Title
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Journal
Molecular carcinogenesis, 61(2)

ISSN
0899-1987

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Publication Date
2022-02-01

DOI
10.1002/mc.23379

Peer reviewed

Published in final edited form as:

Mol Carcinog. 2022 February ; 61(2): 200–224. doi:10.1002/mc.23379.

Metabolic Response to Radiation Therapy in Cancer

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Abstract

Tumor metabolism has emerged as a hallmark of cancer and is involved in carcinogenesis and tumor growth. Reprogramming of tumor metabolism is necessary for cancer cells to sustain high proliferation rates and enhanced demands for nutrients. Recent studies suggest that metabolic plasticity in cancer cells can decrease the efficacy of anticancer therapies by enhancing antioxidant defenses and DNA repair mechanisms. However, there are few robust studies characterizing the metabolic changes induced by radiation therapy in cancer. Extensive study of radiation-induced metabolic changes will lead to a better understanding of radiation response mechanisms as well as the identification of new therapeutic targets. In this review, we will highlight studies that provide information on the metabolic changes induced by radiation and oxidative stress in cancer cells and the underlying mechanisms.

Keywords

Cancer; Radiation therapy; Metabolism; Radiosensitivity; Oxidative stress

Introduction

Cancer is the second leading cause of death in the United States, with approximately 1.8 million cases recorded domestically per year[1,2] and over 19 million cases worldwide[3]. About 50% of cancer patients receive radiation therapy during the course of their treatment, often in combination with surgery[4,5], chemotherapeutics[5–8], or immunotherapy[9,10]. Recent efforts have focused on the unique role of cancer metabolism as a diagnostic tool and therapeutic target[11–14].

Over a century after the discovery of a highly glycolytic metabolism in cancer cells by Otto Warburg, now known as the Warburg effect[15,16], the study of cancer metabolism has

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seen a marked resurgence[13,14,17,18]. Relative to their non-malignant counterparts, cancer cells are in general characterized by an enhanced catabolism of glucose[19], glutamine[20], lactate[21] and fatty acids[22–24], accompanied by pronounced changes in glycolytic flux[19], increased uptake of amino acids, especially glutamine[14,25], fatty acid synthesis[26–28], and redox metabolism[14,29]. Moreover, cancer cell metabolism is highly dynamic in response to various environmental stresses, such as hypoxia[30,31] or cytotoxic therapies such as chemotherapy or radiation therapy[32,33]. Ionizing radiation typically induces cell death via indirect ionization of oxide species that form free radical species and attack the nearby DNA backbone, generating double strand breaks[34,35]. Metabolic radiosensitizers generally operate by depleting reducing equivalents such as the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), in affected cells[36], inhibiting tumor-specific metabolic programs[37], or by decreasing the rate of oxygen consumption in the tumor, resulting in a less hypoxic tumor microenvironment[38–40].

Cancer metabolism has considerable utility both as a diagnostic[41,42] and as a predictor of the overall progression[43–47], chemotherapeutic response[41,48–50] and radiosensitivity[51,52] of tumors, and several chemotherapeutics targeting cancer metabolism have been demonstrated as radiosensitizers in recent trials[53]. Despite the growing interest in cancer metabolism and its extension into the field of radiation therapy, comparably little attention has been paid to the effects of ionizing radiation on cancer cell metabolism, which may be a useful predictor of the tumor response to radiation therapy and offer novel radiotherapeutic targets[54,55].

Here, we will first review methods commonly used in cancer metabolism studies and their relative utility in assessing metabolic changes after radiation therapy. We will further expand on the metabolic alterations that have been reported to be induced by ionizing radiation in cancer cells, and the underlying potential molecular mechanisms. Lastly, we will comment on future directions that could further our understanding of the mechanistic and clinical implications of cancer cell metabolism following radiation therapy, including their potential as prognostic or therapeutic markers.

Section 1: Methods for tracking metabolic changes

Several methods have been developed to investigate the concentration and flux of various cellular metabolites and have proven useful both in basic science and in clinical applications. Here, we will discuss five important methods utilized in cancer metabolism studies, as well as which metabolites each method allows to be studied and the temporal dynamics of each. These methods are summarized in Figure 1.

A Extracellular flux analysis—Extracellular flux analysis studies the oxygen consumption rate and extracellular acidification rate of cells cultured in proprietary multi-well plates, allowing for quantification of the rate of ATP production through mitochondrial and glycolytic sources[56]. Sequential treatment with inhibitors of proteins involved in oxidative phosphorylation (i.e. oligomycin, FCCP, and rotenone) determines a cell's basal respiration rate, ATP-linked respiration, proton leak and maximum respiratory capacity, in addition to oxygen consumption due to non-mitochondrial respiration. Extracellular flux assays are typically performed *in vitro* and, since inhibitors of mitochondrial respiration

are added sequentially, do not typically permit multiple analyses of the same population in the same well, but readily permit analysis of irradiated cell cultures. The analysis is high-throughput, easily multiplexed, and provides useful information not just on the baseline activity of mitochondrial and glycolytic respiration, but also on their response to stimulus.

B Mass spectrometry-based metabolomics—Mass spectrometry-based metabolomics have proven useful for analyzing the absolute levels of multiple metabolites as well as metabolic flux, with applications both *in vivo* and *in vitro*. Among the different types of mass spectrometry, metabolomics uses either separation-based techniques, such as liquid chromatography[57] and gas chromatography (GC), or separation-free techniques, such as Matrix-Assisted Laser Desorption Ionization (MALDI). These techniques each have their own advantages and limitations, which have been discussed extensively elsewhere[58]. What is particularly interesting about metabolomics studies is the possibility of studying treatment-induced changes in pathway flux by performing targeted metabolomics in the presence of stable isotopic tracers[59]. Tracers are chosen based on the pathway studied[60] and may be incubated for different periods of time based on the pathway of interest, and at different timepoints after interventions such as radiation therapy. As an example, the use of uniformly-labeled ^{13}C-glucose allows for carbon tracing through glucose metabolism and downstream pathways, but specific pathways might not be distinguishable. To specifically quantify the glycolytic flux relative to the pentose phosphate pathway flux, 1,2-^{13}C-glucose may be used as it results in metabolites with a different labeling pattern compared to uniformly-labeled ^{13}C-glucose[59]. Among the metabolites that can be quantitatively analyzed are amino acids, lipids, nucleotides, carbohydrates and organic acids, making mass spectrometry-based metabolomics a broad approach to study metabolism. While this technique is widely used *in vitro*, it can also be applied *in vivo*. The latter has been made possible thanks to the development of minimally invasive surgical techniques for isotope infusion[61]. Finally, spatial (or imaging) mass spectrometry that detects the spatial distribution of metabolites in tissues and cells[62] has also been developed and can be applied *in vitro* and *in vivo*. By ionizing select regions of a sample at a time, the identities and positions of metabolites within the sample can be detected with[63] or without[64] metabolic tracers. This technique holds great promise for studying *in situ* metabolism[65]. This is of particular importance in the context of cancer and tumor heterogeneity. The metabolic response to radiation is likely to vary within a tumor, for example based on oxygen availability.

C Nuclear magnetic resonance spectroscopy—Hyperpolarized nuclear magnetic resonance spectroscopy, or similarly, hyperpolarized magnetic resonance spectroscopic imaging (HP-MRSI) typically uses metabolic probes designed with a single carbon-13 (^{13}C) at a known location, which is then subjected to extremely low temperatures and a strong magnetic field[66,67]. This results in a single, highly polarized, ^{13}C-labeled metabolite, whose concentration and conversion to other metabolites can be measured in real-time using conventional MRSI methods[68,69]. Notably, this allows not only for localization and quantification of chosen metabolites, but, owing to the different chemical shifts of downstream metabolites, also allows for determination of the downstream metabolites to which the injected probe was converted over time. Several ^{13}C metabolites have

been developed, including labelled pyruvate[70], alpha-ketoglutarate[71,72], acetyl-CoA[73], and arginine[74], to name a few[75,76], allowing for the study of various metabolic pathways. Selective labeling of particular carbons can be used to specify unique downstream pathways for further study. However, given the rapid decay of spin-polarization experienced by hyperpolarized samples, detectable signal tends to decay quickly after application of ^{13}C probes. This presents logistical challenges, since probes are typically administered concomitantly with imaging, and limits the duration of HP-MRSI experiments. Accordingly, experimental design using hyperpolarized metabolites should take into account the time scale at which metabolic changes are expected, since continuous imaging over a prolonged time course is difficult with hyperpolarized metabolites.

HP-NMR has been successfully used to track metabolite conversion over time in cells cultured in NMR-compatible bioreactors, providing a useful system for studying cancer cell organoids[77], tissue slices[78] or alginate bead cultures of cancer cell lines after ionizing radiation[79]. This method has successfully tracked the concentration and conversion of multiple metabolites in cancer cells, including the conversion of pyruvate to lactate, alanine and bicarbonate[66,80] and the conversion of alpha-ketoglutarate to dextro-2-hydroxyglutarate[71] in cells with mutations in isocitrate dehydrogenase 1 (IDH1)[81]. Similarly, HP-MRSI was used to track metabolism in mouse xenografts and in pilot studies of human tumors, and is the subject of ongoing clinical trials (Table 1). Nuclear magnetic resonance without hyperpolarization of naturally-occurring ^{31}P or ^{1}H has also been used clinically for decades, and is capable of identifying changes in the levels of multiple metabolites after radiation therapy in patient tumors[82–84] or in plasma, urine, and stool[85].

D Measuring oxygenation—Multiple methods exist for imaging oxygenation of tumor tissues, including blood oxygen level-dependent (BOLD) MRI[86], positron emission tomography (PET) with ^{18}F-imidazoles[87] and electron paramagnetic resonance oximetry[88], all of which can provide useful insight into the hypoxic status of heterogenous solid tumors. Considering the frequency with which metabolic radiosensitizers alter the oxygenation status of tumors, imaging of tumor oxygenation[89] can enhance effective studies of changes in tumor metabolism *in vivo*.

