Int J Clin Oncol (2016) 21:626–632
DOI 10.1007/s10147-016-1000-2

INVITED REVIEW ARTICLE

Biological imaging in clinical oncology: radiation therapy based on functional imaging

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Received: 29 April 2016 / Accepted: 29 May 2016 / Published online: 6 July 2016

Abstract Radiation therapy is one of the most effective tools for cancer treatment. In recent years, intensity-modulated radiation therapy has become increasingly popular in that target dose-escalation can be done while sparing adjacent normal tissues. For this reason, the development of measures to pave the way for accurate target delineation is of great interest. With the integration of functional information obtained by biological imaging with radiotherapy, strategies using advanced biological imaging to visualize metabolic pathways and to improve therapeutic index and predict treatment response are discussed in this article.

Keywords Biological imaging · Functional imaging · Radiation therapy

Introduction

Radiation therapy (RT) is commonly used as a part of multiple modality treatments for cancer. In RT, staging, target delineation, treatment planning, and evaluation of response are typically based on computed tomography (CT) and magnetic resonance imaging (MRI) as measures of "anatomical imaging." Recently, the treatment planning paradigm in radiation oncology is beginning to shift toward a more biological and molecular approach as advances in biochemistry, molecular biology, and technology have made functional imaging [positron emission tomography (PET), nuclear magnetic resonance spectroscopy (MRS), optical imaging] of physiological processes in tumors more feasible and practical [1]. This mini-review provides an overview of the role of current imaging strategies in radiation oncology, including functional CT, MRI, and several PET tracers in (1) target delineation, (2) treatment response prediction, and (3) resistant tumor targeting for dose-escalation in radiotherapy.

Target delineation

For several decades, along with the development of three-dimensional conformal radiation therapy (3DCRT) and intensity-modulated radiation therapy (IMRT) techniques [2, 3], CT has been the foundation of RT target delineation and dose calculation.

A special technique called dual-energy CT (DECT) provides information beyond morphological CT, and functional imaging [4, 5]. Physically, DECT is found on different X-ray attenuation coefficients of all materials naturally abundant in humans. The measurement of two different X-ray spectra therefore allows the data to be decomposed into a desired pair of images. The advantages of DECT include (1) fast, robust, quantitative and functional imaging; (2) better tumor detection and more detailed tissue differentiations; and (3) less radiation dose exposure [6]. In the RT planning process, DECT may reduce metal artifacts, thus benefiting target delineation [7, 8].

Dynamic contrast-enhanced CT (DCE-CT) first acquires a baseline image without contrast enhancement followed

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by a series of images over time after an intravenous bolus of contrast agent. The temporal changes in contrast enhancement can be analyzed to quantify a range of physiological parameters that indicate the functional characteristics, such as blood volume, tissue perfusion, and vascular permeability, within tumors and adjacent tissues [9, 10]. DCE-CT may improve the accuracy of RT target volume delineation in liver, lung, and prostate cancer [11, 12]. For example, Jensen et al. [12] successfully used DCE-CT to aid gross tumor volume (GTV) delineation of liver tumors and reduced interobserver variability.

Diffusion-weighted MRI (DWI) takes advantage of the random thermal movement of molecules that is often referred to as Brownian motion. Water movement in the extracellular space is restricted by the surrounding cells, and therefore the diffusion constant (ADC) is an apparent value that is estimated from the slope of relative signal intensity (on a logarithmic scale) against a series of *b* values [13]. RT target volume delineation by DWI has been investigated for various cancers. There is an excellent review of target volume delineation for radical radiotherapy planning for glioma by Whitfield et al. [14]. DWI coupled with CT images is shown to improve accuracy and/or consistency to delineate GTV in esophageal or rectal cancer [15, 16].

Most of the fundamentals of molecular imaging akin to tumor detection are built on an urgent demand for sources to sustain aberrant proliferation of a neoplasm. Glucose, a direct material of cellular energy, is desperately needed for tumor growth. ^{18}F-Fluorodeoxyglucose (^{18}F-FDG), a glucose derivative with a radio-fluorine substitution at the 2′-position, was used to reveal the difference in glucose metabolism between tumor and normal tissues, which allowed outlining the lesion. FDG PET has gradually become more important in RT planning [17]. For example, the benefit of FDG PET in lung cancer may include (1) increasing feasibility to distinguish tumors from collapsed lung tissue (atelectasis) and (2) increased accuracy in lymph node staging [18]. In lymphoma, FDG PET is essential for involved node or involved site irradiation.

Treatment response prediction

Early prediction of tumor response is instrumental for treatment planning and optimization of cancer treatment. Thus, a noninvasive imaging biomarker providing accurate information in monitoring as well as enhancing the therapy efficacy is of great importance.

