Cancer Cell Metabolism: Implications for X-ray and Particle Radiation Therapy

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Abstract

Advances in radiation delivery technologies and immunotherapy have improved effective cancer treatments and long-term outcomes. Experimental and clinical trials have demonstrated the benefit of a combination of radiation therapy and immunotherapy for tumor eradication. Despite precise radiation dose delivery that is achievable by particle therapy and benefits from reactivating the antitumor immune response, resistance to both therapeutic strategies is frequently observed in patients. Understanding the biological origins of such resistance will create new opportunities for improved cancer treatment. Cancer metabolism and especially a high rate of aerobic glycolysis leading to overproduction and release of lactate is one such biological process favoring tumor progression and treatment resistance. Because of their known protumor effects, aerobic glycolysis and lactate production are potential targets for increased efficacy of radiation alone or in combination with immunotherapy. In the following review, we present an overview of the interplay of cancer cell lactate metabolism with the tumor microenvironment and immune cells. We discuss how a deeper understanding and careful modulation of lactate metabolism and radiation therapy might exploit this interplay for improved therapeutic outcome.

Keywords: radiation therapy; tumor metabolism; lactate; immunotherapy; aerobic glycolysis

Introduction

Radiation therapy is a key treatment modality for noninvasive and targeted tumor therapy. It is used for over 50% of patients with cancer, either alone or in combination with surgery, chemotherapy, or more recently, immunotherapy [1]. Advances in delivery technology and types of radiation from x-rays to charged particles have improved dose delivery accuracy to the tumor with reduced exposure to adjacent healthy tissue. Distinct external radiation beam modalities deposit energy in different manners and display distinct tissue irradiation characteristics along their respective paths. While x-ray irradiation deposits energy continuously along the beam path, charged particles deposit most of their energy at the end of their trajectory in a dose deposition pattern termed the *Bragg peak* [2] without exit dose and with lower dose deposition along the path of the beam [3]. Charged particles therefore have the potential to spare healthy tissue and to allow a more accurate targeting of energy deposition within tumor cells, potentially providing opportunities for focused dose escalation. Particle therapy can thus reduce toxicities and risk for secondary tumors due to the exposure of healthy tissues to radiation.

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New immunotherapy drugs have recently provided significant advances in the treatment of chemotherapy-resistant cancers. Based on a blockade of immunosuppressive signals emanating from tumor cells, immune checkpoint inhibitors function to liberate and activate a patient's native immune cells to recognize and destroy the tumor [4, 5]. Notably, sole x-ray therapy to a primary tumor can occasionally be associated with immune-mediated response to distant metastatic sites in a phenomenon termed the *abscopal effect*. In theory, a combinatorial treatment that targets the tumor specifically to increase antitumor efficacy and overcoming resistance while decreasing side effects of radiation would be highly beneficial. In practice, combinations of x-ray radiation therapy and immunotherapy have begun to show promise in preclinical and clinical trials [6, 7]. The mechanistic underpinnings, optimal dose and schedule, and potential for enhancement of efficacy of these combination therapies are understudied. Moreover, the biological and clinical differences in effects of immunotherapy combined with either x-ray or charged particles have yet to be carefully evaluated. Interestingly, metabolic reprogramming and particularly lactate production by tumor cells is thought to regulate both radiation biology and immunotherapy. The following is an overview of the interplay of cancer cell lactate metabolism with the tumor microenvironment and immune cells, focusing on how radiation therapy might exploit this interplay for improved therapeutic outcome.

Aerobic Glycolysis and Lactate Metabolism: Enablers of Tumor Development and Survival

