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Review Article

Imaging radiation response in tumor and normal tissue

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Abstract: Although X-ray computed tomography (CT) and magnetic resonance imaging (MRI) are the primary imaging modalities used in the clinic to monitor tumor response to radiation therapy, multi-modal molecular imaging may facilitate improved early and specific evaluation of this process. Fast and accurate imaging that can provide both quantitative and biological information is necessary to monitor treatment and ultimately to develop individualized treatment options for patients. A combination of molecular and anatomic information will allow for deeper insight into the mechanisms of tumor response, which will lead to more effective radiation treatments as well as improved anti-cancer drugs. Much progress has been made in nuclear medicine imaging probes and MRI techniques to achieve increased accuracy and the evaluation of relevant biomarkers of radiation response. This review will emphasize promising molecular imaging techniques that monitor various biological processes following radiotherapy, including metabolism, hypoxia, cell proliferation, and angiogenesis.

Keywords: Radiation therapy, molecular imaging, tumor microenvironment, tumor response, normal tissue response

Introduction

The goal of molecular imaging is to gain insight into biological functions and mechanisms by visualizing changes in physiological and cellular properties. Observing tissue response to cancer therapy may lead to the discovery of improved cancer treatments that may be tailored to an individual patient's needs. Conventional imaging techniques, including ultrasound (US) and X-ray computed tomography (CT), have been successful in providing an accurate visualization of anatomic features of tumors. Recent developments in molecular imaging probes and techniques, including biologically active PET tracers and specialized magnetic resonance imaging (MRI) pulse sequences, allow for the visualization of cellular properties and microenvironmental changes.

Radiotherapy has a long history in cancer treatment, stemming from Röntgen's discovery of the X-ray in 1895 [1]. Radiation therapy is used in the treatment of a wide range of cancers and is prescribed for over 50% of all cancer patients [2]. Since DNA damage is the mechanism by which ionizing radiation kills cells [3], exposure of normal tissue to radiation commonly limits

the maximum deliverable dose and hence efficacy of this therapy through the induction of treatment-related toxicity [4]. The current clinical standard for evaluating response to treatment is to determine tumor size reduction on CT using a unidimensional measurement, termed Response Evaluation Criteria in Solid Tumors (RECIST) [5]. The RECIST criteria focus solely on tumor size reduction, which provides a limited view of treatment efficacy [6] and does not necessarily correlate with patient survival [7-9]. In addition to CT, other non-invasive imaging methods, including US and MRI, have been used to determine the response of tumors to radiation therapy by monitoring reduction in the size of the primary tumor after treatment [10]. Crucial information about the location of tumors and injury to surrounding areas can also be gained from these modalities. However, they are insensitive to the underlying biological changes that drive tissue response to radiation. Combining multiple modalities may improve outcomes not only by determining changes in tumor size but also by characterizing biological processes.

New clinical criteria have been proposed for assessing tumor volume changes that include biological information gained from PET imaging

Imaging radiation response

Table 1. Methods for visualizing radiation response based on biological processes

Method	Tracer/Technique	Biological process	References
PET	FDG	Metabolism	[11, 13-21]
	FMISO	Hypoxia	[36, 37, 39, 41-47]
	FAZA	Hypoxia	[40, 48-50]
	EF5	Hypoxia	[38]
	⁶⁰ Cu-ATSM	Hypoxia	[51, 52]
	FLT	Proliferation	[63-65]
	¹⁸ F-ISO	Proliferation	[69, 70]
	¹⁸ F-ML-10	Apoptosis	[90, 91]
	¹⁸ F-ICMT-11	Apoptosis	[84-89]
	¹⁸ F-galacto-RGD	Angiogenesis	[115-119]
MRI	¹ H-MRSI	Metabolism	[22-27]
	¹³ C-MRSI	Metabolism	[28-31]
	BOLD	Hypoxia	[58]
	APT	Necrosis	[94, 95]
	DTI	Necrosis	[96, 97]
	DCE	Angiogenesis	[99, 102-106]
US	DCE	Angiogenesis	[110-114]

of glucose metabolism, called PET Response Criteria in Solid Tumors (PERCIST) [5]. The inclusion of PET imaging overcomes some limitations of anatomical imaging by allowing the assessment of early responses to treatment [11]. Whereas anatomical and structural changes have classically been the focus of tumor response to therapy, emerging imaging techniques provide crucial biological information at the molecular level. Molecular imaging provides a means to detect and predict biological responses to therapy (Table 1), allowing for the development of personalized treatment options. Being able to visualize radiation response in both tumors and normal tissues may aid in the determination of treatment efficacy (Table 2). Given the complex nature of the tumor microenvironment and its potential contribution to therapy response, molecular imaging can provide a means to visualize individual components of the microenvironment, including changes in the vasculature, immune response, and stroma [12]. This review highlights the current state of post-therapy biological response imaging and examines future directions for research in this area.