E Positron emission tomography—PET is a non-invasive imaging technique commonly used to visualize patient tumors, in particular with the use of ^{18}F-fluoreodeoxyglucose (FDG). This technique is based on the high glucose uptake by cancer cells relative to the surrounding normal cells. Other PET tracers have also been developed in the context of tumor detection[90]. In addition to its role in clinical oncology, PET has been used in pre-clinical research to measure metabolite uptake *in vivo* and study pathways such as glutamine metabolism[91] (^{11}C-L-glutamine) and fatty acid synthesis[92] (^{11}C-acetate). PET can also be useful for evaluating treatment response *in vivo*. If normalized to tumor size instead of being used to define it, this technique can also be used to determine the uptake of a given tracer per cancer cell or tumor unit[93,94]. However, it provides no indication on the metabolic flux downstream of the tracer. Studies using FDG-PET early in the course of combination therapy have shown prognostic value in several tumor types, implying utility of these imaging methods both before and after radiation therapy[95–98].

Section 2: Changes to major metabolic pathways induced by ionizing radiation

The effects of ionizing radiation on cancer cell metabolism are potentially a reflection of the indirect damages that radiation-induced reactive oxygen species (ROS) cause on macromolecules, leading to disruption of redox homeostasis and 'oxidative stress'. Oxidative stress can be defined as an imbalance between ROS generation and ROS scavenging by cellular antioxidant defenses[99]. If not successfully repaired, the oxidative damage can be lethal. The effectiveness of radiation therapy is in part due to the therapeutic index created by the superior repair capacity of normal tissues relative to cancer cells, with radiation sensitivity in cancer thought to be a function of DNA repair capacities and antioxidant defenses[52]. Rewiring of metabolic pathways so as to provide cells with metabolites for antioxidant defenses and DNA repair is necessary for both these mechanisms. Cancer cells with high metabolic plasticity may therefore be able to better respond to oxidative stress and mitigate the cytotoxic effects of ionizing radiation.

It is important to point out that many studies aimed at investigating the effects of ionizing radiation on cells use oxidizing agents in lieu of radiation to mimic its effects. As an example, although hydrogen peroxide (H_2O_2) does induce oxidative stress[100], its effects are not equivalent to those of ionizing radiation[101,102]. H_2O_2 is converted to water and dioxygen within minutes by the catalase enzyme and studies therefore require high concentrations of H_2O_2 to induce potentially lethal oxidative stress[103]. A recent study using various types of cancer cells showed that while radiosensitivity predicted sensitivity to peroxide, the converse was not always true, as H_2O_2-resistant cancer cells were sensitive to X-ray-induced cell death[101]. Such studies caution against equating observations made under oxidative stress conditions induced by means other than ionizing radiation to those of radiation-induced effects. In this section, we will specifically focus on metabolic changes after oxidative stress induced by ionizing radiation.

A Metabolic response to oxidative stress induced by radiation—Robust antioxidant defenses play a major role in radiation sensitivity. For example, cancer stem cells, notorious for their resistance to therapies, have higher basal activation of ROS scavenging systems, which is correlated to radiation sensitivity[104]. It is reasonable to postulate that cancers with poor responses to radiotherapy may be more likely to have either strong basal antioxidant defenses or a great deal of metabolic plasticity that facilitates rewiring of metabolic fluxes to generate a powerful antioxidant response after radiation. In agreement with this idea, two very recent studies using personalized genome-scale metabolic flux models identified tumor redox metabolism as a major predictor for radiation sensitivity[105,106]. It seems that tumors with poor radiation response reroute metabolism to boost the levels of reducing factors of the cell, such as NADPH and glutathione, thus enhancing clearance of ROS[105]. This implies a dependency on rerouting metabolic fluxes for maintaining antioxidant defenses and survival after lethal oxidative damage, ultimately pointing to metabolic plasticity as a driver of radiosensitivity[53,107,108]. This mechanism of radiation sensitivity is distinct from enhanced DNA repair capacity, although successful DNA repair ultimately depends on enhancing flux through metabolic pathways that generate precursors for DNA building blocks (more below). Considering radiation sensitivity from this angle opens up the possibility that metabolically targeted interventions might improve

radiotherapeutic outcomes. Multiple metabolic pathways feed into antioxidant defenses and DNA repair, including glycolysis, the pentose phosphate pathway (PPP), glutaminolysis, and 1-carbon metabolism (1CM), all of which will be discussed more in detail below.

B bGlycolysis and the pentose phosphate pathway—Glucose is one of the major nutrients (the other being glutamine) which supports the growth and survival of cancer cells. Irradiated breast cancer cells have been shown to increase glucose consumption, accompanied by increased extracellular lactate[109,110]. While the Warburg effect has been well characterized to increase glycolysis in cancer cells at baseline[16,111], changes to glycolytic flux after ionizing radiation may be a function of cellular capacity to transport metabolites[112,113]. Glucose transporters (GLUTs) are responsible for glucose import and GLUT1 expression in particular has been linked to radiosensitivity[112]. Studies have shown HIF-1-dependent upregulation of GLUT1 after ionizing radiation in cancerous[110,114] and normal tissue[115], including under normoxic conditions[115], thus explaining in part the radiation-induced increase in glucose consumption, although the exact mechanisms remain to be elucidated.

The altered glycolytic flux that seems to be induced by radiation is accompanied by a suppression in the enzymatic activity of the M2 isoform of pyruvate kinase (PKM2), which converts phosphoenolpyruvate to pyruvate immediately before pyruvate enters the TCA cycle[109]. Others have shown similar effects on PKM2 with chemically-induced oxidative stress[116], suggesting that inactivation of this redox-sensitive glycolytic enzyme may be a shared response to oxidative stress. The role of PKM2 in reprogramming cancer cell metabolism to promote tumor growth is well established[117,118] and it has provided an explanation for the preferential expression of the M2 isoform in cancer cells. While all other isoforms of PK exist as constitutively active tetramers, PKM2 can shift between enzymatically active tetramers and inactive dimers; tetrameric PKM2 efficiently converts phosphoenolpyruvate to pyruvate[119]. In contrast, kinase-inactive PKM2 blocks pyruvate production, creating a bottleneck in glycolysis that makes upstream glycolytic intermediates available for auxiliary glycolytic pathways, such as the pentose phosphate pathway (PPP) and the serine synthesis pathway (SSP). In proliferating cells, the PPP and SSP provide building blocks necessary for cellular anabolism[29,120,121]. These same pathways also provide the two main reducing equivalents in cells, NAPDH and glutathione (Figure 2). NADPH fuels antioxidant systems and recycles oxidized glutathione to combat oxidative stress so as to restore cellular redox homeostasis[122]. It therefore appears that one advantage of the seemingly paradoxical radiation-induced increase in glucose consumption in breast cancer cells while PKM2 activity is suppressed may be the availability of glycolytic intermediates for funneling into antioxidant pathways subsidiary to glycolysis that can support survival during oxidative stress conditions. In this manner, PKM2 may act as a ‘metabolic switch’ that provides cells with metabolic plasticity in a context-dependent manner[123,124]. A study by Tuttle and colleagues shows that cells with high PPP activity are less sensitive to radiation therapy and our own work (unpublished data) suggests that forced activation of PKM2 can sensitize breast cancer cells[109] as well as glioblastoma tumors to radiation.

Enhanced activity of the PPP after ionizing radiation is possible via the activation of diverse molecular pathways that are responsive to oxidative stress, for example, the activation of the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2), a master regulator of oxidative stress. Under normal conditions, NRF2 is targeted for degradation by its negative regulator Kelch-like ECH-associated protein 1 (KEAP1)[125], but oxidative stress inactivates KEAP1, resulting in accumulation of newly translated NRF2, which can then translocate to the nucleus and initiate transcription of its target genes that contain 'antioxidant response elements' (ARE) largely involved in maintaining cellular redox homeostasis[125,126]. Among NRF2's target genes are a number of metabolic genes that drive the PPP, which generates NADPH (Figure 2). Ionizing radiation activates the NRF2 pathway and protects normal cells (mouse embryonic fibroblasts) from ROS and radiation-induced toxicity. Our own data also shows that cancer cells activate the NRF2 pathway after radiation[127] (Figure 2), and that NRF2 activation is involved in radiation-induced metabolic reprogramming (unpublished data). An additional way of upregulating the PPP is via the redox-sensitive, protein kinase ataxia telangiectasia mutated (ATM). ATM is activated in response to radiation-induced DNA damage[128,129] and prevents radiation-induced cell death by promoting homologous recombination and DNA repair[130]. Evidence exists that ATM can induce the activity of G6PDH, the first rate-limiting, and NADPH-producing enzyme in the PPP (Figure 2). Others have shown that in addition to promoting an antioxidant response, ATM-induced PPP activation also leads to increased nucleotide synthesis via the generation of the sugar backbone for dNTPs, the ribose-5-phosphate (R5P)[131] (Figure 2).

C Pyruvate and lactate metabolism—In addition to its stereotypical role as a waste product of anaerobic glycolysis, lactate serves several important roles in cancer cell metabolism. Given the high demand for NAD^+ imposed by a highly glycolytic phenotype[14], the conversion of pyruvate to lactate serves as a critical mechanism for replenishing NAD^+ levels from the cellular NADH pool[132] (Figure 3). On the other hand, lactate also serves as an important source for gluconeogenesis through the hepatic Cori cycle, and can serve as an important extracellular signaling molecule[133,134], altering the activity of several key metabolic enzymes at high concentrations[135–138]. Moreover, high lactate concentration in the tumor microenvironment has been implicated in alterations of local immune cell populations[139–141], decreased radiosensitivity[142,143], higher cancer cell proliferation[144], metastasis[145–147] and worsened prognosis[145,148–150] in a variety of cancer etiologies.

The radiation-induced increase in extracellular lactate concentration despite the suppressed enzymatic activity of PKM2 in breast cancer cells is unexpected[109], as low PKM2 activity should decrease pyruvate production from glycolysis. One possible explanation is the generation of pyruvate via the oxidative decarboxylation of malate to pyruvate (Figure 3). This reaction is catalyzed by the cytosolic malic enzyme 1 (ME1), a target gene of NRF2[151], or the mitochondrial isoforms (ME2/ME3). It is possible therefore that the activation of the NRF2 pathway by radiation[127] contributes to increased lactate production by upregulating the malic enzyme levels. The conversion of malate to pyruvate by malic enzyme generates NADPH, thus further contributing to the antioxidant defenses, as well as to lipid and cholesterol biosynthesis[152].