The development and sustained growth of a tumor is generally associated with the deregulation of metabolism in cancer cells [8]. Under aerobic conditions, normal cells metabolize glucose to pyruvate via glycolysis in the cytoplasm. Pyruvate is then metabolized to carbon dioxide during oxidative phosphorylation in mitochondria. Under anaerobic conditions, normal cells metabolize pyruvate to lactate for rapid production of energy. In contrast, cancer cells have a preference for metabolizing pyruvate to lactate even under aerobic conditions, leading to increased lactate release into the tumor microenvironment. This "Warburg effect"—named after Otto Warburg who first described the "aerobic glycolysis" switch in cancer cells [9]—is less efficient at producing energy than oxidative phosphorylation and results in continued high uptake and consumption of glucose by cancer cells. Aside from "aerobic glycolysis," transformed cells also produce energy through the upregulation of glutaminolysis, which further stimulates the production and release of lactate [10]. This shift in metabolism, generally accepted as a common phenomenon in cancer, results in the scavenging of glucose from the tumor microenvironment, and high concentrations of lactate resulting in acidification of the microenvironment. Solid tumors also harbor a multitude of nontransformed stromal cell types (eg, immune cells) that use different metabolic programs for their energy production, and can also produce lactate to support tumor cell growth.

Global benefits to the tumor that are produced by a switch to aerobic glycolysis remain elusive, but studies have demonstrated a variety of likely scenarios, including evasion of the immune system, stimulation of neo-vascularization, and selection for drug-resistant cancer cell clones. Interestingly, the activation of oncogenes such as *Ras* [11], *Akt* [12], *Myc* [13], and *Dek* [14] is in and of itself sufficient for increased cellular glucose uptake and glycolysis. Given that oncogene activation is an early event in tumor development, the switch to a glycolytic metabolism and associated benefits to the tumor appear to be equally early events in the disease process. Detection and targeting of this metabolic switch is thus a potential therapeutic approach for new tumor diagnostics and treatments. Historically, lactate had been viewed as a necessary, but inconsequential, byproduct of this metabolic reprogramming in cancer cells. The discovery that lactate itself can directly modify metabolism through the GPR81 receptor [15], however, yields new insights into specific biological consequences of lactate production on carcinogenesis.

Lactate Promotes Multiple Stages of Tumor Development and Metastatic Dissemination

High rates of aerobic glycolysis by cancer cells can lead to a significant increase in lactate in adjacent normal tissue with concentrations ranging from 2 mM/g to 40 mM/g above that of the corresponding cancer tissue [16]. The acidic extracellular environment directly promotes the transformation of nascent tumor cells and metabolic reprogramming therein, while selecting for clones that are resistant to transitory hypoxic conditions during tumor development [17]. These effects on cancer cells are paracrine in that they require the export and uptake of lactate by the MCT receptors 1 and 4 and lactate signaling through its receptor GPR81 (Figure 1). GPR81 is expressed in multiple tumor types [18], and suppression by silencing has been shown to reduce both tumor growth and invasion. Interestingly, silencing of GPR81 leads to a concomitant suppression of MCT1 and

