Targeting Hypoxia, HIF-1, and Tumor Glucose Metabolism to Improve Radiotherapy Efficacy

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Abstract

Radiotherapy, an important treatment modality in oncology, kills cells through induction of oxidative stress. However, malignant tumors vary in their response to irradiation as a consequence of resistance mechanisms taking place at the molecular level. It is important to understand these mechanisms of radioresistance, as counteracting them may improve the efficacy of radiotherapy. In this review, we describe how the hypoxia-inducible factor 1 (HIF-1) pathway has a profound effect on the response to radiotherapy. The main focus will be on HIF-1–controlled protection of the vasculature postirradiation and on HIF-1 regulation of glycolysis and the pentose phosphate pathway. This aberrant cellular metabolism increases the antioxidant capacity of tumors, thereby countering the oxidative stress caused by irradiation. From the results of translational studies and the first clinical phase I/II trials, it can be concluded that targeting HIF-1 and tumor glucose metabolism at several levels reduces the antioxidant capacity of tumors, affects the tumor microenvironment, and sensitizes various solid tumors to irradiation.

Introduction

When ionizing radiation is absorbed in tissue, free radicals are produced as a result of ionizations either directly in the DNA molecule itself or indirectly in other cellular molecules, primarily water (H₂O). This indirect effect leads to the production of reactive oxygen species (ROS), which are molecules that contain chemically active oxygen molecules including superoxide radical anions and hydroxyl radicals, thereby inducing oxidative stress. ROS can diffuse to the DNA to produce similar free radicals. These free radicals, whether they are produced as a result of the direct or indirect mechanism, break chemical bonds and initiate the chain of events that results in DNA damage. Oxygen molecules are able to react with the free radicals to yield a stable change in the chemical composition of the DNA damage. In this way, oxygen chemically “fixes” DNA damage and this is the basic mechanism for the therapeutic use of ionizing radiation in cancer (1). Hypoxia, a pathophysiologic characteristic of solid malignancies (Fig. 1), interferes with the fixation of DNA damage and is therefore a major cause of resistance to irradiation (2–5). A number of therapeutic approaches have been designed to overcome tumor hypoxia (2, 4, 6).

The hypoxia-inducible factor 1 (HIF-1) pathway enables tumor cells to survive by changing glucose metabolism toward a glycolytic phenotype, by inducing angiogenesis and by regulating pH balance and proliferation rate. This review describes how the HIF-1 pathway impacts on radioresistance of solid tumors, with special focus on HIF-1–induced changes in tumor glucose metabolism. Furthermore, inhibition of HIF-1 and glucose metabolism is discussed as a new method to overcome radioresistance.

HIF-1 Pathway

Hypoxia-inducible factor 1

HIF-1 is a heterodimeric protein consisting of an O₂-regulated HIF-1α and a constitutively expressed HIF-1β subunit. Under normoxia, HIF-1α is rapidly degraded, whereas hypoxia leads to stabilization and accumulation of HIF-1α (Figs. 1 and 2A and B; refs. 7 and 8). However, under certain normoxic conditions, HIF-1α expression can be increased; for example, mutations in the von Hippel-Lindau protein stabilize HIF-1α protein and PI3K/AKT/mTOR activity stimulates translation of HIF-1α mRNA (7–11). Moreover, reactive oxygen and nitrogen species inhibit proteasomal degradation of HIF-1α (10, 12). After stabilization of HIF-1α, the heterodimeric protein activates transcription of numerous genes involved in angiogenesis, proliferation, glycolytic tumor metabolism, and pH regulation (refs. 7, 10, and 13; Fig. 2B).

Glycolytic cancer cell metabolism and aggressive tumor behavior

Under aerobic conditions, normal cells generate energy by processing glucose both via inefficient glycolysis and via more efficient mitochondrial oxidation. As hypoxia
decreases the rate of mitochondrial oxidation, tumor cells switch to glycolysis for energy production under hypoxic circumstances (14). This process in which pyruvate, lactate, and hydrogen ions are produced is called anaerobic glycolysis or Pasteur effect (9, 15). However, a hallmark of cancer cells is a high rate of glucose consumption and lactate generation even in the presence of oxygen (aerobic glycolysis or Warburg effect; refs. 9, and 16–18).