Metabolism

Following radiation therapy, tumor cell metabolic activity changes due to decreases in cell

survival and proliferation. Imaging these metabolic changes is one avenue for measuring tumor response to treatment (Figure 1A). The most common radiotracer used in PET imaging is ¹⁸F-fluoro-2-deoxyglucose (FDG), an analog of glucose incorporating a positron-emitting isotope of fluorine (¹⁸F). Because glucose uptake is increased in most cancers, FDG-PET has been widely used to detect elevated glucose metabolism in both primary and metastatic tumors and to determine tumor response to therapy [11]. For example, FDG-PET has been used to monitor the response of locally advanced cervical cancer to chemoradiation [13-16]. FDG is also taken up by macrophages and inflammatory lesions [17]. This characteristic has been examined in patients with non-small cell lung cancer (NSCLC) to determine the response of normal lung and pleura to radiation by evaluating inflammatory changes based

on FDG uptake [18]. FDG uptake in normal tissues within the radiation field was evaluated independently of tumor response using a qualitative scoring system (Figure 1B), and it was found that inflammatory changes in normal tissue stemming from radiation pneumonitis and pleuritis were positively correlated with the tumor metabolic response due to a potential relationship between the radiosensitivities of the tumor and normal tissue.

Despite its wide use in monitoring response to therapy [19-21], FDG lacks tumor specificity. As such, other means of monitoring tumor metabolism are being explored, including magnetic resonance spectroscopic imaging (MRSI), which allows for non-invasive, simultaneous acquisition of biochemical and anatomical properties [22]. Proton (¹H)-MRSI has been applied toward imaging gliomas in order to plan radiation treatments by distinguishing the abnormalities in metabolic properties of these tumors as compared to normal cerebral tissues [23-25]. ¹H-MRSI has also been shown to be effective in monitoring radiation response in brain tumors [22]. Characteristic changes in glioma-associated metabolites, including choline, creatine, lactate, and lipids, have been shown with ¹H-MRSI after radiotherapy, which could be indicative of patient response to treatment [26, 27]. Similar to PET imaging which

Imaging radiation response

Table 2. Methods for determining normal tissue response following radiotherapy

Modality	Tissue	Readout	References
FDG-PET	Normal lung and pleura	Normal tissue uptake within radiation field	[18]
MRSI	Cerebral tissues	Normal metabolites	[23-25]
FLT-PET	Bone marrow	Hematopoietic cell uptake	[64]
DCE-MRI	White matter	K^{trans}	[106]

requires the injection of an exogenous probe, ^{13}C -MRSI uses hyperpolarized agents to increase the detection sensitivity of injected ^{13}C labeled molecules by greater than 10,000 fold [28]. ^{13}C -labeled molecules such as $[1-^{13}\text{C}]$ -pyruvate [29], $[1, 4-^{13}\text{C}]$ -fumarate [30], and $[5-^{13}\text{C}-4-2\text{H}_2]$ -glutamine [31] can be used for imaging metabolism, cell death, and cell proliferation, respectively. The first clinical trial using this technique detected elevated levels of ^{13}C -pyruvate in known areas of prostate cancer, showing its feasibility in examining tumor therapeutic response (**Figure 1C**) [32]. Monitoring changes in metabolic processes following radiation therapy is promising for detecting early treatment response in patients, however it is limited to observation of molecules that can be delivered and metabolized within the lifetime of hyperpolarization, typically 1-2 minutes [32].

Hypoxia

It has been established that hypoxia, or low oxygen, is an important factor in tumor response and resistance to therapy in many cancers [33]. Hypoxia influences tumor cell invasiveness, energy metabolism, gene expression, and metastasis [34]. Visualizing hypoxic areas within tumors as well as changes in oxygen levels after treatment may allow optimized treatment selection, planning, and adjustment (**Figure 2A**). For example, hypoxia-specific radiotracers are being investigated to non-invasively monitor hypoxic tumor populations following treatment [35]. Due to their reduction under low oxygen tension and intracellular binding in hypoxic cells [34, 35], 2'-nitroimidazole-containing compounds, such as ^{18}F -fluoromisonidazole (FMISO), ^{18}F -fluoroazomycin arabinoside (FAZA), and 2-(2-nitro-1*H*-imidazol-1-yl)-*N*-(2, 2, 3, 3, 3- ^{18}F -pentafluoropropyl)-acetamide (EF5), are being used to quantify hypoxia based on tumor-to-plasma or tumor-to-muscle uptake ratios (T/M) [36-38]. These hypoxia markers exhibit similar signal-to-noise ratios (SNR): in head and

neck cancer, for example, T/M ratios range from 1.1-3.2 for EF5 [38] to 1.1-3.0 for FMISO [39], and 1.6-2.4 for FAZA [40]. The widely-used PET tracer FMISO has been utilized to image and quantify hypoxic areas in many types of tumors, including lung, head and neck, and prostate [41-43]. A clinical trial of head and neck cancer patients used FMISO imaging before and after radiotherapy to define hypoxia kinetics over the course of treatment [44]. Another clinical trial conducted in patients with head and neck, gastrointestinal, lung, and uterine cancers successfully showed the feasibility of using FMISO to monitor the reoxygenation of hypoxic areas following radiotherapy [45]. The use of FMISO as a universal hypoxia marker is limited by contradictory results with respect to the correlation of FMISO uptake and hypoxia markers in various tumor types [46, 47], so FAZA is being evaluated as an additional tracer for detecting hypoxia [48]. FAZA has been used to image hypoxia in lung, head and neck, blood, and brain tumors [49]. In NSCLC patients, FAZA was used to monitor changes in hypoxia following chemoradiation. Despite limitations in the number of patients in the study, results indicated that therapy likely decreased tumor cell survival and improved reoxygenation of intratumoral regions [50].