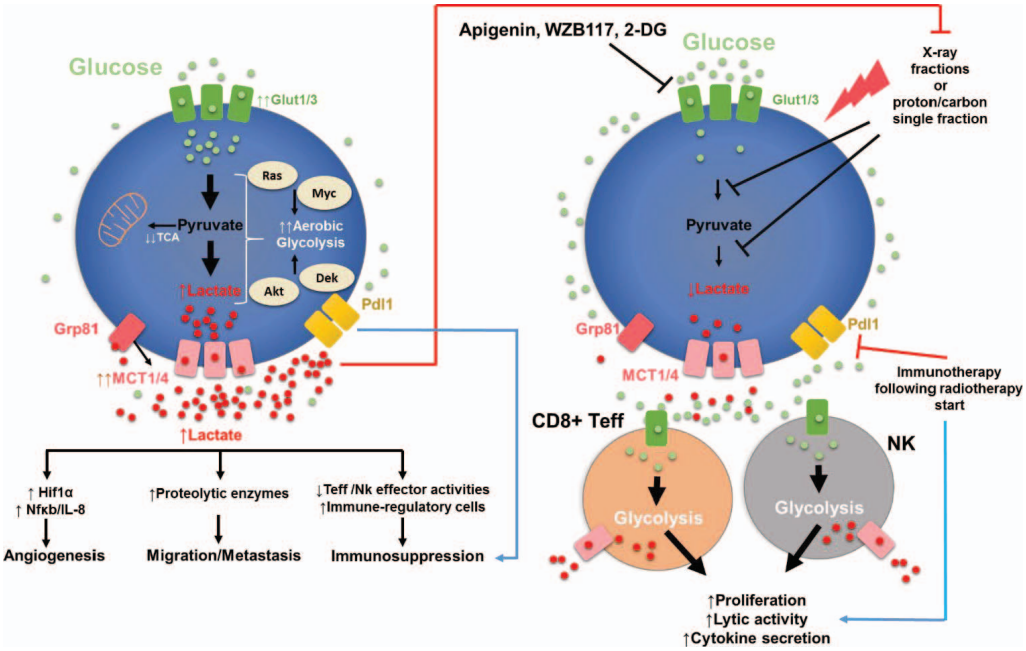

Figure 1. Deregulation of cancer cell metabolism towards high production of lactate to promote tumor phenotypes and potential radiation therapy/immunotherapy combination. Oncogenes including *Ras*, *Akt*, *Myc*, and *Dek* drive increased aerobic glycolysis in tumor cells leading to the conversion of pyruvate into lactate instead of the redirection of pyruvate into mitochondria for the TCA cycle. Export of lactate trough the MCT1/4 transporter promotes the accumulation of lactate and acidification of the extracellular microenvironment. Lactate signaling through GRP81 stimulates the expression of MCT1/4 and capacity for lactate export. Released lactate acts on other cells present in tumor microenvironment stimulating production of VEGF and angiogenesis by endothelial cells, facilitating migration of cancer cells by the production and release of proteolytic enzymes, and diminishing the potency of antitumor immune responses. Lactate content of tumor has been correlated with tumor radioresistance. Lactate directly acts on monocyte to stimulate their differentiation into M2 macrophages. Furthermore, high concentrations of extracellular lactate inhibit the release of lactate from T effector (Teff) or NK cells leading to functional impairments. Depletion of glucose in the microenvironment of cancer cells via high expression of glucose transporter creates a deficit of glucose availability to immune effector cells which impairs their activities via energy production insufficiency. Blockade of GLUT1 activity and glucose transport into tumor cells by apigenin, WZB117, or 2-DG leads to decreased production and release of lactate, increase in glucose in tumor microenvironment, and radiosensitization of cancer cells. Multiple fractions of photon irradiation or a single fraction of particle irradiation is expected to decrease aerobic glycolysis in cancer cells and leads to decreased lactate production and secretion via glycolytic enzyme repression. Decreased lactate and increased glucose levels in the tumor microenvironment creates favorable conditions for immune cell activation and antitumor activities. Immune checkpoint signals are also increased in the tumor and impair proper antitumor immune response. Targeting these immune checkpoints (eg, PDL-1/PD1) increases antitumor immune response. The combination of radiation therapy and its effect on tumor metabolism and the lactate microenvironment in combination with immunotherapy could stimulate the efficacy of tumor treatment. The timing of this combinatorial approach needs to be studied, and immunotherapy might be favored if administered after radiation therapy treatment.

MCT4 expression, and therefore, GPR81 may be a promising molecule for the targeting of lactate transport and signaling in cancer cells. Lactate-mediated acidosis has also been shown to directly stimulate cancer cell motility, in part by increasing the expression and activation of proteolytic enzymes by adjacent cells leading to extracellular matrix remodeling [19] (Figure 1). In line with this role, bicarbonate treatment of mice for a reduction of pH in the tumor microenvironment decreased tumor invasion or metastases [20, 21]. In addition to aggressive local invasion, the acidic microenvironment is also responsible for increased expression of vascular endothelial growth factor (VEGF) by endothelial cells, and angiogenesis [22, 23]. This occurs through the activation of HIF-1α and via NF-kB/IL-8 signaling independently from hypoxia. Notably, these pathways could potentially be targeted with anti-VEGF antibodies or small molecules, mTOR pathway inhibitors, or proteasome-targeted drugs.