HIF-1 initiates transcription of genes that encode transporters and enzymes regulating glycolysis and the pentose phosphate pathway (Fig. 3; refs. 8 and 19). The glycolytic products pyruvate and lactate induce HIF-1 accumulation (20, 21), suggesting a feed-forward mechanism in which HIF-1 causes a glycolytic metabolism with elevated pyruvate/lactate concentrations, which in turn increase HIF-1 activity (Fig. 2B; refs. 19 and 22). Besides HIF-1, activated oncogenes (e.g., RAS and MYC) and the PI3K/AKT/mTOR pathway are involved in the regulation of the metabolic shift to (aerobic) glycolysis (9, 16, 18).

The glycolytic switch may seem counterintuitive, given the less efficient energy production by glycolysis compared with mitochondrial oxidation. However, the glycolytic intermediate glucose-6-phosphate is also used...
in the pentose phosphate pathway. This pathway synthesizes precursors of nucleotides and amino acids, which are macromolecules required for tumor cell growth and proliferation. When cells do not require these macromolecules for metabolism, intermediates of the pentose phosphate pathway (fructose-6-phosphate and glyceraldehyde-3-phosphate) can be recycled back into glycolysis to produce pyruvate and lactate (Fig. 3). During the pentose phosphate pathway, CO₂ is released, leading to extracellular matrix acidification by carbonic anhydrase IX (CAIX; Fig. 3; refs. 18 and 23).

Moreover, glycolytic metabolism in malignancies correlates with radioresistance and will be described in detail later (24, 25), and lactate accumulation predicts for metastases formation during follow-up in different tumor entities (26–28). The higher incidence of metastases could be explained by the fact that the glycolytic products lactate and acid induce secretion of matrix-degrading hyaluronidase and metalloproteinases by tumor-associated fibroblasts, creating a tumor microenvironment favorable for tumor cell migration (9, 15, 29, 30). Furthermore, lactate inhibits the activity of dendritic cells and T cells, which...
contributes to the immunologic escape of tumors (31, 32). Finally, lactate is able to induce angiogenesis (Fig 3; ref. 29). These findings indicate that a glycolytic tumor cell metabolism contributes to aggressive tumor behavior.

HIF-1 and Radioresistance

Activation of the HIF-1 pathway after radiotherapy

Radiation-induced reoxygenation is the process by which the surviving hypoxic tumor cells become better oxygenated after irradiation due to the aerobic population being killed. As a result, the fraction of hypoxic tumor cells falls. This is a highly variable process within tumors, however, with the final level of oxygenation depending on the degree of reoxygenation (1, 33).

This process elicits a cascade of HIF-1–related events. Within hours postirradiation, intratumoral HIF-1 activity decreases due to von Hippel-Lindau–dependent HIF-1α degradation under these reoxygenated conditions (34). However, due to reoxygenation, ROS arise that induce HIF-1α stabilization (12). As a result, HIF-1 expression increases in a hypoxia-independent manner 18 to 24 hours after radiotherapy in in vivo mice studies (34–38). This upregulation endures up to 1 week (38). In an in vivo tumor model, it was shown that some HIF-1 target genes are also upregulated after irradiation, including vascular endothelial growth factor (VEGF) and CAIX (36–38). In these preclinical studies, tumors were irradiated with 3 fraction of 5 Gy or single fractions of 5 to 8 Gy. However, during fractionated radiotherapy in clinical practice, these processes are likely to be much more complex with schedules of 25 to 40 fractions of 1.8 to 2 Gy, delivered over several weeks. Initially, the responses in the tumor microenvironment regarding reoxygenation, ROS, and HIF-1 upregulation may to some extent follow the patterns described earlier. However, with increasing cumulative dose toward the end of the treatment, it is conceivable that there will be a progressive disorganization of the response pathways and disruptions of the microenvironmental structure. It remains to be elucidated whether HIF-1 upregulation by cycles of reoxygenation-induced ROS can be sustained for several weeks.

Several other reoxygenation-dependent mechanisms are also able to upregulate HIF-1 and its downstream targets postirradiation, including depolymerization of cytoplasmic stress granules containing HIF-1–regulated mRNA transcripts (36, 39, 40) and AKT/mTOR signaling (35). Furthermore, nitrogen monoxide (NO), which is a free radical produced by tumor-infiltrating macrophages, prevents HIF-1α from degradation after radiotherapy (41, 42). Without continued treatment and due to renewed tumor progression, after 1 or 2 weeks postirradiation, hypoxia will increase again resulting in an increase in tumor HIF-1 levels (33, 43).