DCE-CT is useful in both target delineation and the prediction of treatment response. Abramyuk et al. [19] performed DCE-CT in 15 patients with HNC before and after chemoradiotherapy (CRT). The increment of transfer coefficient (K_{trans}) and relative tumor blood volume (rTBV) under treatment indicates a poor outcome. DCE-CT also has an important role in lung cancer. In a study by Hwang et al. [20], 75 non-small cell lung cancer (NSCLC) tumors in 65 patients with stable disease according to RECIST after chemotherapy or CRT were investigated. The implementation of DCE-CT predicted the hypermetabolic status of residual tumor in patients with NSCLC after concurrent chemoradiotherapy (CCRT).

Dynamic contrast-enhanced MRI (DCE-MRI), similar to DCE-CT, assesses the changes in signal intensity over time. This imaging follows the intravenous injection of a paramagnetic contrast agent that reduces the T_1 (spin–lattice or longitudinal relaxation time) value of the blood and thereby increases (enhances) the signal intensity on T_1-weighted imaging [10]. Measurements obtained from DCE-MRI before, during, or after RT were related to tumor response or recurrence, mostly in the cervix, head and neck, central nervous system (CNS), and breast [21]. For example, Ng et al. [22] found that the local control could be predicted by pretreatment K_{trans} in oropharyngeal or hypopharyngeal squamous cell carcinoma patients treated with chemoradiation. Furthermore, Shukla-Dave et al. [23] analyzed the significance of pre-treatment DCE-MRI parameters in 74 patients (61 had CCRT and 13 underwent surgery) with squamous cell carcinoma and neck nodal metastases. The result revealed that skewness of K_{trans} was a powerful predictor of progression-free survival (PFS) and overall survival (OS) for stage IV patients. However, in cervical carcinoma, there was no agreement whether the level of signal enhancement in DCE-MRI was crucial in treatment success and survival [24–26]. For example, Donaldson et al. [24] measured the fraction of voxels showing significant signal enhancement and found that high enhancing fraction (EF) at 25 s postcontrast was correlated with poor disease-free survival (DFS). In contrast, Zahra et al. [26] in an analysis found low signal enhancement was related with lower OS and DFS in cervical carcinoma with the utilization of low-enhancing tumor volume (LETV) and low-enhancing tumor fraction (LETF) as input parameters.

Numbers of studies have shown the prognostic potential of the apparent diffusion coefficient (ADC) in predicting treatment outcome after RT among different tumor entities, mostly in prostate, gynecological, head and neck, CNS, and rectal cancer [27]. Liu et al. retrospectively analyzed the pre-RT and post-RT ADC value of patients with prostate cancer receiving RT. The statistical significance in post-IMRT ADC values was noted between patients with and without recurrence ($1.27 \pm 0.14 \times 10^{-3}$ versus $49 \pm 0.12 \times 10^{-3}$ mm^2 s^{-1}; $P < 0.001$). The increase in ADC values observed in prostate cancer after RT leads to a good prognosis [28]. In a prospective cohort study conducted by Harry et al. [29], 20 women with advanced

cervical cancer receiving CCRT were included. ADC values and the change in ADC after 2 weeks of therapy showed a consequential connection with eventual tumor response as determined both by MR imaging ($P = 0.048$) and clinical assessment ($P = 0.009$). The value of ADC, a prediction of treatment response, is consistently utilized for CNS tumors, including primary tumors as well as brain metastases [30, 31]. In metastatic brain lesions treated with RT alone, Farjam et al. [31] reported the diffusion abnormality index (DAI) drastically decreased from pre-RT to 2 weeks after starting RT in the responsive tumors compared with the progressive ones ($P < 0.0002$). DWI was also capable of evaluating the treatment outcome in head and neck cancer [32–34] and rectal cancer [35–37]. In a study to assess DFS in head and neck squamous cell carcinoma (HNSCC) treated with CRT using the ADC value [33], 78 patients were retrospectively studied and ADC was calculated with two sets of two *b* values: 0–750 s/mm^2 (ADC750) and 0–1000 s/mm^2 (ADC1000). The result indicated that tumor volume (sensitivity, 73 %; specificity, 57 %) and lymph node ADC1000 (sensitivity, 71–79 %; specificity, 77–79 %) were significant but independent predictors of DFS. Lambrecht et al. [35] evaluated the ADC value of DWI before, during, and early after initiating preoperative CRT in local advanced rectal cancer. Significantly high values of pathologically complete remission (pCR) were displayed in cases with low ADC pretreatment and higher ADC change during or after RT.