The source and rate of lactate usage in cancer cells has been the subject of a number of studies[19,21]. Notably, even cells with net lactate production import exogenous lactate in *in vivo* rat glioma models, using it both as an energy source and to generate alanine and glutamate[153,154]. Production of lactate through lactate dehydrogenase A (LDHA)[148] and transport of lactate via monocarboxylate transporter (MCT) families have been correlated with cancer prognosis[150,155,156]. Several key cancer-associated signaling pathways also influence lactate production, including Ras, Akt, Myc, and the Dek oncogene[157]. The Myc signaling pathway upregulates LDHA[149,158] and cellular MCTs[159]. Lactate itself has an effect on several key signaling pathways, upregulating expression of HIF-1α, an upstream regulator of GLUT1[160,161], and many other glycolytic enzymes, including LDHA[162] (Figure 3).

Several studies have found that the interconversion between lactate and pyruvate is highly responsive to chemotherapeutics[113,163–165] and radiotherapy[79,163,164] in both three-dimensional alginate cultures[79,113] and mouse xenografts[163–165]. The lactate-to-pyruvate ratio after radiation shows significant temporal dynamics, with a marked decrease in lactate levels and lactate-to-pyruvate ratio shortly after irradiation[79,166,167] and increased lactate levels over time[166,168], which may imply differences in tumor reducing potential over time after ionizing radiation[167,169,170]. Radiation can upregulate expression of MCT1 after 8 hours in an AMPK/NFkB-dependent manner, providing a mechanism for the radiation-induced decrease in lactate-to-pyruvate ratio[171]. MCT inhibition radiosensitizes small cell lung cancer cells *in vitro* and decreases tumor growth[172]. Interestingly, studies of tumor oxygenation following MCT inhibition found a notable increase in tumor oxygenation, arguing that radiosensitization of MCT-inhibited tumors may stem from loss of the hypoxic core[173]. Similar mechanisms targeting inhibition of the mitochondrial pyruvate carrier (MPC) found that inhibition of extracellular lactate uptake and accumulation of intracellular pyruvate radiosensitized spheroid cultures by decreasing oxygen consumption and decreasing the size of the hypoxic core independent of extracellular lactate or glucose concentrations[40].

Pyruvate and lactate levels are strongly influenced by the expression of the bidirectional cell membrane monocarboxylate transporter family, including MCT1[174,175] (Figure 3). Studies of siRNA-mediated MCT knockouts in three-dimensional alginate cultures found that the lactate-to-pyruvate ratio was predominantly a function of MCT expression regulating pyruvate influx, rather than LDHA expression or glycolytic flux[113]. This implied that the lactate-to-pyruvate ratio in cancer cells may be a function of transport rather than synthesis. MCT1 expression was found to rate-limit the conversion of pyruvate to lactate in non-irradiated cells[113]. Together with observations that ionizing radiation alters expression of MCT1, this provides an independent mechanism by which lactate production can increase while the lactate-to-pyruvate ratio decreases in irradiated cells. Given the importance of both lactate synthesis and transport following ionizing radiation, further studies are necessary to gain more insight into this seemingly competitive utilization of lactate by irradiated cancer cells, paying particular attention to alterations of signaling pathways and the temporal dynamics of post-irradiation metabolic responses.

D Mitochondrial metabolism—Despite the heavy reliance on Warburg metabolism in many cancer cells, mitochondrial metabolism remains a significant source of energy production and oxygen consumption, which may have significant effects on radiation response[176,177]. Interestingly, ionizing radiation upregulates mitochondrial oxygen consumption, ATP production and ROS generation within 24 hours after irradiation[178–182], possibly due to mTOR-dependent upregulation of oxidative phosphorylation[183]. Inhibition of this mitochondrial increase in ATP production after ionizing radiation has been demonstrated to radiosensitize lung cancer cells[181], in addition to other mitochondrial radiosensitizers that may also work to decrease oxygen consumption.

Mitochondria are also responsible for approximately 90% of intracellular ROS generation[184], implying that mitochondrial ROS generation could play a significant role in radiosensitivity. Several key antioxidant proteins with important roles in radiation response, including SOD2, thioredoxin reductase (TXNRD) and glutathione peroxidase, are frequently upregulated in tumors and are downstream targets of the mitochondrial KEAP1-NRF2 pathway[185,186]. Several mitochondrial genes are upregulated in response to ionizing radiation in both directly irradiated and bystander cells, including ATP synthases MT-ATP6 and MT-ATP8, and NADH dehydrogenases MT-ND1, MT-ND5 and MT-ND6[187,188]. Murine cancer models also show upregulation of superoxide dismutase after radiation[188], consistent with upregulation of genes involved in radiation-induced oxidative stress response[189].

Ionizing radiation has also been shown to affect mitochondrial dynamics, inducing mitochondrial fission in normal fibroblasts in a DRP1-dependent manner[190,191], consistent with upregulation by ionizing radiation of the upstream regulators of DRP1, AKT and mTOR[192,193] and increased mitochondrial localization of DRP1[190]. In cancer cells, metabolic reprogramming typically leads to higher cytoplasmic expression of DRP1 at baseline, paired with accumulation of both functional and defective mitochondria [194–197]. Accordingly, mitochondrial autophagy to remove defective mitochondria is correlated with tumor malignancy[198] and strongly tied to loss of mitochondrial membrane polarization, which is induced by mitochondrial ROS. Inhibition of mitophagy after ionizing radiation increased rates of cellular autophagy and slowed growth in a HeLa cell culture model[199], consistent with similar reports connecting inhibition of mitophagy to increased radiosensitivity in *in vitro* and *in vivo* nasopharyngeal carcinoma models[200]. Mitochondrial fission is frequently implicated in other stress responses[201], including ultraviolet radiation, genotoxic stress, and nutrient deprivation[202]. Interestingly, oxidative stress following brief administration of hydrogen peroxide stimulated mitochondrial fusion in human umbilical vein epithelial cells[203], implying different mitochondrial dynamics in response to the duration and severity of stress presented[204]. After ionizing radiation, increased mitochondrial fission has been implicated in calcium release, and mitotic catastrophe via dysregulation of centromeres independent of apoptotic pathways[190,205], with inhibition of DRP1 increasing the rate of mitotic catastrophe[190].

E Amino acids and one-carbon metabolism—Amino acids constitute a necessary fuel for tumor growth. Glutamine, the second major nutrient for cancer cells after glucose, plays an important role in the cancer cell biology. Upon entry in the cell via ASCT2 transporter, glutamine is converted to glutamate and serves as a nitrogen source for amino

acid and nucleotide biosynthesis. Glutamate is particularly important as it can either be converted to α-ketoglutarate (αKG), and enter the tricarboxylic acid (TCA) cycle, or serve as one of the main building blocks in glutathione synthesis (Figure 4). Cancer cells from various origins are highly dependent on glutamine[206–208] and therapeutic targeting of glutamine metabolism is an area of significant interest.

Multiple targets associated with glutamine metabolism have also been associated with radiosensitivity[209–211]. However, very few studies have investigated the effects of ionizing radiation on glutamine metabolism in cancer. Previous work has demonstrated that matched cancer cells selected for radiation resistance upregulated glutamine synthetase relative to parental lines[211]. The authors noted an increase in glutamine anabolism while glycolysis, OCR, and ATP production were reduced, but did not study the direct effect of ionizing radiation on glutamine metabolism. Our own data also suggest that ionizing radiation leads to an enhanced consumption of glutamine 24 hours after radiation[109], most likely as a response to oxidative stress to generate more glutathione[210] (Figure 4). There is also evidence that glutathione levels are increased by radiation in the context of cancer[57,212]. In addition to glutamate, glutathione is made from cysteine and glycine, the latter of which can either be imported or synthesized from serine. While cells can take up free serine from their environment or synthesize it *de novo* from glucose, some cancer subtypes become addicted to *de novo* production of serine[213–215]. The serine synthesis pathway in combination with one-carbon metabolism generates both NADPH and the precursors for reduced glutathione synthesis. Interestingly, levels of phosphoserine aminotransferase 1 (PSAT1), which converts 3-phospho-hydroxypyruvate and glutamate to 3-phospho-serine and αKG, have been shown to increase after ionizing radiation (Figure 4), and targeting PSAT1 leads to radiation- and glutamine-deprivation sensitivity in lung cancer cells[216]. Although there is a lack of studies on serine metabolism after radiation, the field is starting to recognize the role of SSP in redox metabolism[217–219].

One-carbon metabolism, responsible for maintenance of methylation reactions in the cell, is heavily implicated in cancer and frequently altered by ionizing radiation (Figure 4). It is well established that cancer cells maintain significant alterations in both DNA[220,221] and histone methylation[222,223], implicated in carcinogenesis[224], proliferation[225,226] and drug resistance[227] of several cancers. Importantly, one-carbon metabolism is also closely tied to synthesis of purines and pyrimidines via the folate cycle. While a recent review has already covered radiation-induced alterations to methylation and one-carbon metabolism in depth[228], we will summarize important points here.

One-carbon metabolism involves the conversion of folate from circulating monoglutamates[229] or the import of extracellular folate by reduced folate carrier (RFC) transporters[230], with outputs capable of generating purines, thymidylates, and the S-adenosylmethionine and S-adenosylhomocysteine responsible for DNA and histone methylation. Importantly, homocysteine is also a precursor for glutathione synthesis, implicating one-carbon metabolism in glutathione-mediated oxidative responses. Mechanistically, antifolate chemotherapeutics have been shown to be effective via inhibition of nucleotide synthesis[231,232].

Total body irradiation of normal mice resulted in an increase in expression of several key enzymes in the folate cycle, but a decrease in expression of the rate-limiting enzyme methylenetetrahydrofolate reductase 1 (MTHFR1), resulting in a net decrease in total folate levels in liver[233], plasma and bone marrow samples[234]. Direct oxidation of folate by ionizing radiation has also been observed, potentially contributing to a general decrease in folate levels in irradiated animals[235]. Cancer cells also seem to be vulnerable to depletion of methionine, the precursor to S-adenosylmethionine, a key player in both the folate and methionine cycles[236]. Despite their influence on several key signaling pathways and the longstanding use of chemotherapeutic antifolates, little literature exists detailing changes to folate metabolism after ionizing radiation in cancer cell models. Future studies investigating changes in folate metabolism after ionizing radiation, especially with respect to nucleotide synthesis and methionine metabolism, may be beneficial.

F Nucleotide metabolism—Since ionizing radiation primarily affects cells through oxidative damage to DNA, the demand for de novo nucleotide synthesis to aid in DNA repair is high in irradiated cells. Independently of ionizing radiation, proteins involved in the metabolism of nucleic acids are frequently altered in cancer cells[237,238], and are often predictive of cancer prognosis[239–241]. Ionizing radiation has been shown to differentially upregulate enzymes involved in nucleotide synthesis in some cell lines but not others[242–244], with a greater change in expression observed in less radiosensitive cell lines.

