumor cells obtain additional lactate (and ketone bodies) by stimulating autophagy in cancer-associated fibroblasts.

Lactate and ketone bodies use monocarboxylate transporters (MCTs) to cross cell membranes.⁸⁴ Among isoforms known to transport lactate, we found that MCT1 predominantly facilitates lactate uptake by oxidative tumor cells,⁴⁶ whereas MCT4 was known to preferentially convey lactate release from glycolytic tumor cells.⁸⁵⁻⁸⁷ These activities are best reflected by the fact that MCT1 is highly expressed in oxygenated tumor areas in both experimental and clinical tumors,⁴⁶ whereas MCT4 correlates with hypoxia *in vivo*.⁸⁸ Importantly, MCT1 is also found in hypoxic tumor areas (ie, pimonidazole-positive areas in clinical samples of head and neck tumors)⁸⁸ where it could lag behind fluctuations in tumor pO₂ or conveys, as a bidirectional passive transporter, lactate for export, or both.

Based on its key role in the coordination of lactate shuttle in tumors and because of its localization at the surface of cells adjacent to the tumor vasculature, we targeted MCT1 with the experimental inhibitor α -cyano-4-hydroxycinnamate (CHC) to disrupt metabolic symbiosis (Fig. 3).⁴⁶ The strategy successfully retarded the growth of mouse tumors and human tumor xenografts as long as tumor cells expressed MCT1, which was recently confirmed by others.⁸⁹ MCT1 inhibition caused an eradication of the hypoxic tumor cell compartment, which we attributed to glucose starvation in this compartment following a switch from lactate-fueled respiration to glucose-fueled glycolysis in the oxygenated tumor cell compartment.⁴⁶ Thus, the death of hypoxic tumor cells was indirect as the therapeutic target was primarily expressed in oxygenated tumor cells. MCT1 inhibition also blocked tumor angiogenesis by interfering with MCT1-dependent lactate signaling in tumor cells and at the tumor vasculature.⁸⁹⁻⁹² It is surprising that others failed to observe antitumor effects of CHC *in vivo*.⁹³ In our study,⁴⁶ one of the most striking effects of CHC was that it induced tumor reoxygenation. Indeed, when switching from lactate to glucose, surviving cells also switched from OXPHOS to aerobic glycolysis (the Crabtree Effect), resulting in oxygen sparing. To further eradicate MCT1-expressing cells, in a proof-of-concept experiment, we combined MCT1 inhibition with a single dose of X-ray in Lewis lung carcinoma-bearing mice.⁴⁶ In this experiment, chronic MCT1 inhibition was observed to have more than additive effects in inducing tumor growth retardation compared with either individual treatment: MCT1 inhibition or radiotherapy alone slowed down tumor growth, but their combination induced tumor shrinkage.

Although a strain of MCT1^{-/-} mice has been reported to be embryonically lethal⁹⁴ (the nature of the developmental defects has not been reported but could involve brain and heart abnormalities), systemic MCT1 deficiency was reported in adult humans who experienced muscle cramps under intensive exercise but were asymptomatic at rest.⁹⁵ This observation crucially suggests the existence of a therapeutic window to treat tumors with MCT1 inhibitors $\pm$ radiotherapy. Accordingly, in 2011, AstraZeneca launched a first clinical trial combining phases I and II to evaluate the safety and efficacy of their

proprietary MCT1/2 inhibitor AZD-3965 in advanced solid tumors (<http://science.cancerresearchuk.org/>). The results of this trial will not only allow the determination of the safety profile of this drug but could also provide useful information about the therapeutic potential of other drugs exploiting the same target.

An alternative to MCT1 inhibition could be to block MCT4,⁹⁶ but this approach is expected to be confronted with the difficulty of reaching the target transporter in hypoxic tumor areas remote from perfused blood vessels. To our knowledge, no specific MCT4 inhibitor has been reported to date.

NO Donors and NO Synthase Induction

The 1998 Nobel Prize in Physiology and Medicine was awarded to Furchgott, Ignarro, and Murad for their studies on NO, a radical gas and a potent vasodilator. The radiosensitizing properties of NO were first discovered in 1957 in hypoxic bacteria.⁹⁷ Currently, it is accepted that NO can radiosensitize tumors by 3 mechanisms: vasodilation,³⁶ cytochrome *c* oxidase inhibition (as with insulin),^{51,98,99} and direct radiosensitization (wherein NO mimics the ability of oxygen to stabilize DNA damage in hypoxic environments).¹⁰⁰⁻¹⁰³ It is therefore difficult to ascribe a predominant mechanism to the radiosensitizing effect of a given NO donor. As NO has a short biological half-life and may also cause a steal effect, the development of strategies to effectively and selectively deliver NO to tumors has been challenging, as has been extensively reviewed elsewhere.³⁶