HIF-1 and vascular radiosensitivity

Apart from the radiosensitivity of tumor cells as an important determinant of tumor response (44), the degree of radiation-induced microvascular destruction might contribute to tumor cell death (45–48). Radiation-induced endothelial apoptosis starts at exposure to single-dose radiation of 8 to 10 Gy, being maximal at 20 to 25 Gy (45). Tumor blood flow decreases after a single dose of 10 to 15 Gy with subsequently indirect killing of tumor cells. Doses higher than 15 to 20 Gy cause a marked and lasting deterioration of the vasculature (49). In the clinical situation, radiation-induced vascular damage may thus contribute to tumor response in case of high-dose hypofractionated treatment regimens (45, 49). The effect of standard fractionation using 1.5 to 2 Gy per fraction on endothelial cells may be minor (49), albeit that this will also depend on the total dose delivered.

During fractionated radiotherapy, the tumor microvasculature is protected through action of HIF-1. HIF-1 upregulation stimulates tumor cells to produce VEGF and other proangiogenic factors, which induce angiogenesis and protect the microvasculature from radiation-induced endothelial apoptosis (36, 45, 50–52). Moreover, vasculogenesis is responsible for the recovery of tumor blood flow and the recurrence of glioblastomas after ionizing radiation in an in vivo tumor model (43). Irradiation induces influx of bone marrow–derived cells. HIF-1–dependent upregulation of stromal-derived factor 1 (SDF1) and VEGF plays a crucial role in the recruitment of these bone marrow–derived cells (43, 53). These cells promote neovascularization, thereby restoring the radiation-damaged vasculature and stimulating regrowth of surviving tumor cells. Furthermore, VEGF and SDF1 activate resident endothelial cells (10). In short, HIF-1 is responsible for vascular protection, recovery of tumor blood and nutrient supply, and tumor recurrence postirradiation by stimulating angiogenesis and/or vasculogenesis (Fig. 4).

High HIF-1α expression correlates with poor locoregional control and an increased risk of tumor-related death in patients with head and neck, cervical, and prostate cancer treated with radiotherapy (54–59). Preclinical research strongly suggests that HIF-1–mediated vascular protection is also responsible for the tumor resistance to irradiation in patients (36, 37, 60).

HIF-1 inhibition, tumor microenvironment, and response to radiotherapy

Several existing classes of drugs such as anthracyclines (doxorubicin), epidermal growth factor receptor (EGFR) inhibitors (cetuximab), and topoisomerase 1 inhibitors (topotecan) inhibit HIF-1 activity and tumor xenograft growth (10).

Topotecan inhibits HIF-1α mRNA translation (61) and recently, the effect of this compound on the tumor microenvironment was studied in 16 patients with advanced solid tumors expressing nuclear HIF-1α (62). After treatment, nuclear HIF-1α expression decreased, becoming undetectable in 4 of 7 available patients. Furthermore, the HIF-1 downstream targets glucose transporter 1 (GLUT1) and VEGF decreased after HIF-1 inhibition. Decreased tumor blood flow on dynamic contrast-enhanced magnetic resonance imaging was seen in 7 of 10 patients after 1 cycle of
These results indicate that HIF-1α inhibition lowers the level of HIF-1α and the downstream targets and affects tumor vascularization in patients. Only one patient in this study had a partial response lasting for 6 cycles of topotecan, which was accompanied by both a reduction in HIF-1α level and a decreased tumor blood flow (62).

Newly developed drugs with HIF-1 inhibitory capacity are YC-1 (3-[5-hydroxymethyl-2-furyl]-1-benzyl indazole) and PX-478 (S-2-amino-3-[4'-N,N-bis(chloroethyl)amino]phenyl propionic acid N-oxide dihydrochloride). PX-478 is an agent that inhibits HIF-1α by reducing HIF-1α mRNA levels, by inhibiting HIF-1α translation, and, to a lesser extent, by inhibiting HIF-1α deubiquitination (63). YC-1 induces HIF-1α protein degradation (64), and it inhibits the translation of HIF-1α mRNA via suppression of the PI3K/AKT/mTOR pathway and Akt/NF-κB signaling (11). Furthermore, YC-1 regulates HIF-1α functional activity at the posttranslational level (65).

In in vivo mice studies, YC1-treated stomach, renal, and cervical carcinomas are smaller and less vascularized. These tumors express lower levels of HIF-1α and VEGF with higher hypoxic fractions compared with control tumors (34, 66). Apparently, YC-1 halts tumor growth by blocking HIF-1 activity and subsequent vascularization (66). Treatment of tumor-bearing mice with radiotherapy followed by YC1 suppresses upregulation of HIF-1 activity, dramatically increases radiation-induced vascular destruction, and delays tumor growth (34, 36). Adversely, administration of YC-1 before radiotherapy increases tumor hypoxia and...
suppresses the therapeutic effect of irradiation (34). Also, PX-478 radiosensitizes glioma tumor xenografts through inhibition of HIF-1-dependent proangiogenic signaling (38).