PET imaging with copper-60 diacetyl-bis (*N* 4-methylthiosemicarbazone) (^{60}Cu -ATSM) is also being evaluated to improve upon the lower SNR of the previously mentioned hypoxia markers. T/M ratios between 3.0-3.5 have been defined in patients with NSCLC and cervical cancer [51, 52]. In both cases, it was found that uptake of ^{60}Cu -ATSM was variable based on the extent of hypoxia within tumors and that a decrease in T/M ratios correlated to patients who had responded to therapy. However, this technique suffers from time and tumor dependent variation in uptake and retention, which limits its use as a general hypoxia marker [53, 54].

Imaging radiation response

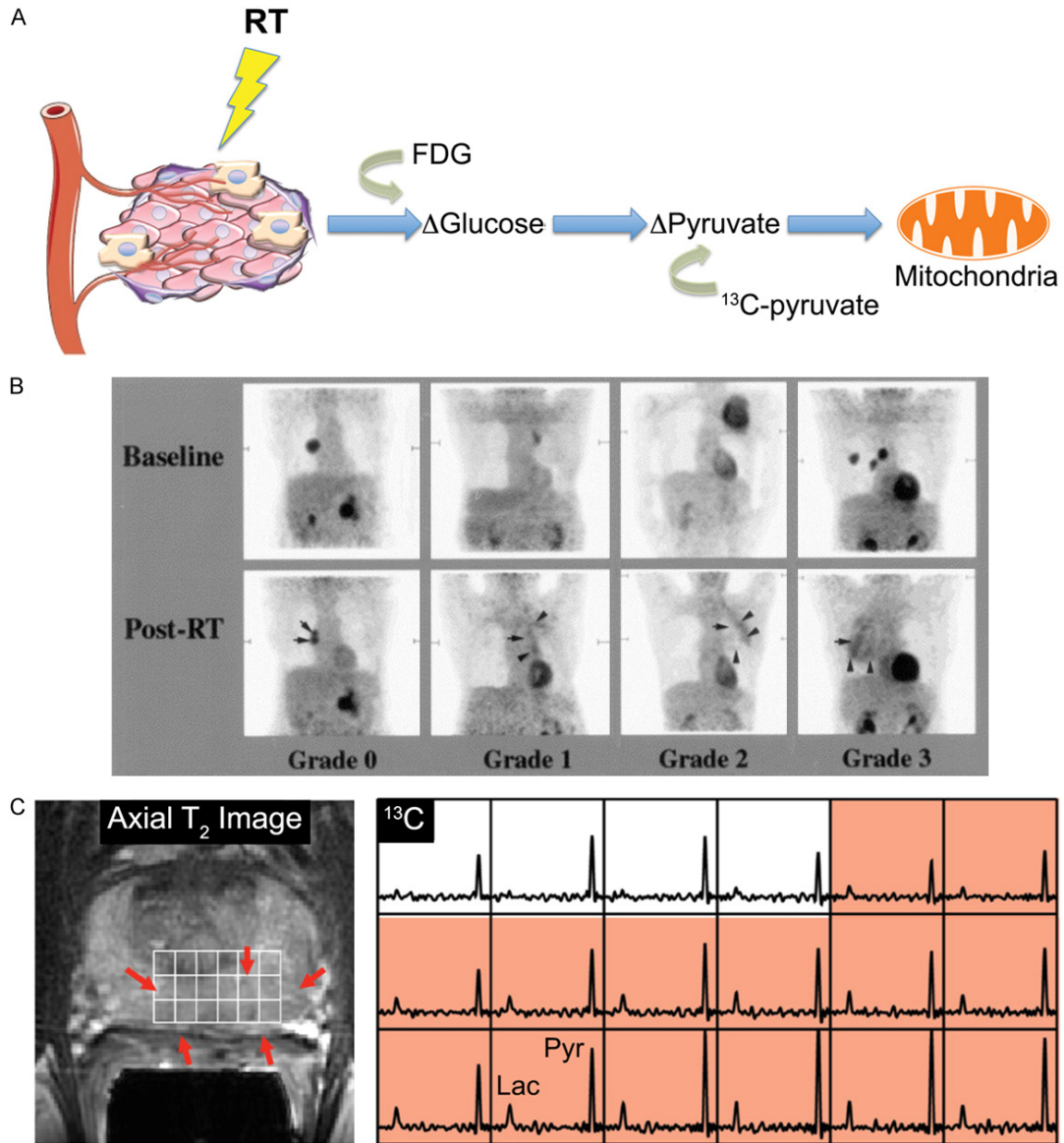


Figure 1. Imaging metabolism. A. The complex tumor microenvironment, consisting of tumor cells, vasculature, stromal cells, and immune cells, plays a critical role in response to therapy. Techniques such as FDG-PET that use glucose analogs to observe cellular uptake or ^1H or ^{13}C MRI that monitor metabolites can evaluate metabolic changes in the tumor microenvironment following radiotherapy (RT). B. FDG-PET scans performed in 4 different patients with NSCLC before (Baseline) and 6-12 weeks after radiotherapy (Post-RT) are shown. Horizontal arrows highlight the original tumor site, oblique arrows designate a new tumor site, oblique arrowheads show pleural reaction, and vertical arrowheads indicate parenchymal lung changes. A qualitative scoring system was devised to evaluate normal tissue FDG uptake for the determination of radiation-induced changes in inflammation. Grade 0 shows no normal tissue