Magnetic resonance spectroscopy shares the same general principles and equipment as its widely used partner, MRI. Nevertheless, MRI builds images using signals from ^{1}H nuclei in tissue water (and sometimes lipid), present at concentrations of approximately 35 M, MRS is used to measure signals from magnetic nuclei (usually ^{1}H, but ^{31}P has also been extensively studied) of tissue metabolites such as choline, creatine, and lactate that are present at much lower concentrations (typically of the order of a few mM) [38]. In RT, MRS serves as a noninvasive predictor for the response of tumors and treatment outcome in glioma and prostate cancer [39–42]. The potential benefit of MRS was reported in a study on 18 patients with glioblastoma who had three-dimensional MR spectroscopic imaging along with T_2- and T_1-gadolinium-enhanced MR images at simulation and at boost treatment planning after 17–20 fractions of RT. All patients received standard radiotherapy with concurrent temozolomide followed by adjuvant temozolomide. The result showing an increase in mean or median Cho/NAA values at the third-week RT scan stood a great chance of early progression ($P < 0.01$) [39]. In a prospectively enrolled study conducted by Crehange et al. [42], 24 patients with localized prostate cancer treating with IMRT with or without long-term adjuvant hormonal therapy (LTHT) underwent 3-T MRS and prostate-specific antigen (PSA) assays. It was concluded that low normalized choline in the peripheral zone, 6 months after radiation, predicts which patients attained a PSA $\leq$0.5 ng/ml at 1 year.

^{18}F-FDG PET is highly sensitive and specific in evaluating the treatment response of various cancers [68]. Among them was lymphoma, being the first type of tumors to be investigated with PET/CT. A recent study conducted by Radford et al. [43] suggested that consolidation of involved-field RT could be omitted in newly diagnosed patients in stage IA or in stage IIA Hodgkin's lymphoma because of the negative result of FDG PET after three cycles of ABVD. In local advanced rectal cancer, 15–27 % of the patients experience a pCR after neoadjuvant CRT, which raises the concern whether invasive surgery could be avoided in a selected cohort of patients with complete clinical response after preoperative RCT. In addition, the importance of early changes in FDG uptake on the prediction of pCR and the role of ^{18}F-FDG PET/CT over RCT need further research. Regardless, quantitative and qualitative ^{18}F-FDG PET/CT measurements are equally effective in the assessment of pCR after CRT [44].

3′-Deoxy-3′-^{18}F-fluorothymidine (^{18}F-FLT), another radiolabeled nucleoside analogue recently used as a specific radiotracer, has superseded ^{18}F-FDG in describing the status of cell proliferation. It is documented that the level of deoxythymidine triphosphate will be elevated for the formation of the nucleus via salvage pathway during rapid cell division [45–47]. ^{18}F-FLT PET, hence, is extremely useful in the detection, localization, and staging of tumors. For example, the ability of ^{18}F-FLT PET to detect early changes in tumor proliferation after chemoradiotherapy was testified in esophageal carcinoma. With ^{18}F-FLT instead of FDG PET, a higher specificity for depicting early reductions in tumor proliferation that precede tumor size changes after chemoradiotherapy was shown [48].

Several studies reported that the decline in ^{18}F-FLT tumor uptake during RT or CCRT was a reliable determinant of treatment outcome in patients with HNSCC [49–51], NSCLC [52, 53], and glioma [54]. Nyflot et al. [50] observed an apparent reduction of ^{18}F-FLT and ^{64}Cu-ATSM (discussed in the next section) take-up in HNSCC after RT combined with bevacizumab treatment. In NSCLC, a similar drop of ^{18}F-FLT uptake was noted after 1–2 weeks of RT [52]. Everitt et al. [53] found that the level of ^{18}F-FLT had decreased earlier than that of ^{18}F-FDG after chemo-RT on NSCLC patients, making it possible for this concurrent therapy to have more influence on proliferation rather than cellular glucose metabolism.

Resistant tumor targeting for dose escalation

There is still a risk of treatment failure even when treatment planning is well organized. A promising strategy to

increase tumor control rate without undesired radiation exposure of normal tissues would be delivery of additive dose to the radio-resistant tumor regions with biological imaging [55]. Noninvasive imaging tools such as functional MRI and PET coupled with tracers such as ^{18}F-fluoroazomycin arabinoside (^{18}F-FAZA) and ^{62,64}Cu-diacetyl-bis [*N*(4)-methylthiosemicarbazone] (^{62,64}Cu-ATSM) are discussed next.