De novo nucleotide synthesis is a 10-step process catalyzed by six enzymes[245], taking inputs from ribose 5-phosphate produced by the pentose phosphate pathway (Figure 2), glycine, glutamine, and aspartate, with a rate-limiting step catalyzed by ribonucleotide reductase[246], a homologue of which is a downstream target of p53[247,248]. Likewise, contributions from other metabolic pathways to nucleotide synthesis are altered by ionizing radiation: the rate-limiting enzyme of the pentose phosphate pathway, glucose-6 phosphate dehydrogenase (G6PD), is a downstream target of the key DNA repair kinase ATM[249,250], and alterations to glutamine metabolism are discussed elsewhere in this review. Interestingly, radiosensitization of nasopharyngeal carcinoma cells by inhibition of glutamine synthetase showed that this radiosensitization was dependent on the activity of *de novo* pyrimidine and purine synthesis, with glutamine synthetase-knockdown cells demonstrating significantly lower levels of homologous recombination, implying that a significant portion of nucleotide synthesis in irradiated cells is dependent on glutamine metabolism[211].

Similarly, nucleotides can be generated via salvage pathways, which add activated ribose-5 phosphate to bases to form nucleoside monophosphates. In particular, given the high need for NAD+ in cancer cells[251], the key salvaging enzyme nicotinate phosphoribosyltransferase (NAPRT) is widely upregulated in cancer cells[252]. While nucleic acid salvage pathways also require many of the same intermediates as the synthesis pathway discussed above, membrane transporters can import hypoxanthine, guanine, and adenine, which are capable of being converted to inosine-, guanosine-, and adenosine 5’-monophosphate. Such nucleotide salvage pathways are likely upregulated by ionizing radiation; for example, deoxycytidine kinase, which is responsible for phosphorylation of deoxyadenosine, deoxyguanosine, and deoxycytidine, is upregulated by the master DNA damage regulator ATM[253,254]. Inhibition of *de novo* nucleotide synthesis forces cancer cells to rely on

these nucleotide salvage pathways, increasing the efficiency of radiolabeled nucleotide analogues[255,256], implying that both *de novo* nucleotide synthesis and scavenging may play an important role in cancer cell nucleotide metabolism after radiation-induced DNA damage. Recent studies have demonstrated that purine, but not pyrimidine levels correlate inversely with radiosensitivity, as inhibition of guanylate synthesis radiosensitized glioblastoma cells and addition of exogenous purines rescued this effect[257]. While little is known about the difference between purine and pyrimidine metabolism after irradiation, prior studies have shown that decreased proliferation of nucleotide-depleted cells can be rescued by addition of exogenous thymine[258], implying that differences in purine and pyrimidine metabolism may be contextually relevant. Additionally, investigation of expression of enzymes involved in nucleotide synthesis after ionizing radiation showed increases in pyrimidine synthetic pathways, but not purine pathways[242]. While concentrations of nucleotides broadly affect the synthesis of the other nucleobases[259], a specific need for purine synthesis is consistent with observations that purine and GTP levels predict aggressiveness of glioblastoma[260,261]. As DNA repair, requires contributions from both purine and pyrimidine pools, especially through homologous recombination, the disparate need for purine synthesis may point at a significant need for GTP in irradiated cells, though this mechanism requires further study[257].

G Fatty acids/lipids—While the prominence of the Warburg effect has led to considerable study of sugar metabolism in cancer cells, lipid metabolism is also significantly altered[262], with upregulation of lipogenic pathways predictive of increased cancer incidence[263–265] and increased proliferation[265,266], seemingly in an AKT-mTOR-dependent manner[267]. In addition to rewiring their own lipid metabolism, cancer cells can also scavenge lipids from surrounding cells[268,269]. Accordingly, irradiation of prostate cancer induces detectable, significant, and durable changes in several fatty acids in the plasma and stool of patients given pelvic radiation therapy, with further studies planned to investigate the prognostic potential of changes to short-chain fatty acids in these samples[85].

Inhibition of stearoyl-CoA desaturase 1 (SCD1), the rate-limiting enzyme in the formation of monounsaturated fatty acids, decreased clonogenic survival after ionizing radiation in hepatocellular carcinoma cell culture, while overexpression decreased genomic instability as measured by comet assay[270]. Additionally, radiosensitivity of hepatocellular carcinoma can be decreased by addition of monounsaturated fatty acids[270], and radiosensitivity of colorectal cancer cell culture and xenografts can be diminished by exogenous addition of cholesterol[271]. Ionizing radiation was found to alter expression of several key regulators of lipogenesis, upregulating AMPK, an upstream regulator of the key mediator of cholesterol synthesis SREBP1 for 6–24h after irradiation (Figure 5). This alteration was accompanied by an increase in cholesterol and decrease in triacylglycerol levels[271]. Interestingly, inhibition of stress-responsive cholesterol synthesis in glioblastoma after combination therapy with ionizing radiation and dopamine receptor antagonists did not significantly increase radiosensitivity in cell culture, but did significantly improve median survival and reduce proliferation in murine xenograft models[272]. Ionizing radiation has also been shown to upregulate the long-chain-fatty-acid CoA ligase ACSL4 (Figure 5), which is responsible for esterification of CoA onto fatty acids[273], with ASCL4 inhibition decreasing cancer cell ferroptosis after radiation[274].

While the molecular mechanisms governing alterations to lipid metabolism after ionizing radiation are unclear, possible mechanisms include interplay between monounsaturated fatty acid levels, AKT phosphorylation[275], and p53 activity[276,277]. Moreover, despite the utility of lipids as a reservoir for the production of ATP, CoA, and NADPH in cancer metabolism, comparably little is known about the alterations to redox metabolism following radiation, especially in light of Akt-dependent acetyl-CoA production being utilized in *de novo* production of fatty acids in cancer[262,278,279]. Accordingly, inhibition of fatty acid synthesis in matched lines of different radiosensitivity *in vivo*[280] showed that the less radiosensitive cells had an increased sensitivity to PPP inhibitors, and elevated NADPH levels, and displayed greater radiosensitivity following inhibition of fatty acid synthase (FASN)[281].

In addition to its well-documented effects on nucleotide backbones, ionizing radiation has pronounced effects on both lipid backbones[282,283] and on lipogenesis in normal cells[284,285], while studies in cancer cells are lacking. Typically measured using proton magnetic resonance spectroscopy (1H-MRS), studies of lipogenesis in irradiated cancer cells have found an increase in production of both saturated and unsaturated lipid levels after ionizing radiation[286,287]. Cervical cancer biopsies from patients undergoing radiotherapy showed increases in lipid and fatty acid -CH_2 levels predictive of apoptosis in irradiated cells, while changes to creatine, taurine, glucose, or lactate metabolism were not individually associated with apoptosis[286], consistent with prior observations of changes in lipid levels in necrotic cancer tissue[287].

Specifically, considerable research has focused on sphingomyelinase (ASMase)-mediated hydrolysis of sphingomyelin to form ceramide after ionizing radiation[288], which induces a translocation of ASMase from the lysosomes to the plasma membrane[289,290], followed by increased activity of ceramide synthase[291] (Figure 5), resulting in significant increases to ceramide concentrations after ionizing radiation and a consequent increase in ceramide-derived lipid rafts[290]. Accordingly, ceramide has emerged as a useful biomarker of irradiated cells[288,292,293], with ceramide accumulation shown to result in G_0/G_1 cell cycle arrest[294,295] and apoptosis[296–298] via permeabilization of the mitochondrial outer membrane[299] after ionizing radiation. Interestingly, p53 appears necessary for ceramide accumulation after ionizing radiation in cells with intact p53 independent of ASMase activity[300]. Both mitochondrial apoptosis and de novo synthesis of ceramide are dependent on Bcl-2 in p53-positive cells[301], but ceramide-derived growth suppression appears to be p53-independent[302].

Recent studies have also investigated the potential for radiation-induced oxidation of lipids to stimulate ferroptosis (Figure 5), an erastin and RSL3-dependent form of cell death independent of canonical apoptotic or necrotic pathways triggered by lipid peroxidation[274,303,304]. Accordingly, a decrease in available redox scavengers such as glutathione radiosensitize cells in a ferroptosis-dependent manner[305,306], as does inhibition of SLC7A11 (xCT), a glutamate-cystine antiporter, in line with cysteine's role as a scavenger and necessity for glutathione synthesis[274,307–309]. Similarly, *de novo* lipogenesis suppresses ferroptosis[310].

H Radiation dose fractionation and metabolic response—Clinical radiotherapy is usually delivered across fractionated doses as opposed to a single high dose. This approach provides several clinical benefits, including improved radiation response of formerly hypoxic and reoxygenated tumor regions and facilitated repair of sublethal DNA damage in surrounding normal tissues[35,311]. While many different fractionation plans exist clinically, the metabolic response of tumor cells irradiated in fractionated doses may be significantly different from the metabolic responses of cells given single high doses, which may therefore offer new opportunities for interventions directed at tumor metabolism during a course of radiation therapy. Numerous studies have documented the effects of fractionated radiotherapy on hypoxia and repopulation in the tumor microenvironment[312–315], and the metabolic effects of those stimuli have been thoroughly discussed elsewhere[316,317].

Notably, gene expression patterns in cells exposed to fractionated radiation can differ significantly from responses in cells given single equivalent doses, with significant changes in timing and magnitude of responses. For example, irradiated 3-dimensional prostate cancer cultures upregulated AKT within 2 hours of a multifractionated dose regimen, but did not show the same response following an equivalent single dose, resulting in enhanced sensitivity to an AKT inhibitor only when administered after fractionated radiation[318]. These changes occur rapidly after fractionated radiotherapy, and altered signaling in integrin expression has been shown to continue for up to a month after fractionated radiotherapy, implying sustained changes in signaling after fractionated radiotherapy[319,320]. Biological context also informs response to fractionated radiotherapy, with prostate cancer xenografts showing significantly different activation of several important signaling pathways (e.g. interferon-related genes, STAT1) to fractionated radiation than the same cell line cultured *in vitro*[321].