Briefly, increasing NO concentrations in solid tumors can be achieved either by the use of NO donors or by the stimulation of endogenous NO production. If administered systemically, nonselective NO donors such as sodium nitroprusside, isosorbide dinitrate, and S-nitrosocaptopril produce unpredictable influences on radiotherapy, depending on the type of tumor, its size, and vascular arrangement with host tissues.¹⁰⁴⁻¹⁰⁷ A way to circumvent the problem is selective tumor delivery, which can be achieved by exploiting microenvironmental parameters common to most solid tumors. One of these characteristics, low extracellular pH, promotes the conversion of nitrites to NO.³⁰ Consequently, a systemic delivery of nitrites efficiently increases tumor oxygenation and its response to radiotherapy. Another tumor parameter is hypoxia, which triggers hemoglobin (Hb) deoxygenation and a conformational change (R to T state), facilitating the release of cysteine-bound NO and its subsequent transport to the vascular wall.²⁶ In clinical settings, inhalation of the NO-donor gas ethyl nitrite (ENO) can increase the NO load of Hb in RBCs,¹⁰⁸ and the selective release of NO in tumors is supported by *in vivo* studies that have carefully explored the biology of naked S-nitrosylated Hb.²⁹ Both ENO¹⁰⁸ and nitrites¹⁰⁹ are already in clinical use for pathologies other than cancer. Because of their apparent safety, existing clinical use, and biological activities, they obviously deserve clinical evaluation as tumor radiosensitizers.

As an alternative to NO donor administration, endogenous NO production can be stimulated selectively in tumors. One of the most promising approaches is the use of insulin, by stimulating endothelial NO synthase as described before and in Fig. 2. Another method relies on the use of ionizing radiation itself. Indeed, low doses of X-rays were found to enhance endothelial NO synthase expression and activity and, thereby, to modulate the efficacy of fractionated radiotherapy.^{33,34} This offers a rationale to fine-tune fractionation protocols to optimally target the reoxygenation windows induced by each previous doses. Yet another approach exploits the chronic inflammation usually associated with cancer: lipid A analogues were found to activate macrophages, so that they directly produce NO or, via cytokine production, stimulate its production in neighboring cells.^{103,110,111} NO then diffuses to tumor and tumor-associated cells where it is made available as a radiosensitizer.

In conclusion, NO-mediated tumor radiosensitization is a feasible and safe therapeutic option for radiotherapy as long as NO delivery or production is transient and restricted to the tumor tissue. NO has multiple modes of action that include the stabilization of DNA damage (through an effect similar to that of molecular oxygen), which could offer a valuable advantage over other radiosensitizing approaches. ENO gas breathing, in particular, holds important expectations and could readily be evaluated in clinical trials in combination with radiotherapy, potentially concomitantly with hyperoxic gas breathing.

MAPK Pathway Inhibition

Among other MAPK pathways, the ERK pathway positively regulates tumor cell proliferation, differentiation, and migration, and confers resistance to apoptosis.¹¹² It is activated by a cascade of phosphorylations initiated at tyrosine kinase receptors. The signal is transduced by Ras GTPase and Raf to MEK and then ERK kinases to ultimately activate effector kinases, phosphatases, transcription factors, and cytoskeleton proteins associated with a wide range of prosurvival processes. Evidence supporting the importance of this pathway in tumor biology is provided by its constitutive activation in approximately one-third of cancers.¹¹² Among the many ERK pathway inhibitors developed to date, some were recently shown to modulate tumor cell metabolism in such a way so as to reduce cellular oxygen consumption. They could thus be exploited in combination with radiotherapy. By targeting upstream signaling, the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor Gefinitib and the farnesyl transferase inhibitor L-754,832 were both shown to be able to decrease tumor hypoxia in experimental models of tumors with EGFR expression and constitutively activated (mutated) H-Ras, respectively.^{113,114} Although the exact molecular mechanisms underlying these responses are still elusive, Biaglow et al.¹¹⁵ have shown that oncogenic H-Ras modulates cell metabolism. For example, the oncogenic transformation of rat embryonic cells with H-Ras decreased cellular respiration and promoted glycolysis, whereas transduction with H-Ras and *v*-Myc stimulated OXPHOS and radioresistance. In independent

studies, others using radiolabelled glucose and oximetry reported that the transfection of immortalized human bronchial epithelial cells with oncogenic H-Ras increased TCA cycle activity and oxygen consumption.¹¹⁶ A recent study¹¹⁷ from our groups confirmed the ability of MAPK inhibitors to improve tumor oxygenation in vivo and extended the paradigm to downstream kinases. It showed in 2 distinct experimental models that the Raf inhibitor Sorafenib and the MEK inhibitor PD0325901 are able to increase tumor pO₂, which was further exploited with Sorafenib that increased the radiation response of fibrosarcoma (FSa-II) mouse tumors. Interestingly, in addition to inducing metabolic oxygen sparing, Sorafenib, through a yet unknown mechanism, also enhanced tumor perfusion.¹¹⁷

Although important basic questions still remain and pre-clinical studies are needed to optimize treatment regimens, there is already positive clinical evidence supporting the use of MAPK inhibitors with radiotherapy. In a phase III trial, 424 patients with advanced head and neck carcinoma received radiotherapy ± the specific EGFR inhibitor Cetuximab.¹¹⁸ Results at 5 years showed improved survival in the radiotherapy + Cetuximab arm (overall survival: 46.6%; median survival: 59 months; and *n* = 211 patients) compared with radiotherapy alone (overall survival: 36.4%; median survival: 29.3 months; and *n* = 213 patients). However, these data should be cautiously interpreted as they may well reflect the well-known inhibition of prosurvival pathways by Cetuximab rather than an oxygen effect.