Functional MRI performs excellent RT dose escalation by brachytherapy or external beam boosting. Mason et al. [56] delivered a boost dose by DCE-MRI-guided high-dose-rate (HDR) brachytherapy without violating urethral and rectal dose constraint. The focal boost treatment plans increased median D90 from 17.6 to 20.9 Gy and median V150 from 27.3 % to 75.9 %. Dyk et al. [57] delineated the GTV and delivered a higher dose by combining MRI/DWI and HDR brachytherapy. Total dose delivery to the GTV from MRI/DWI-guided HDR is strongly correlated with local tumor control. In the study among patients who had GBM, Einstein et al. conducted a prospective phase II trial using selective MRS-targeted functional SRS boost to high-risk tumor volumes. This treatment is feasible, with tolerable toxicity and patient survivals higher than in historical controls [58]. A single blind randomized phase III trial (NCT01168479) was also conducted to investigate the benefit of a focal lesion ablative microboost in prostate cancer with MRS imaging. Patients allocated to the standard arm receive a dose of 77 Gy in 35 fractions to the entire prostate and patients in the experimental arm receive 77 Gy to the entire prostate and an additional integrated microboost to the macroscopic tumor of 95 Gy in 35 fractions. To delineate the macroscopic tumor within the prostate, different MR imaging techniques including MRS were used. This study is still ongoing.

Hypoxia, the state of oxygen deprivation, is a hallmark of cancer. It upregulates the expression of hypoxia-responsive elements and in turn contributes to chemo-radioresistance in tumors. The gold standard to evaluate the level of oxygen is an invasive method using Eppendorf polarographic electrodes; however, practical utilization of this technique is confined to well-defined superficial neoplasms. Unfortunately, it is unable to provide the three-dimensional distribution of an hypoxic area. ^{18}F-FAZA belongs to the class of nitroimidazole derivatives as well as ^{18}F-fluoromisonidazole (^{18}F-FMISO), which is the first studied hypoxia probe in PET. The mechanism with respect to the accumulation of radiolabeled nitroimidazole derivatives in hypoxic cells is grounded on changes in mitochondrial respiratory chain in hypoxic and normoxic cells. Briefly, if O_2 is not present, the $-NO_2$ group on the imidazole ring tends to accept an electron released from the mitochondrial respiratory cycle to form $-NO_2^-$, which can be reacted with another electron to produce a 2-electron reduction product and sequentially reduced to $-NH_2$, resulting in cellular retention because of covalent bonding to the macromolecule. Basically, ^{18}F-FAZA has a few advantages over ^{18}F-FMISO, such as better specific activity, specificity, tumor-to-background ratio, and more chemically stability when injected into the living subjects. Trinkaus et al. [59] and Bollineni et al. [60] have reported the application of ^{18}F-FAZA PET in monitoring the hypoxic fraction of tumor growth in NSCLC patients. Servagi-Vernat et al. [61] reached a similar conclusion in a clinical study using ^{18}F-FAZA PET to identify the hypoxic area in HNSCC of patients during the course of radiochemotherapy. These reports suggest that change in the hypoxic volume between pre- and mid-treatment is crucial to dose-escalation protocols.

Another class of PET hypoxia radiotracers, ^{62,64}Cu-ATSM, a prognosis predictor as well as ^{18}F-FAZA, has been demonstrated to accumulate in tumors [62–64]. The apparent retention of ^{64}Cu-ATSM in hypoxic cells may result from the elevated bioreductive enzymes under a low oxygen condition. The lack of oxygen impairs the electron transfer chain, leads to increased presence of cellular NADH and NADPH, and causes the release of radioactive copper from ATSM that is irreversibly trapped within hypoxic cells, but not in cells under normal oxygen supply condition. The in vitro and in vivo therapeutic efficacy of ^{64}Cu-ATSM in eliminating tumor cells and prolonging the survival time of tumor-bearing hamsters have been reported by Obata et al. [65] and Lewis et al. [66], respectively. Recently, Yoshii et al. [67, 68] found remarkable retention of ^{64}Cu-ATSM in CD133$^+$ cells, which are regarded as cancer stem cell-like cells associated with radio-chemoresistance and metastasis. However, the natural high level of accumulation of radioactive copper in liver, a well-known critical organ for RT, raised a concern about harmful effects on the liver [69]. Yoshii and her colleagues [70] developed a method by using penicillamine, a drug for Wilson's disease, to reduce radiation dose-escalation in liver with no significant effect on tumor uptake. Being tested in phantoms and a tumor model, Cu-ATSM-based dose-painting has proved to be feasible to deliver a higher dose of radiation to the hypoxic tumor subvolume to overcome inherent hypoxia-induced radioresistance without compromising normal tissue sparing [48, 71].

Conclusion

Biological imaging with functional CT/MRI and PET tracers offers a variety of tactics for tumor detection and visualization, as well as describing the biological aspects of tumor pathophysiology and radiation sensitivity. Although thriving and promising, the potential of imaging modalities in treatment outcomes remains unconfirmable [72]. Further

studies on dose-painting are essential in this emerging field of RT. It is thrilling to see biological imaging laying bare the individualized RT strategy.

Acknowledgments The authors acknowledge the help of Vivian Wu and Vicky C. Lin in manuscript preparation and editing.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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