Since radiotherapy is typically delievered in fractionated regimens, it is also important to note that the size and frequency of fractions can be informed by metabolic response as measured by the metabolic imaging methods discussed above. For example, recent clinical trials (NCT01507428, NCT01576796, NCT02473133) track the effects of modifying fractionation patterns to introduce more fractions to NSCLC patients whose tumors do not show metabolic improvement as measured by mid-treatment PET imaging[322–324]. Given the clinical benefits of fractionated radiotherapy and the differential dynamics of metabolic response over time, future radiation therapy studies of cancer cell metabolism and stress responses after ionizing radiation should investigate the responses to fractionated radiation.

I Effects on normal cells—Despite recent technical and technological advances in clinical radiation therapy, normal tissue toxicity remains a significant dose-limiting factor[325]. To this end, several studies have aimed to identify easily-assessable metabolic markers of normal tissue toxicity after ionizing radiation, either at a systemic level by measuring changes in blood[326,327] or urine[328] metabolomes, or in specific organs of interest[329]. These studies in both animal models[328,330] and humans[327], aside from their value in predicting normal tissue toxicity, have revealed interesting differences in metabolic response to radiation between normal and cancerous tissue, resulting in a series of metabolite panels useful for uniquely predicting radiation-induced normal tissue damage[331–333].

Normal tissue irradiation produces characteristic metabolite profiles that can serve as useful predictors of damage to normal tissue or of associated inflammation in multiple models[326], including total body irradiation[334,335], lung cancer[336], head and neck cancer[337], and glioblastoma[338]. Studies of metabolic changes induced in normal tissues have typically focused on some of the metabolites also upregulated in cancer cells after ionizing radiation, including phospholipids and triglycerides[339,340], nucleic acids[341,342], and precursors to nucleotide synthesis[333]. Modification of metabolic pathways in normal cells can be highly dependent on radiation quality[343], dose rate[344,345], and timing[346,347], with significant alterations in pyruvate metabolism and nucleic acid synthesis precursors detected in cells exposed to doses as low as 10 cGy[347] and increasing in proportion to registered dose[344,348].

Additionally, several studies have investigated radiotoxicity to tissues in the field of irradiated tumors. For example, cardiomyocyte metabolism is primarily mitochondrial[349] and cardiac tissue is often damaged by thoracic radiation therapy[350]. Rat hearts treated with a single dose of 2 Gy showed dysregulation of several metabolic proteins isolated from mitochondrial fractions, including proteins involved in the electron transport chain, glycolysis, and lipid metabolism[351]. Interestingly, murine cardiac models of high-dose radiation have showed downregulation and decreased activity of multiple parts of the electron transport chain, paired with increased activity of pyruvate dehydrogenase E1α[352,353]. These data contrast with observations from irradiated cancer cells, which show a HIF-1α-dependent upregulation of mitochondrial complex proteins after comparable doses of X-rays or gamma irradiation[182,354], highlighting the differential impact of metabolic reprogramming in cancerous tissue.

Section 3: Changes to the tumor environment

A Tumor microenvironment—Irradiation of a solid tumor inevitably affects all the cells in the tumor microenvironment (TME)[355], but the metabolic response of TME cells to radiation is largely unknown. Some evidence suggest that radiation therapy will affect stromal cells, such as cancer-associated fibroblasts (CAFs), so as to promote a metabolic re-wiring and increased glutamine consumption in cancer cells in a paracrine manner[356]. Radiation could also potentially have direct effects on the metabolism of cells in the tumor microenvironment, or their metabolic phenotype could be indirectly affected by the metabolic changes of the cancer cells induced by ionizing radiation. For example, as discussed above, ionizing radiation can induce an increase in lactate production and secretion[109,110,357,358]. The sudden increase in lactate concentration following ionizing radiation in the microenvironment is likely to impact the surrounding cells. Apart from serving as a fuel source for cancer cells, lactate participates in tumor acidification, which plays a role in local invasion[359], metastasis[360], therapy resistance[361], immune escape[362], and angiogenesis[363]. Intratumoral lactate levels are capable of modulating the count and activity of several immune cell populations in the TME. For example, T_{reg} cells, which suppress effector T cells in the TME[139,364,365], preferentially suppress antitumor immunity in low glucose, high lactate conditions[139]. Interestingly, inhibition of lactate dehydrogenase prevented lactate-mediated decreases in T-cell cytotoxic activity, implying that T-cell lactate metabolism within the tumor microenvironment has a strong effect on antitumor cytotoxicity. High lactate can decrease antitumor T-cell cytotoxic activity up to

50% in a spheroid model, with cytotoxic activity restored 24 hours after removal of the exogenous lactate[140]. Similarly, high lactate decreases rates of monocyte migration and macrophages polarize towards an immunosuppressive M2-like phenotype in tumors with high lactate[141]. More recent evidence has suggested that MCT1-mediated lactate export is required for T_{reg} function intratumorally, but not in peripheral T_{reg} cells[139]. Similarly, inhibition of lactate transport in intratumoral cytotoxic T lymphocytes decreased T-cell cytotoxicity in a tumor spheroid model[140], in agreement with the above observations that high concentrations of exogenous lactate are sufficient to enhance the immunosuppressive activity of the T_{reg} population. Increased lactate secretion after radiation therapy can also affect myeloid-derived suppressor cells (MDSCs) in pancreatic cancer[358]. In this study, the authors demonstrate that radiation-induced lactate secretion in pancreatic cancer cells promotes the activation of immunosuppressive, pro-tumoral MDSCs via induction of HIF1[358].

Cancer-associated fibroblasts (CAFs) are another important cell population found in the TME, and are known to contribute to tumor growth and metastasis[366], and radiotherapy is one of many factors shown to activate CAFs[356,367–369]. Interestingly, radiation-activated CAFs have been shown to induce metabolic reprogramming in colorectal cancer cells[356]. By using conditioned media from irradiated CAFs, the authors demonstrated that radiation-activated CAFs induce an increase in glucose metabolism, lactate release, and glutamine consumption, while the expression of many metabolic genes was enhanced, including xCT responsible for glutamine transport. These changes happened in a paracrine manner through IGF1 secretion by CAFs[356]. Although this study did not investigate radiation-induced metabolic changes in the CAFs themselves, there is evidence that CAFs also undergo metabolic reprogramming to provide the cancer cells with additional sources of carbon[370], such as glutamine[371] or alanine[372]. As radiation therapy activates CAFs[356,367–369], it is reasonable to postulate that ionizing radiation can induce metabolic reprogramming in activated fibroblasts that will further sustain cancer growth. Another study demonstrated that radiation therapy induces high inducible nitric oxide synthase[139] expression and increased levels of nitric oxide (NO) secretion in CAFs, which in turn enhanced iNOS/NO signaling in pancreatic cancer cells, contributing to an acidic microenvironment[368]. Interestingly, NO is involved in metabolic reprogramming by promoting nuclear translocation of PKM2 and macromolecules biosynthesis through accumulation of glycolytic intermediates in ovarian cancer[373]. Epigenetic changes can also be responsible for metabolic reprogramming in CAFs, via induction of HIF1 and the subsequent expression of glycolytic target genes[374]. This is of particular interest because, as we will discuss below, radiation can induce HIF1 expression in cancer cells[375,376].

B Role of hypoxia—Hypoxia is an indisputable player in radiation response[377], due to the direct effects of molecular oxygen in facilitating oxidative damage after ionizing radiation[34] and the hypoxia-induced shift in cell metabolism, resulting in suppression of the oxygen-consuming oxidative phosphorylation while enhancing glycolysis[378]. Under hypoxic conditions, hypoxia-inducible factor (HIF) is activated secondary to both lower oxygen availability[379] and mitochondria-generated ROS[380]. Of note, while HIF-1β is constitutively expressed, the activity of the HIF-1 pathway is largely dependent on the

oxygen-reliant modification and stability of HIF-1α, the main effector of cellular response to hypoxia[381]. Surprisingly, given the importance of hypoxia on radiation sensitivity, studies investigating the effect of ionizing radiation on the HIF1 pathway are sparse, but there is some evidence that ionizing radiation activates the HIF-1 pathway in cancer cells[375,376]. Part of the mechanism of HIF1 activation by ionizing radiation has been attributed to the ROS generated from tumor re-oxygenation after radiation[357]. HIF1 is a positive regulator of a number of glycolytic enzymes, including lactate dehydrogenase A (LDHA) and pyruvate dehydrogenase kinase 1 (PDK1)[162,382]. While PDK1 inhibits pyruvate dehydrogenase (PDH), which forms acetyl-CoA from pyruvate, LDHA generates lactate from pyruvate. Therefore, following HIF1 activation, pyruvate is routed away from the TCA to form lactate. Another important HIF1 target is GLUT1[383], through which HIF1 can regulate glucose import. This could explain in part the enhanced glucose consumption observed in irradiated cancer cells[109,110,357], although activation of HIF1 pathway by ionizing radiation seems to only occur under hypoxic conditions *in vivo*[357] while the radiation-induced increase in glucose consumption is observed in cancer cells propagated under normoxia *in vitro*[109]. Only a few studies have investigated the effect of ionizing radiation on the HIF1 pathway in cancer cells and these are limited to specific cancer cell lines[358,375,376]. It is likely that the pre-irradiation metabolic state of a cancer cell will dictate its metabolic response after radiation. From the limited studies available it seems that ionizing radiation induced metabolic changes may resemble those of hypoxia[109,110,357]. It remains to be determined whether the radiation-induced metabolic changes on cancer cells under hypoxic conditions are different from those of normoxic cells.

Section 4: Metabolic radiosensitizers

A recent review has summarized radiosensitizing drugs with potential metabolic mechanisms[53] and several radiosensitizers associated with rewired glucose metabolism have been documented. Most notably, 2-deoxyglucose (2-DG) has been shown to radiosensitize prostate, pancreatic, and cultured HeLa cells[384,385] via multiple proposed mechanisms, including decreased glutathione levels[384] or autophagy induction via AMPK stimulation[386]. 2-DG treatment in combination with radiation therapy in glioma patients was well tolerated and increased patient survival in a Phase I/II clinical trial[387,388], though the relatively short biological half-life and high drug concentrations involved in such studies may be problematic without development of improved therapeutics with similar mechanisms[389]. Inhibitors of the glucose transporter GLUT1 have also been demonstrated to have potential as radiosensitizers[37,390]. Other metabolic pathways, including amino acid and fatty acid metabolism, have been targeted for radiosensitization. Notably, the conversion of arginine to citrulline by arginine deiminase-polyethylene glycol (ADI-PEG 20) has been shown to increase radiosensitivity via arginine depletion, especially in cells lacking expression of argininosuccinate synthetase[391]. Given the heightened requirement for fatty acid synthesis in irradiated cells, inhibition of fatty acid synthase (FASN) has potential as a radiosensitizer. While several FASN inhibitors exist[392–394] and have radiosensitized diverse cancer types in preclinical studies[281,395–397], none have shown efficacy *in vivo*, likely due to the ability of cancer cells to scavenge fatty acids from the tumor microenvironment after ionizing radiation[397]. This suggests that the efficacy of treatments involving FASN inhibition may be significantly improved by co-targeting of fatty acid scavenging. These studies demonstrate

the potential of therapeutics aimed at targeting metabolites especially significant to cancer metabolism, especially in the context of metabolic alterations following radiation therapy.