Cancer Metabolism and Lactate Production Diminish Antitumor Immune Responses

Major progress in cancer treatment has been achieved with immunotherapy, highlighting the importance of the immune response in tumor surveillance and rejection. The basis of checkpoint-targeted immunotherapy relies on blockade by immune inhibitory factors such as PDL-1/PD1 or CTLA4 expressed directly by cancer cells or by immune regulatory and effector cells [4, 5]. However, expression of immune modulatory factors is only one of many strategies for immune evasion by cancer cells. The abovementioned aerobic glycolysis metabolism switch and high lactate production, in addition to suppressing tumor

phenotypes directly, are also responsible for evasion of the antitumor immune response (Figure 1). Effector CD8 T cells responsible for the destruction of cancer cells undergo a glycolytic metabolic switch to sustain high proliferative activity and to produce cytokines and immune effector molecules [24]. The high capacity of cancer cells to take up glucose creates a deficit of glucose in the tumor microenvironment. Low glucose levels in the tumor inhibits proliferation, cytokine production, and cytolytic activity of effector T cells [25, 26]. Thus low levels of glucose in the tumor microenvironment are in part responsible for reduced CD8 T-cell antitumor activity. High prevalence of regulatory T cells (Tregs) is often detectable in the tumor mass and these cells diminish the inflammatory response. Interestingly, Tregs show greater reliance on oxidative phosphorylation than on glycolysis [27, 28]. Low levels of glucose in the tumor environment may therefore tilt the developing immune cell populations towards Tregs rather than T effector cells, creating an imbalance towards immune suppressor and away from immune effector response. The lactate-rich acidic tumor microenvironment also decreases the efficacy of the antitumor response by the immune system. High concentrations of lactate are responsible for decreased lactate export from activated T cells and decreased T-cell glycolysis, leading to attenuated interferon gamma production [29, 30]. Acidosis reduces T-cell activity directly [31] and T-cell activity is restored by buffering the tumor environment to physiological pH values [32, 33]. In addition to directly suppressing effector T cells, lactate can indirectly impair T-cell function by promoting alternative M2 differentiation in tumor-associated macrophages [34, 35]. M2 macrophages suppress tumor-infiltrating lymphocytes by producing anti-inflammatory cytokines such as IL-10, and increasing angiogenesis and tumor vascularization [36].

While there is strong evidence for impairment of T-cell function by lactate and low pH, the data documenting an effect on antigen processing and presentation by Major histocompatibility complex (MHC) class I (HLA-ABC) are more sparse and frequently supportive of an increase in antigen presentation by tumor cells and antigen-presenting cells. Tumor cells frequently escape immune-mediated destruction by downregulating surface expression of MHC class I [37], and in contrast to the suppressive effect of lactate and low pH on T cells, these factors increase expression of genes involved in antigen processing and presentation by tumors [38]. In some, but not all studies, low pH and lactate can also improve antigen processing and presentation by dendritic cells, which can augment priming and activation of tumor-specific T cells, an effect mediated by acid-sensing ion channels [39–41]. A similar increase in MHC class I is seen on monocytes exposed to lactate [42], an effect that is antagonized by hypoxia. Lactate also increases expression of MHC class II and its associated antigen-processing pathways on tumor neutrophils and monocytes, an effect that can stimulate antitumor CD4+ helper T cells [43]. However, the overall effect of lactic acidosis in the tumor microenvironment is immune suppressive, and targeting aerobic glycolysis and lactate production in tumors might be a useful approach to arm the host with antitumor immunity.

Interplay Between Radiation Therapy and Lactate Production, an Opportunity for Combinatorial Treatments

Among the available therapeutic avenues for cancer treatment, radiation is often used as a primary or adjunctive strategy. More than half of cancer patients receive radiation therapy, and the success of such an approach in each case depends upon tumor- and patient-specific sensitivities. X-rays have been used for decades in the clinic as the main radiation source. As photons without mass or charge, x-rays deposit maximum energy near the point of entry into irradiated tissue, and continue to deposit dose distal to the target, leading to substantial exposure of healthy tissues. In contrast, proton beams and other charged particle radiation types allow for more precisely targeted radiation therapy. Charged particles release less dose along the path of the beam towards the tumor, maximum energy at the precise location of the Bragg peak [3], and no dose after the end of range. The depth of the Bragg peak is dependent on the incident energy of the charged particles. Particle therapy systems deliver many Bragg peaks in order to generate a spread-out Bragg peak that can effectively target the tumor.