PX-478 was tested in 40 patients with advanced solid tumors in a phase I dose-escalation trial (NCT00522652; ref. 67). Only a limited number of severe events were reported, including severe thrombocytopenia without bleeding (n = 1), anemia (n = 1), acute renal failure (n = 1), hypotension (n = 1), and elevated liver enzymes (n = 1). A relatively high proportion of patients (39%) achieved stable disease. This single-agent study excluded patients who received radiation therapy within 4 weeks before entry into the study. Therefore, no conclusions can be drawn about potential interactions of PX-478 treatment with radiotherapy.

As HIF-1α is not expressed in all cancer cells in a tumor, inhibition of HIF-1 activity is probably not effective as monotherapy. Therefore, clinical trials should combine HIF-1 inhibition with radiotherapy and should focus on optimal patient selection. For example, tumors showing little or no hypoxia cannot undergo radiation-induced reoxygenation and HIF-1 activation (37). Furthermore, establishing optimal timing of HIF-1 inhibitory treatment relative to fractionation of PX-478 treatment with radiotherapy can be drawn about potential interactions of PX-478 treatment with radiotherapy.

In conclusion, redox adaptation is an important mechanistic concept that explains why cancer cells become resistant to radiotherapy (69). Several preclinical studies have shown that tumor glucose metabolism is likely to be involved in alterations of the cellular redox status and radioresistance. Interfering with glucose metabolism of tumor cells to reduce the levels of antioxidant metabolites could therefore improve response to radiotherapy (29).

Modulating tumor glucose metabolism and the cellular redox status to overcome radioresistance

Tumor glucose metabolism can be targeted at several levels, directly via inhibiting enzymes and transporters involved in glucose metabolism and indirectly by anti-HIF-1 therapy (71). HIF-1 inhibition results in metabolic changes with a decreased rate of glucose uptake and lactate production in vitro (76) and an increase in oxygen consumption, reflecting enhanced mitochondrial oxidation in in vivo mice studies (76, 77). As ROS are produced during mitochondrial oxidation, these metabolic alterations could enhance the therapeutic efficacy of radiotherapy (69).
2-Deoxyglucose competes with glucose for transmembrane transport. Hexokinase converts 2-deoxyglucose into 2-deoxyglucose-6-phosphate, which cannot be further metabolized within the cancer cell. Treatment with 2-deoxyglucose is cytotoxic and radiosensitizes human glioma, cervical carcinoma, and pancreatic carcinoma cells (72, 78, 79). The combination of radiotherapy with 2-deoxyglucose shows a trend toward increased growth delay of pancreatic carcinomas in an \textit{in vivo} tumor model, relative to either agent alone (72). This cytotoxic and radiosensitizing effect of 2-deoxyglucose is mediated by disruptions in glutathione metabolism and decreased NADPH content (72, 79), which reflects the close link between tumor glucose metabolism and an altered cellular redox status. However, glycolysis inhibitors may improve the therapeutic efficacy of radiotherapy not only through disruptions in the cellular redox status, but also by depletion of ATP levels (71). Inhibition of GLUT1 and hexokinase decreases ATP levels resulting in reduced cancer cell viability \textit{in vitro}, which inhibits tumor growth in \textit{in vivo} tumor models (80, 81). Addition of ATP rescues GLUT1-inhibited cancer cells (81), suggesting that glycolysis inhibition has an anticancer effect partially through ATP depletion.

Phase I/II clinical trials examined the effect of the combination of 2-deoxyglucose with hypofractionated radiotherapy in patients with cerebral glioma (82, 83). 2-Deoxyglucose was given 30 minutes before every fraction of radiotherapy in a dose-escalation regimen (83). This treatment strategy was well tolerated without severe acute toxicity and did not lead to any apparent increase in the late radiation damage to normal brain tissue (83). This regimen resulted in a moderate increase in survival with a significantly improved quality of life (82). Daily administration of 2-deoxyglucose alone and in combination with docetaxel has very recently been tested in lung, breast, gastric, pancreatic, and head and neck cancer in a phase I dose-escalation trial (NCI00096707), but results are not yet available. One other 2-deoxyglucose study has been terminated due to slow accrual (NCI00633087) and a third trial has been cancelled before enrollment of patients because of logistic reasons (NCI00247403).