Metformin, frequently implicated in several relevant metabolic pathways in cancer, including AMPK, mTOR, MAPK, and PI3K signaling, has been the subject of considerable recent study in combination with chemo- and radiation therapy. Interestingly, a recent Phase II clinical trial of metformin combined with paclitaxel and carboplatin in non-diabetic NSCLC patients[398] found no significant increase in progression-free survival, despite models suggesting metabolic response in the majority of similar tumors[399]. Patients in this study demonstrated increased glucose uptake in the majority of tumors, despite preclinical evidence suggesting that metformin decreases glucose consumption via inhibition of PKM2[400] and demonstrated utility of metformin as a monotherapy both *in vitro* and *in vivo*[401]. Several mechanistic explanations for metformin's inability to increase overall survival in this trial are plausible. Metformin's antineoplastic activity may be more important than its effects on cytotoxicity, or the cytotoxic effects of metformin may be mitigated by altered local immune function after ionizing radiation[402]. Further study of metabolic flux alterations after metformin treatment, especially in *in vivo* models, may help to elucidate the components of the whole-organism response. Such understanding may enable combination therapies directed at the metabolic context of chemotherapy-treated tumors and tumor-infiltrating lymphocytes after radiation.

Since cellular metabolism is a significant consumer of oxygen and local oxygen is required for the cytotoxic effects of radiation, several metabolic radiosensitizers have functioned by decreasing the size of the hypoxic core of *in vivo* tumors[38,39,403]. Radiosensitization brought on by these radiosensitizers in three-dimensional cultures with differential oxygen tension likely stems in large part from an increased capacity for oxygen-mediated DNA damage in newly-oxygenated tissues. Consistent with the importance of redox biology in cancer cell radiosensitivity, pharmacological alteration of cellular redox pools can significantly alter radiosensitivity. For example, decreased glutathione levels, either through inhibition of glutaminase[209,404] or modification of the glutamine-cysteine antiporter xCT[309], have been demonstrated to alter radiosensitivity inversely with glutathione concentration. Similarly, thioredoxin reductase inhibitors radiosensitize via ROS accumulation[405]. While drugs targeting glutaminase have been shown to increase radiosensitivity *in vitro*, *in vivo* application has suffered from either poor potency or poor bioavailability[406]. The glutaminase inhibitor CB-839 increased radiosensitivity in head and neck squamous cell carcinoma xenograft models[407] and has been tested in early phase clinical trials of renal cancer alongside the VEGFR inhibitor cabozantinib[408] (NCT02071862), demonstrating potential for clinical applications of metabolic radiosensitizers aimed at altering metabolism.

Conclusions

In light of the centrality of of radiation therapy to multidisciplinary cancer care and the rapidly expanding body of work surrounding cancer metabolism, it is critical to gain additional insight both into the capacity for radiotherapy to alter cancer cell metabolism and the ways in which metabolism impacts the efficacy of radiotherapy. In this review, we outline methods capable of tracking relevant alterations to cancer cell metabolism

that are compatible with radiobiological studies, including *in vitro* models and metabolism-directed imaging modalities transferrable to clinical studies. These methods informed numerous studies into radiation-induced alterations to signaling pathways and metabolite concentrations in cancer cells, as well as studies investigating alterations to the tumor microenvironment after ionizing radiation. Studies of cancer metabolism before and after ionizing radiation offer significant prognostic and therapeutic potential.

Acknowledgements

This study was supported by National Cancer Institute CA251872 (to E.V) and CA222493 (to A.H.K). The authors report no conflicts of interest.

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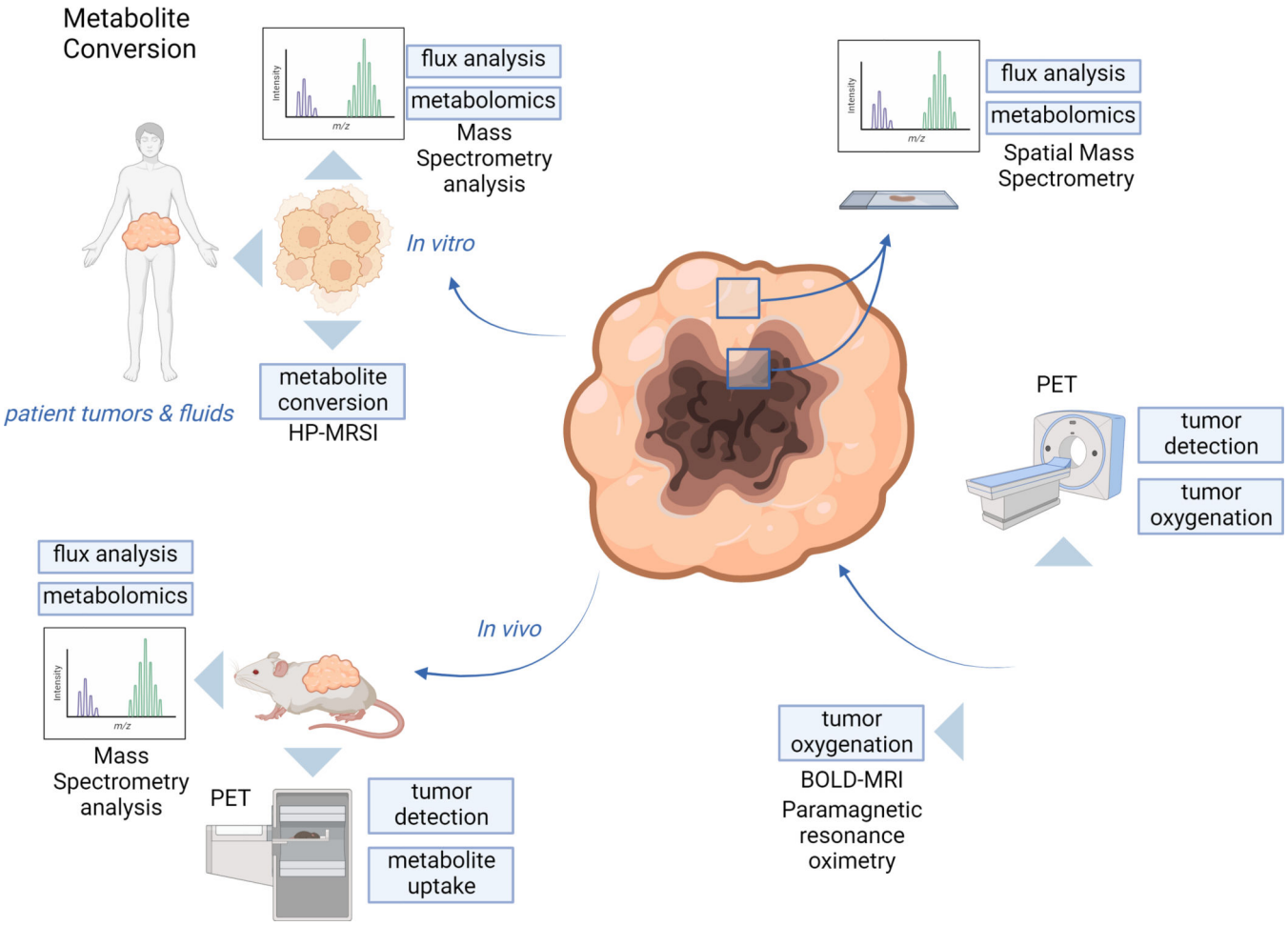

Figure 1. A summary of methods for the study of cancer cell metabolism and its alterations after radiation.

Methods are grouped by their utility *in vivo* or *in vitro*, with suggested applications listed in blue boxes. Created with BioRender.com.