abnormalities within the treatment volume. Grade 1 denotes activity in the pleural reflections and soft tissues without parenchymal lung changes. Grade 2 indicates increased parenchymal uptake of equal or lower intensity than normal soft tissues while Grade 3 shows uptake in the right lung parenchyma of higher intensity than surrounding normal soft tissues. Reprinted with permission from [18]. C. ^{13}C -MRSI study in prostate cancer patient who received 0.43 mL/kg of hyperpolarized [$1\text{-}^{13}\text{C}$]pyruvate. T_2 -weighted image through the malignant region (left) with corresponding ^{13}C -MRSI spectral array (right). Pink shading represents tumor. Lac = lactate; Pyr = pyruvate. Adapted from [32].

Imaging radiation response

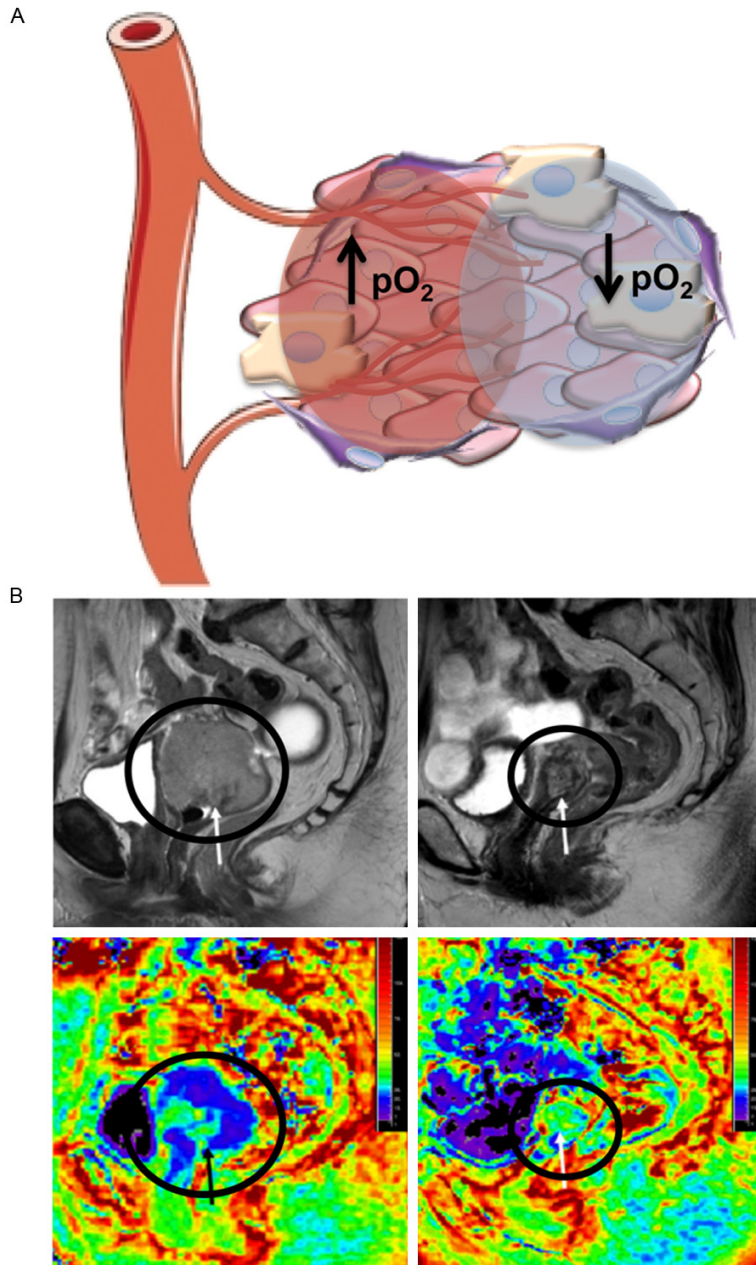


Figure 2. Hypoxia imaging. A. The tumor microenvironment exhibits heterogeneous levels of oxygenation, which can vary based on proximity to the vasculature: higher oxygen concentrations near vessels (red circle) and lower concentrations at greater distances (blue circle). Areas of hypoxia are resistant to radiotherapy. PET and MRI methods to image hypoxia levels before and after treatment are being explored. B. T₂w and BOLD R₂* MRI images of a patient with advanced cervical cancer undergoing concurrent chemoradiotherapy (left panels, before therapy; right panels, 1 month after therapy). T₂-weighted imaging (top panels) shows the size decrease of a cervical tumor (arrows, circles) following treatment. Color-coded R₂* maps (bottom panels) demonstrate increases in R₂* values post-therapy. Reprinted with permission from [58].