Resistance to radiation can in theory be overcome by dose escalation. However, in the case of x-ray treatment, this is limited by radiation tolerance of healthy tissues and related toxicities. Radiation by protons or other charged particles allows for more accurate dose delivery to the tumor while sparing healthy tissues. In this way, particle radiation therapy represents an opportunity for increasing the dose while minimizing side effects. However, escalating dose can be a concern for long-term outcome. Our knowledge of biological effects of x-ray radiation on normal and tumor tissues is extensive owing to decades of laboratory research and clinical use. The related biological consequences of particle therapy are surprisingly understudied owing to the limited numbers of radiation facilities with associated molecular, cellular, and animal research opportunities, and the body of research data available to date. This lack of knowledge narrows optimal clinical applications of particle therapy, which largely depend on the related experiences from x-ray studies. For instance, treatment planning for proton therapy is based on a radiobiological effectiveness (RBE) of 1.1 when compared to megavoltage x-rays. RBE is defined by in vitro and in

vivo experimental models, and while many patients have been successfully treated by using this simplified conversion, several studies have shown that RBE varies significantly by tumor type and genetic background [44–48]. A deeper understanding of the biology of particle therapy may advance more effective applications, and potentially individualized use in patients. In addition, identification of factors that contribute to tumor radioresistance might lead to the development of sensitization strategies that may differ between particle and x-ray treatments. When coupled with the dose delivery accuracy of particle therapy, such strategies may represent safer, more efficacious opportunities for maximizing long-term outcomes. New targets are required for improving the activity and specificity of therapy, and ideally primarily include those that are specifically regulated in cancer, but not normal cells. In line with that notion, several studies have demonstrated that aerobic metabolic reprogramming of tumor cells in general, and lactate production in particular, can favor tumor radioresistance. Experimental in vivo data have shown that tumors with a high lactate content gain radiation resistance [49, 50]. The antioxidant potential of lactate [51] may explain this phenomenon, as lactate has a protective role for the tumor during radiation exposure. High tumor lactate content has also been linked to poor outcome, metastasis, and recurrence in patients with head and neck or cervical cancer treated by radiation therapy [52, 53]. Radiosensitization of tumors has been achieved by targeting glycolysis in cancer cells in different experimental models. Targeting of glucose uptake into cancer cells by inhibition of GLUT1 expression or function via genetic chemical approaches such as apigenin [54], WZB117 [55], and 2-DG [56] have been shown to sensitize cancer cells to x-ray radiation in vitro and in vivo in models of breast and head and neck cancers (Figure 1). However, those studies were conducted in immune-deficient models and the impact on immune antitumor activity remains to be investigated. Relevant molecular reasons for tumor sensitization following a glycolytic block remain unclear but suppression of DNA repair activity has been observed by using such a strategy. It is possible that similar metabolism-based therapies, such as using the glucose analog 2-DG or inhibitors of enzymes involved in aerobic glycolysis, may additionally enhance the antitumor immune response (Figure 1).