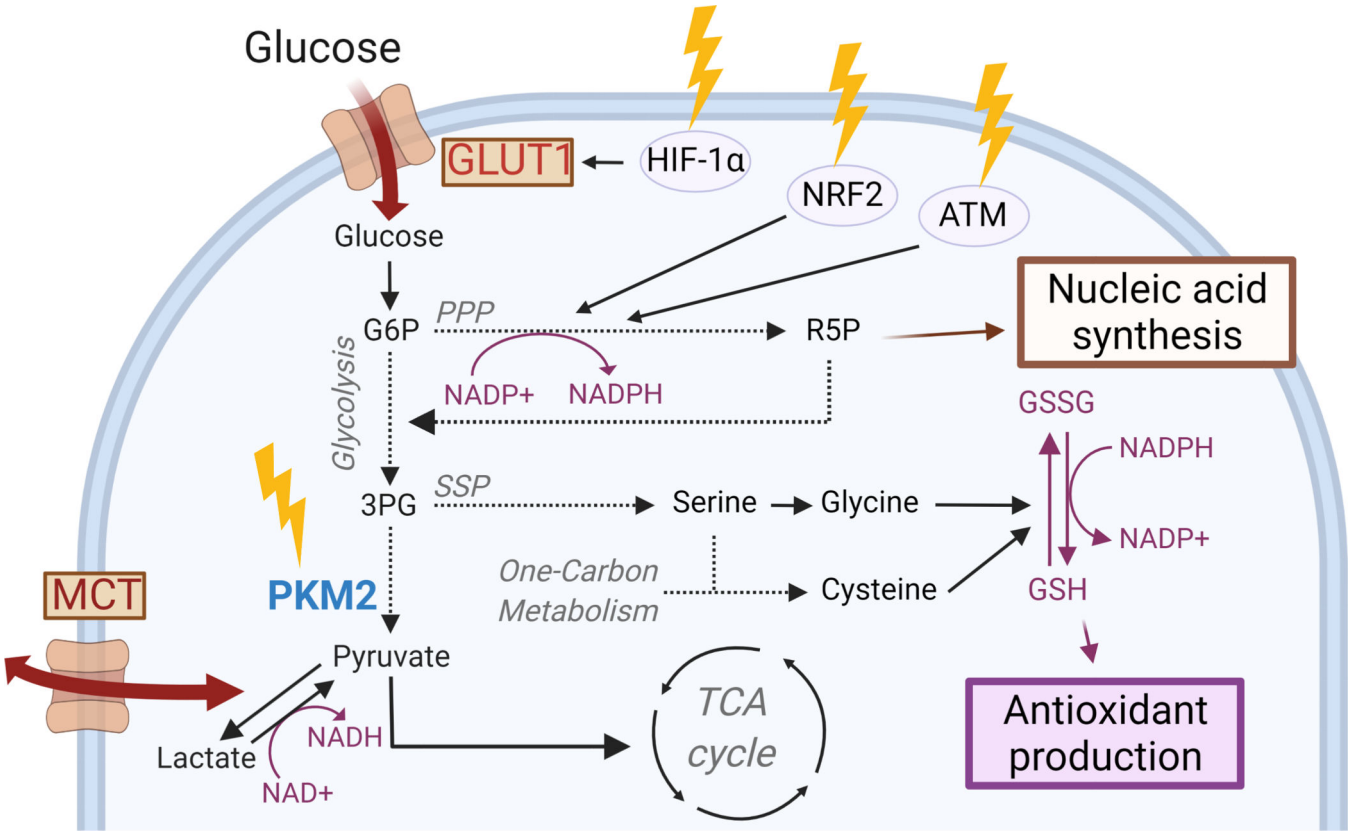

Figure 2. Radiation increases glucose metabolism to enhance antioxidant production and nucleic acid synthesis.

Ionizing radiation (yellow arrows) decreases PKM2 activity (in green) while increasing GLUT1-mediated glucose uptake and MCT-mediated exchange of pyruvate and lactate (in red), providing intermediates for nucleic acid synthesis and antioxidant production. Created with BioRender.com.

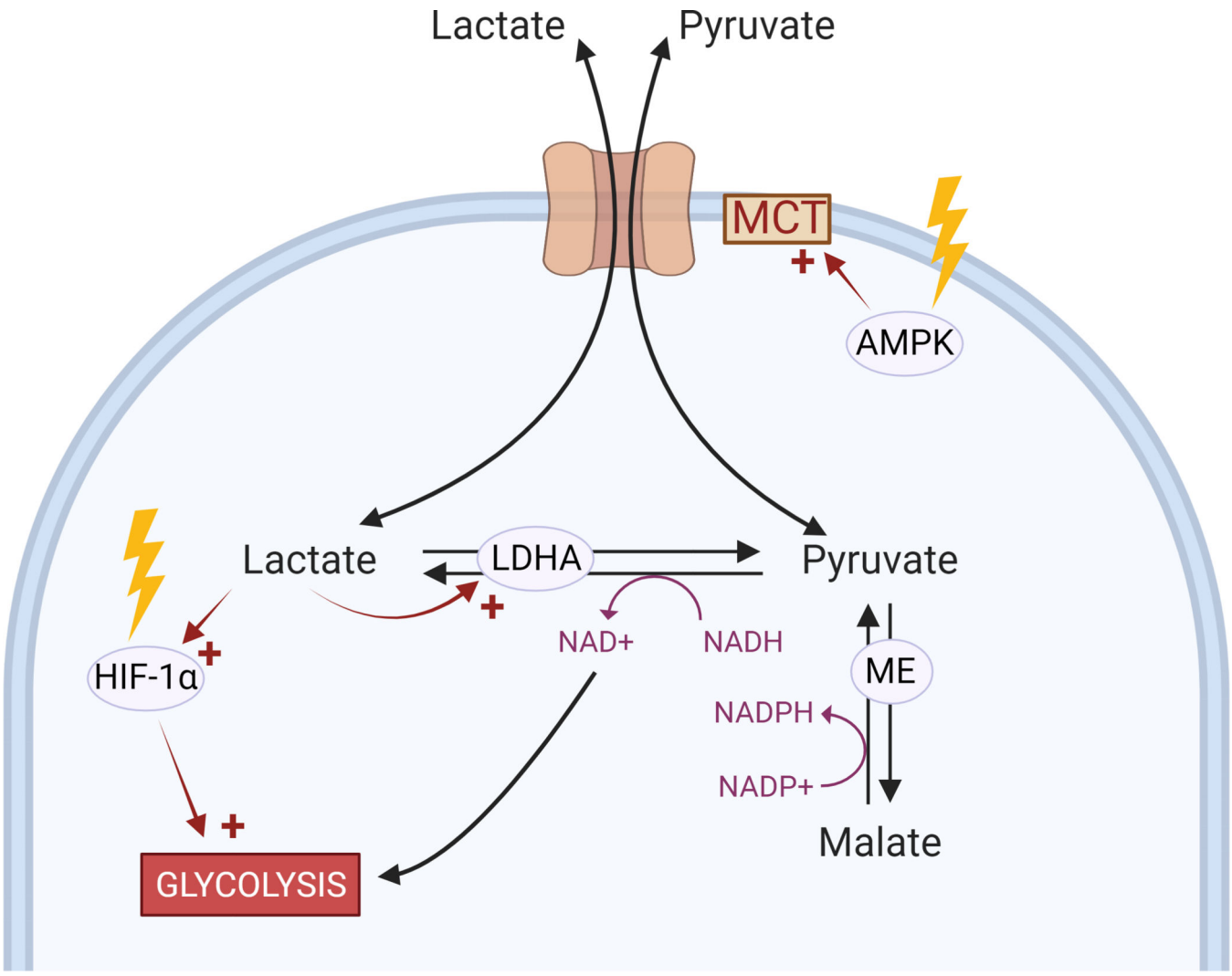

Figure 3. Radiation affects the lactate to pyruvate ratio via MCT transporters.
Ionizing radiation (yellow arrows) activates several pathways (indicated by red arrows) implicated in lactate production and transport. Created with BioRender.com.

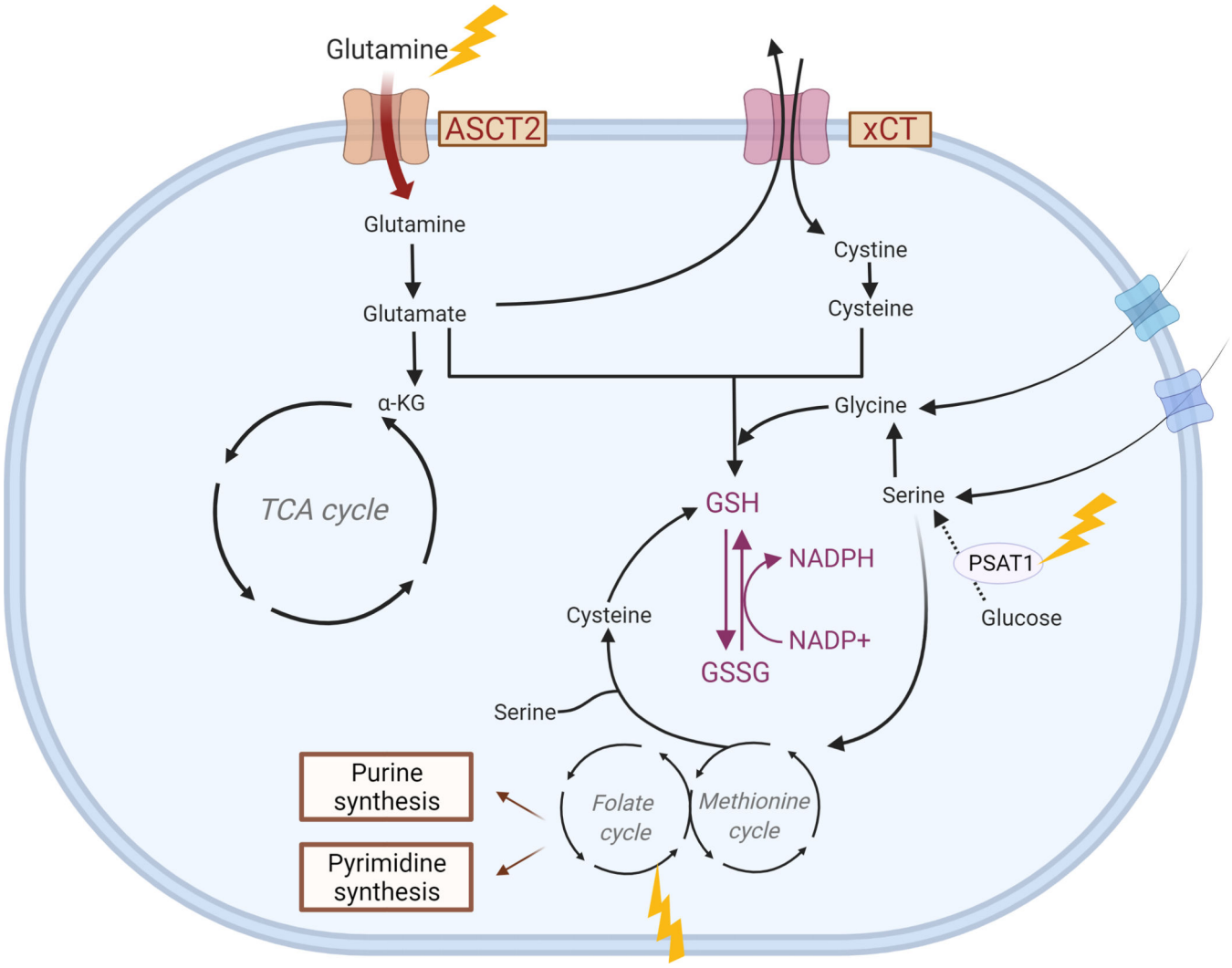

Figure 4. Effects of radiation on amino acids and one-carbon metabolism.

Ionizing radiation (yellow arrows) affects expression of several pathways (in red text) associated with amino acid synthesis and redox metabolism. Other important signaling pathways that provide intermediates for these reactions are also shown. Created with BioRender.com.