Another target of tumor glucose metabolism is the pentose phosphate pathway, which is closely connected to glycolysis via the glycolytic intermediate glucose-6-phosphate. Lowering the amount of glucose-6-phosphate through inhibition of hexokinase may reduce flux into the pentose phosphate pathway. The enzyme transketolase (TKT) has a key role in the regulation of the pentose phosphate pathway (84). Transketolase-like protein-1 (TKTL1) is significantly overexpressed in different human cancers and is responsible for 60% to 70% of transketolase activity in human hepatoma and colon cancer cells (84, 85). TKTL1 increases the generation of fructose-6-phosphate and glyceraldehyde-3-phosphate, accompanied by significantly enhanced levels of pyruvate and lactate \textit{in vitro}. These data indicate that TKTL1 overexpression increases glycolysis (Fig. 4; ref. 22). Human colon carcinoma cells with suppressed TKTL1 activity display reduced cell proliferation, lactate production, glutathione content, and NADPH/NADP+ ratio, as well as an increase in ROS-induced apoptosis. Lower amounts of these antioxidants may alter the ability of cells to detoxify ROS (86). The cellular redox status can also be modulated by directly targeting glutathione. Glutathione depletion followed by radiotherapy results in large areas of apoptosis and significantly enhances the sensitivity of xenografted head and neck squamous cell carcinomas and non–small cell lung carcinomas to irradiation (87, 88).

Besides the alteration of the cellular redox status as described earlier (75), Sonveaux and colleagues identified another mechanism explaining the radiosensitizing effect of MCT1 inhibition (89). Lactate produced by glycolytic hypoxic tumor cells can be taken up through MCT1 and used as a fuel for mitochondrial oxidation by aerobic tumor cells (89–92). The use of lactate instead of glucose by oxygenated cells saves glucose for the hypoxic cells to support glycolysis. MCT1 inhibition blocks lactate uptake and forces aerobic cells to use glucose for their energy metabolism. As a consequence, hypoxic cells located at large distance from the vasculature are deprived of glucose and die. MCT1 inhibition induces extensive necrosis, decreases tumor hypoxia, and delays tumor growth in \textit{in vivo} tumor models. Combining MCT1 inhibition with irradiation further delays tumor growth. Radiotherapy may complement MCT1 inhibition by eliminating the remaining oxygenated tumor cells in the vicinity of blood vessels (89).

CAIX inhibition with indanesulfonamide (11c) also results in a significantly slower tumor growth in a colorectal carcinoma tumor model. Tumor growth is further delayed by combining 11c with single-dose radiotherapy. However, \textit{in vitro} incubation of colorectal carcinoma cells with 11c before irradiation does not enhance the effect of radiotherapy. Targeting CAIX under hypoxia reduces the rate of extracellular acidification and proliferation and induces apoptosis of colorectal carcinoma cells (93). Therefore, CAIX inhibition might kill hypoxic cells, whereas radiotherapy attacks normoxic cells, resulting in the therapeutic enhancement of the combined treatment strategy in the colorectal carcinoma tumor model. However, the effect of intracellular acidification on tumor metabolism and the cellular redox status has not yet been determined.

In conclusion, blocking tumor glucose metabolism at several levels has been shown to decrease the amount of antioxidant molecules and to radiosensitize different solid tumors in preclinical studies. The administration of the glycolysis inhibitor 2-deoxyglucose combined with hypofractionated radiotherapy shows promising results in the first phase I/II clinical trials without severe toxicity. However, daily use of antiglucose metabolism treatment for several weeks, which might be useful as an additive treatment in combination with daily-fractionated radiotherapy, could cause more severe side effects. For example, the brain is highly dependent on glucose for its energy metabolism. Therefore, more clinical studies examining the efficacy and...
side effects of antitumor glucose metabolism treatment are required.

Conclusions and Future Perspectives

This review describes that the HIF-1 pathway is involved in the tumor-protective response to radiotherapy, both via vascular protection postirradiation and via enhancing the tumor antioxidant capacity through initiating a glycolytic tumor metabolism. Targeting HIF-1 and tumor glucose metabolism affects the tumor microenvironment, induces metabolic alterations, and sensitizes various solid tumors to irradiation.

Future studies should examine whether HIF-1 and glucose metabolism inhibitors are useful in clinical practice. Special attention should be paid to the effect of HIF-1 inhibition on the glycolytic and redox status of tumors, and optimal timing of these inhibitory treatments in relation to radiotherapy. Furthermore, validation of imaging techniques, which establish HIF-1 expression and glucose metabolism and predict response to radiation, is required for optimal patient selection.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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