As an alternative to using PET, blood oxygenation level-dependent (BOLD) MRI is being

used to assess tumor hypoxia in patients [55, 56]. BOLD MRI utilizes paramagnetic deoxyhemoglobin as an endogenous hypoxia marker, as the deoxyhemoglobin blood concentration alters the transverse relaxation rate R_2^* ($1/T_2^*$) and can indicate the oxygenation status of tissues adjacent to perfused microvessels [57]. In a clinical study of cervical cancer patients pre- and post-chemoradiation therapy, it was found that R_2^* and consequently tumor hypoxia increased following treatment, potentially indicating therapeutic efficacy through decreases in vascular permeability and blood flow (**Figure 2B**) [58]. In addition, no changes in the normal uterine myometrium before and after treatment were detected. Although there are limitations in correlating BOLD signal intensity to absolute pO_2 levels in tumors [59], recent pre-clinical work in rat prostate tumor models has combined T₂*-weighted BOLD and T₁-weighted tissue oxygen level-dependent MRI to provide complementary evaluations of tumor oxygenation to better evaluate radiation response [60], which should be further validated in clinical studies.

Cell proliferation

Monitoring cell proliferation is an additional method of determining the success of cancer therapies: a decrease in proliferation can be attributed to the killing of rapidly proliferating cancer cells (**Figure 3A**). ¹⁸F-fluoro-3'-deoxythymidine (FLT), a thymidine analog, is being used to detect cell

proliferation kinetics through phosphorylation by thymidine kinase 1, an enzyme involved in

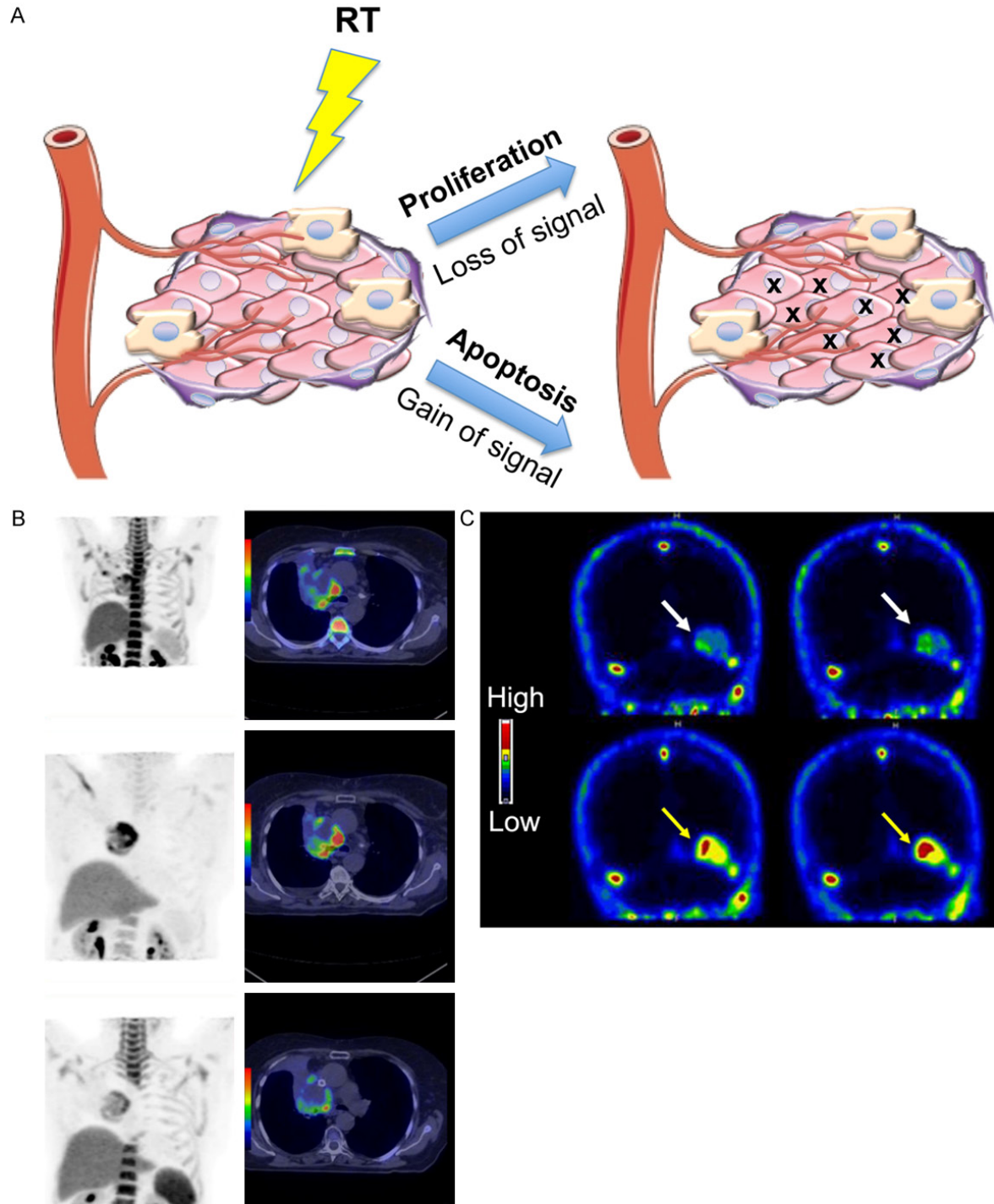
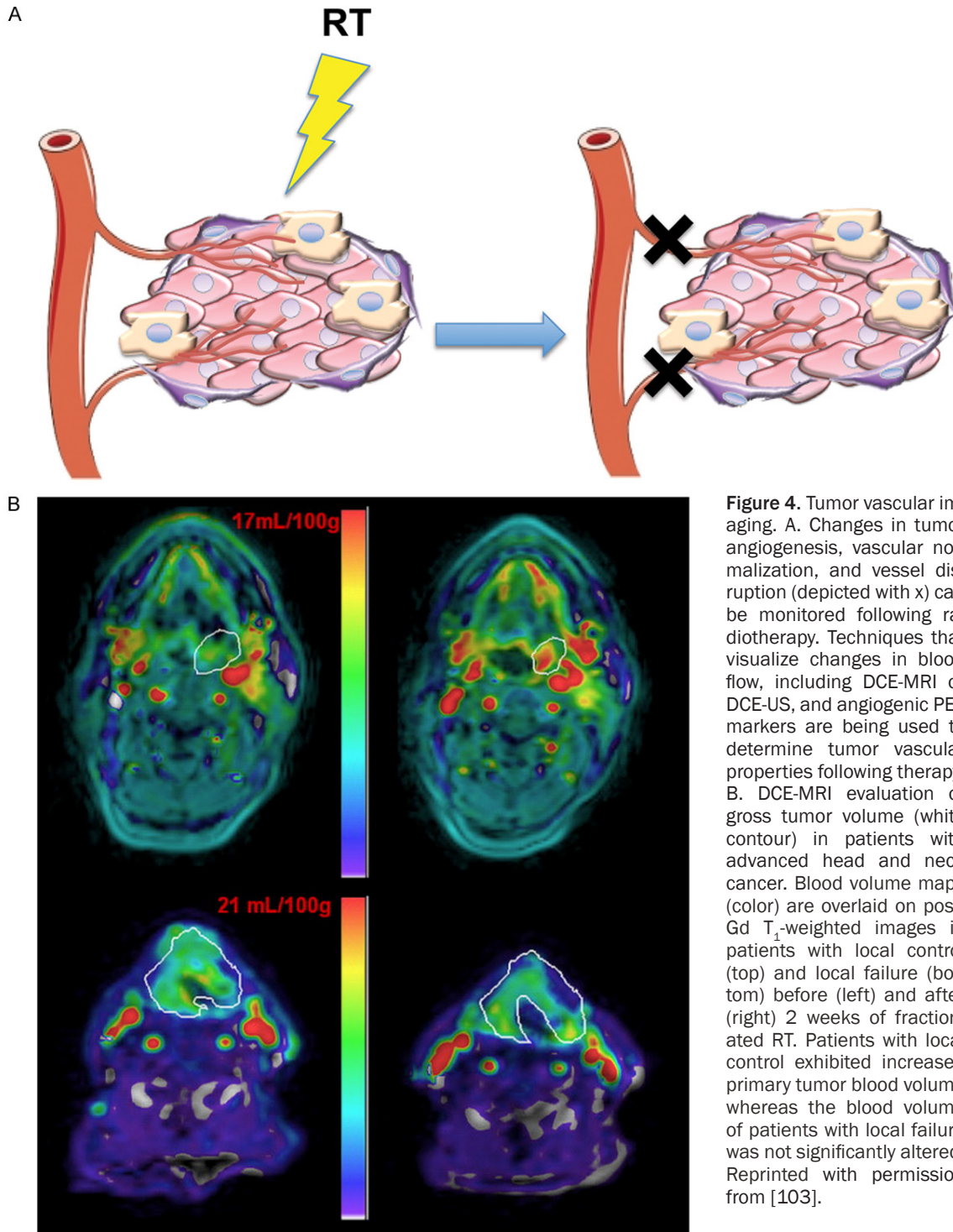