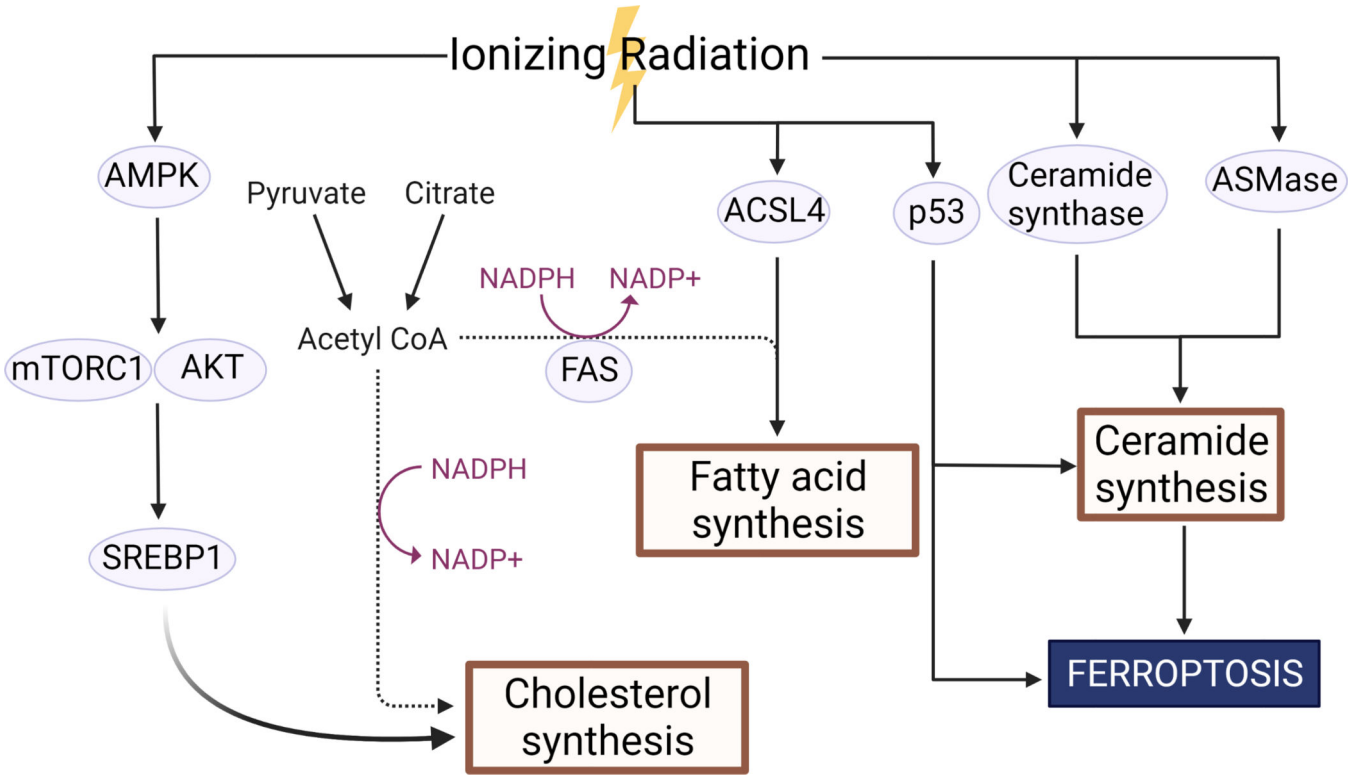

Figure 5. Ionizing radiation affects fatty acid and lipid metabolism.

Ionizing radiation affects several pathways associated with the synthesis of cholesterol, fatty acids, and ceramides. Created with BioRender.com.

Table 1.

A summary of clinical trials involving HP-MRSI.

Status and descriptions of clinical trials were taken from clinicaltrials.gov on 2 August 2021.

Trial ID	Status	Phase	Etiology	Purpose	Metabolites studied
NCT04540107	Active	Phase II	Glioma	Investigate whether changes in levels of HP-pyruvate, lactate, and bicarbonate predict tumor progression in low-grade glioma patients	Pyruvate, lactate, bicarbonate
NCT04258462	Active	Phase II	Renal	Investigate association between HP-Pyruvate-to-lactate conversion and renal tumor histoloy. Predict benign renal tumors versus renal cell carcinoma and grading	Pyruvate, lactate
NCT04565327	Active	Phase II	Pancreatic	Evaluation of early treatment response	Pyruvate, lactate
NCT04019002	Active	Phase I	Glioblastoma	Detection of early response to standard therapy	Pyruvate, lactate
NCT03933670	Active	Phase II	Prostate	Associate intratumoral pyruvate-to-lactate and pyruvate-to-glutamate conversion with Gleason grade	Pyruvate, lactate, glutamate
NCT03739411	Active	Phase I	Glioma	Assess safety of hyperpolarized metabolites, evaluate lactate-to-pyruvate response to standard RT/temozolomide	Pyruvate, lactate
NCT02526368	Active	Early Phase I	Prostate	Determine metabolite levels that accurately detect Gleason 4 component cancer	Pyruvate, lactate, urea
NCT04589624	Active	Phase I	Thryoid	Assess early metabolic changes in response to radiation and/or systemic therapy	Pyruvate, lactate
NCT04346225	Active	Phase II	Prostate	Assess rates of conversion and intratumoral heterogeneity of metabolites, determine if changes from baseline conversion predict clinical outcomes	Pyruvate, lactate, glutamate
NCT04044872	Active	Phase IV	Cardiotoxicity in breast or thoracic tumors	Determine if radiation-induced cardiac injury disrupts mitochondrial metabolism, determine prognostic value of mitochondrial pyruvate flux on cardiotoxicity	Pyruvate, lactate, bicarbonate, glutamate
NCT03830151	Active	Phase I	Glioma	Assess correlation between pyruvate-to-lactate conversion and Ki-67 levels in tumor	Pyruvate, lactate
NCT03581500	Active	Phase II	Prostate	Assess sensitivity and specificity of pre-therapy HP-MRSI for detecting localized prostate cancer	Pyruvate, lactate
NCT02421380	Active	Phase II	Sarcoma, Prostate, Breast, Brain	Test reproducibility of HP-MRSI in vivo dynamics	Pyruvate, lactate
NCT04656431	Closed due to accrual	Phase I	Primary central nervous system lymphoma	Investigate connections between pre and post-treatment lactate signal and NF-kB	Pyruvate, lactate
NCT03685175	Active	Phase I	Cardiotoxicity in breast tumors	Test correlation between doxorubicin-induced cardiotoxicity and aerobic cardiac metabolism	Pyruvate
NCT03759704	Active	Phase 0	Musculoskeletal sarcoma	Determine if pyruvate-to-lactate conversion correlates with malignancy or tumor grade	Pyruvate, lactate, bicarbonate, glutamate
NCT04286386	Active	N/A	Prostate	Assess reproducibility of pyruvate-to-lactate conversion, assess correlation between conversion and tumor grade	Pyruvate, lactate

Trial ID	Status	Phase	Etiology	Purpose	Metabolites studied
NCT03067467	Active	N/A	Glioma, meningioma, brain metastases	Determine whether ratio of metabolites indexes balance between glycolytic and mitochondrial metabolism	Pyruvate, lactate, bicarbonate
NCT01229618	Completed	Phase I	Prostate	Determine time course and signal-to-noise ratio of hyperpolarized pyruvate in cancer and benign tissue	Pyruvate, lactate
NCT02450201	Terminated	Early Phase I	Prostate Cancer	Evaluate reproducibility of pyruvate signal in patients treated with androgen depletion	Pyruvate, lactate
NCT02647983	Withdrawn (Lack of participants)	Phase I	Prostatic Neoplasms	Ability to predict Gleason grade using metabolic profile	Pyruvate, lactate, alanine, bicarbonate
NCT02844647	Recruiting	Phase I	Prostate Cancer	Evaluate pyruvate conversion in bone-metastatic castration-resistant prostate cancer	Pyruvate, lactate, bicarbonate
NCT02911467	Terminated	Phase I	Prostate Cancer	Investigate baseline intratumoral peak lactate-to-pyruvate ratio in androgen signaling inhibitor refractory and responsive tumors	Pyruvate, lactate
NCT02913131	Terminated	Phase I/II	Prostate Cancer	Measure percent change from baseline lactate-to-pyruvate ratio in patients treated with PI3K/mTOR inhibitors	Pyruvate, lactate
NCT02947373	Completed	Phase I	Pediatric Brain Tumors	Categorize adverse events, describe imaging quality in HP-MRSI of pediatric tumors	Pyruvate, lactate
NCT03121989	Recruiting	Phase I	Breast Cancer	Determine diagnostic accuracy of 13C pyruvate, distinguish tumor necroses from viable tumor cells in patients given neoadjuvant chemotherapy	Pyruvate, lactate
NCT03129776	Recruiting	Phase I	Uterine Cervical Neoplasms	Correlate MRSI and 18FDG-PET images in cervical cancer	Pyruvate, lactate, fluorodeoxyglucose
NCT03279250	Completed	Phase II	Prostate	Investigate proportion of patients with pathological complete response via HP-pyruvate imaging	Pyruvate
NCT03324360	Recruiting	Phase I	Brain Metastases	Characterize metabolic features of intracranial metastasis, predict tumor aggressiveness	Pyruvate
NCT03526809	Unknown status	N/A	Ovarian, breast, prostate, pancreatic, hepatic, renal, brain tumors	Correlate hyperpolarization images with tumor histology, biochemistry and genetics. Detect change in metabolism after therapy. Determine correlation between HP and FDG-PET images	Pyruvate, fluorodeoxyglucose
NCT03565367	Suspended (Logistics)	Phase I	Central nervous system, including metastases	Assess frequency and sensitivity of lactate and bicarbonate signal in malignant brain tumors after HP-pyruvate injection	Pyruvate, lactate, bicarbonate
NCT03687645	Unknown status	N/A	Prostate, renal, breast cancer, lymphoma	Determine rate of pyruvate-to-lactate conversion, correlation of rate with genetic markers and to predict response	Pyruvate, lactate
NCT03849963	Recruiting	Early Phase I	Brain Cancer	Evaluate sensitivity and feasibility of HP-MRSI for imaging oxidative metabolism and neurotransmitter synthesis	1–13C and 2–13C pyruvate, bicarbonate, lactate, glutamate
NCT04698564	Not yet recruiting	Phase II	Prostate Cancer	Determine diagnostic accuracy and utility of 13C pyruvate, compare metabolic MRI to standard MRI imaging	1–13C, 2–13C, 1,2–13C pyruvate “and its metabolites”
NCT04772456	Recruiting	Phase I	Glioma	Determine utility of metabolic MRI over standard MRI in diagnosis of glioma	1–13C, 2–13C, 1,2–13C pyruvate “and its metabolites”