Other Treatments

Paclitaxel

Paclitaxel (PTX) is a potent anticancer agent widely used for the treatment of solid tumors particularly of the breasts and ovaries.¹¹⁹ Milas et al.^{120,121} suggested that PTX also induces an enhancement of tumor radioresponse by improving the oxygenation of hypoxic tumor cells. Accordingly, we have recently shown that a micellar formulation of PTX (M-PTX) enhances tumor oxygenation in vivo, via a dual mechanism involving inhibition of cellular oxygen consumption (as a result of cell death or respiration impairment or both) and increased tumor perfusion.¹²² A radiotherapeutic gain (significant increase in tumor regrowth delay) was evidenced 24 hours after M-PTX administration to hepatocarcinoma-bearing mice.

Arsenic Trioxide

Arsenic trioxide (As₂O₃) is a chemotherapeutic drug used for the treatment of acute promyelocytic leukemia.¹²³ Probably because of its inhibitory activity on PDH, but also as it stimulates reactive oxygen species production and glutathione oxidation, As₂O₃ was shown to inhibit the respiration of leukemia cells¹²⁴ and to decrease oxygen consumption in solid tumors in vivo.⁵⁵ The resulting increase in tumor oxygenation has been shown to be sufficient to radiosensitize those tumors.

Conclusions

Second only to surgery, radiotherapy (alone or in combination with chemotherapy) is the most important line of treatment for many types of solid tumors. However, its clinical use is limited by 2 major issues: toxicity to normal tissues and tumor radioresistance. In the past decades, many efforts have been made to widen the therapeutic window, by improving both aspects. Normal tissue preservation has been enhanced with the development of radiation (hyper)fractionation, stereotactic irradiation, improved imaging for the definition of tumor margins, radioprotective agents, and targeted radiolabeled drugs. To radiosensitize tumors, several agents have already been tested and validated preclinically as powerful means to selectively improve radiation-induced tumor cell killing. Owing to the oxygen effect, the availability of molecular oxygen and its homogenous distribution in tumors at the time of irradiation is the key for therapeutic success. Unfortunately, most solid tumors are hypoxic and subject to fluctuations of oxygenation over time, creating radioresistant zones. Preclinical research currently offers 3 main options to modify this feature. First, oxygen delivery can be increased with agents aimed to augment RBC count, hyperoxic gas breathing, or agents that decrease blood viscosity, cause tumor-specific vasodilation, or modify or normalize the vascular architecture.³ Several of these treatments are currently the focus of clinical evaluation. Second, tumor oxygenation can be increased through reducing the consumption of oxygen with metabolic modulators. Finally, the oxygen effect of stabilizing radical DNA damage can be performed by NO independently of oxygen, even under hypoxia.³⁶

This review focused on the use of drugs targeting tumor cell metabolism. Hyperthermia⁴⁷ and antiinflammatory drugs⁵⁶ could by some aspects be included in this list and have been the subject of recent reviews. There are several important points to be learned from this discussion. First, numerous preclinical data are available showing that targeting cell metabolism is a valid option to improve tumor oxygenation and radiosensitivity, thus confirming the predictions of mathematical modelization.⁴²⁻⁴⁴ Second, the vast majority of the drugs and treatments identified to date are already in clinical use, often for diseases unrelated to cancer. Third, based on preclinical data, combining these agents with radiotherapy can reasonably be considered to be clinically safe. However, to date, only a minority of these adjuvant treatments have been evaluated in clinical settings with radiotherapy. None are in widespread clinical use for this indication. One potential reason for this is inherent to the technical difficulties associated with approaches such as heat therapy (homogeneous dose distribution) and the use of insulin (continuous blood monitoring). Another reason is perhaps the scarcity of conclusive information made available to the clinician, which we hope this review would help to solve. Yet another potential reason is the general lack of interest of the industrial world for old drugs that are in the public domain and, therefore, cannot be patented. Finally, it is obvious that before evaluating the potential benefit of these interventions in large clinical trials, there is a clear need to demonstrate their efficacy in smaller

trials, particularly showing that they are producing the expected changes in the tumor microenvironment. In this respect, the use of novel imaging biomarkers able to track changes in tumor metabolism and oxygenation would be beneficial to evaluate the effects of treatments. Although the measurement of tumor oxygen consumption in vivo is still challenging and limited to preclinical models,¹²⁵⁻¹²⁷ it is likely that, in the future, the recent advances in the imaging of tumor oxygenation¹²⁸⁻¹³⁰ would be important to define windows of reoxygenation to optimize treatment protocols.

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