Figure 3. Cell proliferation and apoptosis imaging. A. Radiotherapy induces cell death in the tumor microenvironment, which can be monitored by PET either through the loss of cell proliferation or the increase in apoptosis. Tracers such as FLT and ^{18}F -ISO can be used to visualize decreases in cell proliferation while ^{18}F -ICMT-11 and ^{18}F -ML-10 have been used in the clinic to monitor apoptosis. B. FLT uptake in patients with NSCLC. Coronal ^{18}F -FLT PET (right panels) and transverse ^{18}F -FLT PET/CT (left panels) show baseline (top), 2 week (middle), and 4 week (bottom) response during chemo-RT. A reduction in proliferation was shown within 4 weeks of treatment. Adapted from [65]. C. Visualization of the effect of whole brain RT in two patients with brain metastases. White arrows show baseline ^{18}F -ML-10 uptake and yellow arrows indicate response after 10 fractions of RT. Uptake increased following RT, indicating induced apoptosis. Adapted from [90].

Imaging radiation response



the pyrimidine salvage pathway of DNA synthesis that has 3-4 times higher activity in malignant cells compared to benign cells [61, 62]. Recent studies have evaluated the use of FLT-PET to image radiation response in patients with head and neck cancer or NSCLC over the course of chemo-radiotherapy regimens [63-

65]. In patients with head and neck cancer, FLT uptake in tumors significantly decreased in the first 4 weeks of therapy but remained the same in the surrounding normal tissues [63]. Patients with NSCLC exhibited heterogeneous patterns of tracer uptake and proliferation within individual tumors throughout the study, which

Imaging radiation response

could be used to alter treatments based on individual responses [64]. A phase II trial confirmed that FLT-PET can be used to monitor early changes in cell proliferation as a response therapy in NSCLC patients (**Figure 3B**) [65]. The group also examined the proliferative response in the surrounding normal bone marrow [64]. A reduction of tracer uptake was observed in all cases, suggesting a decrease in the number of proliferating bone marrow cells. These findings illustrate the potential of monitoring cell proliferation to determine the extent of normal tissue damage after treatment.