Combining cancer metabolism–targeting drugs with immunotherapy may need a careful consideration of dose and schedule for both radiation and cancer drugs, with detailed examination of tumor response and immune surveillance. For instance, one study has shown in both transplanted and spontaneous immunocompetent models of mouse mammary tumors that the optimal timing of the combination of immunotherapy with photon radiation depends on the type of immunotherapy used [57]. Biological responses to single-agent and combined therapies require further in vivo analyses. Coupling x-ray radiation with immunotherapy has shown promising results in preclinical and clinical studies [6, 7]. Interestingly, one study has demonstrated that fractionated, but less so single, exposure to x-ray radiation leads to the suppression of glycolytic enzyme expression and decreased lactate production in the tumors [58]. The timing of immunotherapy coupled with conventional radiation therapy needs to be re-evaluated and outcomes might be improved if several fractionations preceded immunotherapy and were applied when lactate levels are at their lowest point. Tumor lactate content and glycolytic activity can be followed in the clinic by magnetic resonance imaging. Thus metabolic imaging during treatment might be of interest to study the window of opportunity for radiation-immunotherapy combinations. Effects of particle radiation therapy on cancer metabolism are currently underexplored and to our knowledge, only one report has investigated the effects of single-dose photon or carbon radiation on HeLa cell metabolism [59]. Interestingly, this study has shown that a single dose of carbon ion radiation leads to decreased glycolytic activity and lactate production in HeLa cells. This effect was not observed with a single dose of x-ray radiation. Since treatments used the same dose of x-ray and carbon ion radiation, it is unclear if the observed effect is linked to increased cell kill due to higher RBE or a specific response to carbon ion radiation. Nevertheless, in line with these results, our preliminary unpublished data of lymphoma cell lines show that a single dose of proton and carbon, compared to photon radiation and at the same dose, increases the levels of glucose and decreases the levels of lactate in culture medium, indicating a potential shutdown of glucose uptake and lactate production. In the same series of investigations, we did not observe differences in glucose or lactate levels after a single dose of photon radiation, consistent with previous observations. These studies were executed by using simple in vitro model systems and need to be further explored in a more physiological context in 3D organoid or in vivo models. Regardless, these data indicate fundamental differences between the biological effects of photon versus particle therapy on the glycolytic activity of cancer cells. As discussed above, glucose uptake and scavenging from the microenvironment, and the lactate content of the tumor environment, represent a challenge for the antitumor immune response. The impact of radiation therapy on tumor metabolism and lactate production could be exploited for combination therapy approaches based on considerations of both effects on tumor metabolism and optimization of immune stimulation. There is currently a need for fundamental knowledge of the effects of different radiation types on cancer metabolism and immune surveillance. These studies should be performed by using different tumor types and donor backgrounds, and in appropriate in vivo settings. Since the effects on cancer cell metabolism might vary depending on

radiation dose fractionation and molecular responses, there is also a need for comparison of different types of radiation that are used in the clinic to define the most effective combination therapies.

Conclusion

Metabolic reprogramming in cancer cells was originally attributed to impaired mitochondrial activity but can occur by other means such as increased glycolytic enzyme expression following oncogene activation. Cancer cells can be induced to return to oxidative phosphorylation, and targeting mitochondria with this goal is being actively explored as a new approach to cancer therapy [60]. Accumulative data have shown that the metabolic switch towards aerobic glycolysis and lactate production represents an advantage for tumor development and invasion, and might be the result of clonal selection in progressive malignancies. Together with the possibility that similar metabolic reprogramming stimulates tumor proliferation and angiogenesis, targeting glycolysis or lactate production could be an appealing strategy for radiation sensitization. Radiation therapy has been a pillar of cancer therapy and standard of care for patients with cancer. More recently, immunotherapy based on blockade of immune checkpoints has emerged as a major advance in cancer therapy as a first- or second-line treatment. For both radiation and immunotherapy, treatment failures and side effects are common events. These need to be fully understood in order to broaden applicability and maximize long-term outcomes. Limitations to current approaches using photon radiation, including treatment toxicity and tumor resistance, might be overcome by the use of proton or carbon ion therapy, in combination with a targeting of aerobic glycolysis in the tumor. On the one hand, blocking cancer metabolism could be a promising avenue for disrupting lactate production and release into the tumor environment. On the other hand, because immune effector cell function relies on high glycolytic activity, the resulting therapeutic effects on the tumor and immune cell death or exhaustion may not produce a stable response. The triad of radiation therapy, immunotherapy, and metabolic inhibition could represent an important opportunity for increasing treatment efficacy. Combination therapy regimens, particularly involving particle therapy, should be examined in different tumor types. For example, a need for fewer fractions to decrease lactate production observed with proton and carbon radiation opens up the possibility of downscaling fraction number and total dose in such cases. It is possible that particle radiation could prove to be superior to conventional photon radiation in efficacy and toxicity, and may provide a foundation for optimal combinations of radiation and immunotherapy.

ADDITIONAL INFORMATION AND DECLARATIONS

Conflicts of Interest: The authors have no conflicts to disclose.

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