Assessing proliferation based solely on FLT, or any thymidine analog uptake, is not feasible because these molecules accumulate as a function of the thymidine salvage pathway but do not necessarily reflect proliferation through the *de novo* thymidine pathway. They will therefore underestimate proliferation due to their inability to distinguish between proliferative cells in the G1, G2, and M phases and quiescent cells [66, 67]. Other tracers are being developed that can determine proliferative status more accurately, including ligands for the sigma-2-receptor, a validated cell proliferation biomarker [68]. One such proliferation tracer, N-(4-(6, 7-dimethoxy-3, 4-dihydroisoquinolin-2(1H-yl)butyl)-2-(2-¹⁸F-fluoroethoxy)-5-methylbenzamide (¹⁸F-ISO-1), was tested in a preliminary clinical trial for evaluation of its biodistribution and dosimetry, and it was found that the PET results correlated to Ki67 expression, a well-established marker of proliferation, in tumor biopsies [69]. Although ¹⁸F-ISO-1 has been used to monitor response following chemotherapy in mouse mammary tumors [70], more studies are needed to assess how changes in uptake of this agent may report on response following radiotherapy in patients.

Apoptosis

The ability to evade apoptosis, or programmed cell death, is a defining characteristic of cancer cells [71]. The link between apoptosis and radiation response has been studied extensively but remains a controversial topic. By evaluating the proportion of cells undergoing apoptosis following irradiation, some studies suggest that apoptosis may play a significant role in the response of tumors to radiotherapy [72, 73]. However, studies by Brown and Attardi conclud-

ed that while there is evidence of apoptosis following radiation treatment, cell death through mitotic catastrophe is the predominant mode of cell death [74]. Apoptosis nonetheless continues to be a focus of radiation research; recent studies have reinforced the observation that apoptosis is induced by radiation in breast cancer while noting an inverse correlation between apoptosis and DNA damage [75]. Huang and colleagues have also demonstrated that caspase-3 may regulate other secreted factors that can stimulate the proliferation of neighboring cells after the induction of apoptosis by radiation [76]. Taken together, these observations indicate that apoptosis occurs in a subset of tumor cells following radiotherapy and monitoring tumor cell apoptosis may be a valuable method of determining tumor response to treatment [77-82].

Unlike the previously mentioned PET techniques targeting cell proliferation, metabolism, and hypoxia, imaging apoptosis after therapy would provide a positive contrast (an increase in signal) following a successful therapy (**Figure 3A**). Because activation of caspase-3 drives the degradation of DNA into fragments during apoptosis, it is widely used as a biomarker for apoptosis [83]. A radiolabeled isatin sulfonamide, ¹⁸F-(S)-1-((1-(2-fluoroethyl)-1H-[1, 2, 3]-triazol-4-yl) methyl)-5-(2(2, 4-difluorophenoxy-methyl)- pyrrolidine-1-sulfonyl) isatin (¹⁸F-ICMT-11), has been identified as a caspase-3-specific PET tracer [84]. In pre-clinical models, ¹⁸F-ICMT-11 had increased tumor retention in murine lymphoma xenografts, which corresponded to drug-induced apoptosis and indicated early response to treatment [85, 86]. The tracer is currently being tested in clinical trials and has been shown to be safe with a favorable dosimetry profile in healthy subjects [87]. Further studies regarding the specificity of the tracer *in vivo* need to be done given the potential for non-specific binding of the probe to other nucleophilic cysteine proteases, which could limit its use in the clinic [88, 89]. Another PET tracer, 2-(5-fluoro-pentyl)-2-methyl-malonic acid (¹⁸F-ML-10), has been used to visualize apoptosis of brain metastatic cells in patients following whole-brain radiation therapy (**Figure 3C**) [90]. ¹⁸F-ML-10 was shown to have high signal-to-background ratios (between 1.75 to 20.45) in tumors as compared to healthy brain tissue and a statistically significant correlation

Imaging radiation response

to response as evaluated by MRI after radiotherapy. This apoptotic tracer also facilitated early detection of response to therapy. Another advantage of this tracer is the ability to distinguish between apoptotic and necrotic cells through the loss of signal upon cell membrane rupture [91].

Necrosis

Differentiating between necrosis and recurrent tumors after radiation has been a long-standing challenge in the treatment of brain tumors [92, 93]. New MRI techniques have emerged that exploit inherent biological properties, including amide proton transfer (APT) MRI, that can detect amide protons of low concentration proteins [94]. Since malignant gliomas have higher protein and peptide content than normal tissue, differentiation of glioma and radiation necrosis with molecular APT-MRI has been shown to be feasible in a pre-clinical model [95]. However, clinical validation is necessary to optimize these techniques for use in patients. In addition, diffusion tensor imaging (DTI) using MRI can measure 3D diffusion of water *in vivo*. This technique has been used to create apparent diffusion coefficient (ADC) maps in order to discriminate necrotic areas from glioma recurrence following radiotherapy [96]. DTI-MRI has also been coupled with ^1H -MRS to track brain injury as a result of nasopharyngeal carcinoma radiation therapy [97]. Distinguishing viable tumor cells from radiation-induced necrosis is crucial in predicting and monitoring tumor response to therapy.

Tumor vascularity

Angiogenesis and abnormal vasculature are known to play important roles in tumor metastasis and response to therapy [98]. Monitoring changes in tumor vascularity, angiogenesis, and vessel normalization is another method of evaluating radiation response (**Figure 4A**). Dynamic contrast-enhanced (DCE)-MRI is a functional technique that is capable of quantifying vascular perfusion, microvessel density, and permeability by evaluating changes in signal intensity over time [99]. DCE-MRI allows for the differentiation of tumors compared to normal tissues due to their marked differences in terms of interstitial volume and permeability [100, 101]. In rectal cancer, DCE-MRI showed differences in microvessel properties after

radiotherapy [102]. DCE-MRI has also been used to assess differences in tumor blood volume in head and neck cancer following chemoradiotherapy (**Figure 4B**) [103]. The technique has also been used to analyze changes in tumor blood flow following fractionated radiotherapy in glioblastoma multiforme (GBM) due to its ability to analyze blood-brain barrier permeability as well as to visualize the characteristic abnormal vasculature [104]. Early changes in cerebral blood volume (CBV) and thus tumor perfusion following treatment were detected. In addition to visualizing changes in tumor blood flow, the technique has also been used to distinguish recurrent gliomas from radiation injury [105]. Quantitative microcirculatory values derived from pharmacokinetic modeling were significantly different in tumors, necrotic regions, and normal white matter. For example, the transfer constant between intra- and extravascular and extracellular space (K^{trans}) was significantly lower in normal white matter and highest in the recurrent glioma group. DCE-MRI may be a promising method of imaging changes in blood flow following radiation treatment as well as determining treatment efficacy with the potential to individualize and modify treatment based on early patient response [106].

Changes in tumor vascularization can also be detected through US. US contrast agents such as gas-filled microbubbles can be injected intravenously to detect a material with different acoustic properties from the tissue [107]. For example, cyanoacrylate microbubbles targeting angiogenic and inflammatory markers have been used to assess changes in tumor vascularization following radiation therapy in a pre-clinical prostate cancer model [108]. In addition, the dynamic contrast-enhanced (DCE)-US technique evaluates microvessel density and perfusion [109]. By utilizing sulfur hexafluoride microbubbles stabilized by a phospholipid shell, changes in vascularization following anti-cancer treatment, including combination radio- and chemotherapy, were observed in patients with a broad range of tumors [110]. DCE-US imaging of a patient with advanced renal cell carcinoma showed decreases in contrast uptake and thus tumor vascularity following chemotherapy treatment [111]. Many clinical trials have moved forward evaluating tumor response to chemotherapy [112-114], and future use of this technique for determining radiation response is reasonable.

Imaging radiation response

PET tracers that can evaluate molecular markers of angiogenesis rather than hemodynamic characteristics, including radiolabeled arginine-glycine-aspartate (RGD) peptides, have also been used to evaluate vascular changes following treatment [115]. The most commonly used tracer for this purpose is the ^{18}F -galacto-RGD peptide that binds to $\alpha_v\beta_3$ integrins [116, 117]. Clinical trials have successfully correlated ^{18}F -galacto-RGD uptake to immunohistochemical staining of $\alpha_v\beta_3$, and have shown the feasibility of using this marker for imaging angiogenesis [118, 119]. The tracer, however, cannot distinguish between endothelial cells and tumor cells that express the integrin or between vasculature in benign and malignant lesions [116].

Future perspectives

Overall, imaging techniques that take biological considerations into account are imperative for understanding how the complex and heterogeneous tumor microenvironment influences response to therapy. Imaging biological processes from metabolism, hypoxia, proliferation, apoptosis, necrosis, and tumor vascularity enable the evaluation of the response of individual microenvironmental components directly related to therapy. Current trends focus on obtaining biological information in more detail to evaluate the underlying mechanisms of tumor structural and functional changes. Because each technique has its own limitations be it sensitivity, specificity, or patient-to-patient variation, it will likely be necessary to use complementary techniques to obtain a more balanced perspective of the response rather than using one technique or one biological process as a universal solution. In addition to determining changes in the tumor itself, assessing surrounding damage or normal tissue response is also crucial for the full evaluation of therapeutic response. Establishing early response to treatment is also a key for evaluating treatment efficacy, which has been shown in the clinic with MRSI, PET, or MRI techniques that assess metabolism, cell proliferation, apoptosis, and vascular properties.

Clinical imaging has progressed significantly from planar X-ray films to high resolution 3D functional imaging. Beyond visualizing tumor size and morphology, molecular imaging pro-

vides valuable information about the tumor microenvironment and may be used to predict and assess clinical outcomes following radiation therapy. Advances in PET, MRI, and US techniques have allowed for a deeper understanding of the biological response to treatment. A large challenge, however, lies in establishing the molecular landscape following initial treatment to assist physicians in decision making for further treatment options. Standardization of metrics for image acquisition and quantitative response assessment are also essential for realizing the full potential of molecular imaging. Progress in molecular imaging may allow for personalized medicine by designing patient-specific therapies based on accurate monitoring of patient response to treatment.

Disclosure of conflict of interest

None.

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Imaging radiation response

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