Monitoring early responses to irradiation with dual-tracer micro-PET in dual-tumor bearing mice

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Abstract

AIM: To monitor the early responses to irradiation in primary and metastatic colorectal cancer (CRC) with $^{18}$F-fluorothymidine ($^{18}$F-FLT) and $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) small-animal position emission tomography (micro-PET).

METHODS: The primary and metastatic CRC cell lines, SW480 and SW620, were irradiated with 5, 10 and 20 Gy. After 24 h, the cell cycle phases were analyzed. A dual-tumor-bearing mouse model of primary and metastatic cancer was established by injecting SW480 and SW620 cells into mice. micro-PET with $^{18}$F-FLT and $^{18}$F-FDG was performed before and 24 h after irradiation with 5, 10 and 20 Gy. The region of interest (ROI) was drawn over the tumor and background to calculate the ratio of tumor to non-tumor (T/NT) in tissues. Immunohistochemical assay and Western blotting were used to examine the levels of integrin β3, Ki-67, vascular endothelial growth factor receptor 2 (VEGFR2) and heat shock protein 27 (HSP27).

RESULTS: The proportion of SW480 and SW620 cells in the G2–M phase was decreased with an increasing radiation dose. The proportion of SW480 cells in the G2–M phase was increased from 43.23% ± 4.50% to 87.09% ± 5.46% ($P < 0.001$) and that of SW620 cells in the S-phase was elevated from 43.57% ± 2.65% to 66.59% ± 2.73% ($P = 0.021$). In micro-PET study, with increasing dose of radiation, $^{18}$F-FLT uptake was significantly reduced from 3.65 ± 0.51 to 2.87 ± 0.47 ($P = 0.008$) in SW480 tumors and from 2.22 ± 0.42 to 1.76 ± 0.45 ($P = 0.026$) in SW620 tumors. $^{18}$F-FDG uptake in SW480 and SW620 tumors was reduced but not significantly ($P = 0.582$, $P = 0.633$ vs $F = 0.273$, $P = 0.845$). Dose of radiation was negatively correlated with $^{18}$F-FLT uptake in both SW480 and SW620 tumors ($r = -0.727$, $P = 0.004$; and $r = -0.664$, $P = 0.009$). No significant correlation was found between $^{18}$F-FDG uptake and radiation dose in SW480 or SW620 tumors. HSP27 and integrin β3 expression was higher in SW480 than in SW620 tumors. The T/NT ratio for $^{18}$F-FLT uptake was positively correlated with HSP27 and integrin β3 expression ($r = 0.924$, $P = 0.004$; and $r = 0.813$, $P = 0.025$).

CONCLUSION: $^{18}$F-FLT is more suitable than $^{18}$F-FDG in monitoring early responses to irradiation in both primary and metastatic lesions of colorectal cancer.
Key words: $^{18}$F-fluorothymidine; $^{18}$F-fluorodeoxyglucose; Irradiation; Positron emission tomography; Colorectal cancer

INTRODUCTION

Radiation therapy has long been used for curative or palliative management of colorectal cancer (CRC) patients\[1-2]. However, some patients undergoing radiotherapy might present primary CRC lesions as well as metastatic lymph nodes. The current method for assessing the response of a solid tumor to radiotherapy is to measure the change in tumor size on anatomical imaging modalities. It takes weeks to months to detect the change, so it is difficult to evaluate early responses to therapy \textit{via} morphological means. Noninvasive methods for monitoring early responses to radiotherapy would be of great value in individualized treatment.

Positron emission tomography (PET) is a quantitative molecular imaging technique that allows for noninvasive \textit{in vivo} imaging and quantification of biological processes\[3-8]. $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) is the most widely used PET tracer and has become an indispensable staging modality for many types of cancer. However, $^{18}$F-FDG may be unsuitable for monitoring the response after radiotherapy, because increased uptake can appear in inflammatory lesions and fibrosis\[8-9].

$^{18}$F-fluorothymidine ($^{18}$F-FLT) is a pyrimidine analogue that uses the salvage pathway of DNA synthesis to reveal cell proliferation. $^{18}$F-FLT has been found useful for noninvasive assessment of the proliferation rate of several types of cancer, such as colorectal, esophageal, breast and laryngeal cancer. Imaging and measurement of proliferation with $^{18}$F-FLT-PET could be a noninvasive tool to monitor the response to anticancer treatment\[9-10].

Recently, many studies claimed that PET has a special promise as a biomarker for anticancer treatment, and can be used longitudinally and provide information on the whole body or tumor. Early identification of cancer patients who are responding \textit{or} resistant to radiotherapy may lead to individualized therapeutic approaches and improved clinical outcomes\[11-12]. Yang et al\[12] found that tumor uptake of $^{18}$F-FLT was reduced significantly at 24 h after radiation with 10 Gy and 20 Gy compared with $^{18}$F-FDG. At 48 h after irradiation, $^{18}$F-FLT uptake was further reduced, but $^{18}$F-FDG uptake was reduced slightly. So, $^{18}$F-FLT-PET may be a promising imaging modality for monitoring the early effects of radiation therapy.

Bearing those in mind, we wondered whether $^{18}$F-FLT could be used to reflect the early response to irradiation and compared $^{18}$F-FLT and $^{18}$F-FDG-PET in a possible early response in CRC primary or metastatic lesions. We chose two kinds of human CRC cells, SW480 and SW620\[13-15], derived from CRC primary and lymph-node metastatic lesions, respectively, in the same patient to create a dual-tumor-bearing model. PET with $^{18}$F-FLT and $^{18}$F-FDG was performed before and 24 h after increasing doses of irradiation. The radioactivity uptake in SW480 and SW620 tumors was investigated with small-animal (micro)-PET.

MATERIALS AND METHODS

Chemicals

RPMI1640, Leibovitz’s L15 medium, and fetal bovine serum (FBS) were obtained from PAA Laboratories GmbH, Linz, Austria. All the other chemicals were of reagent grade. Cell cycle and cell apoptosis kits were from Nanjing Keygen Biotechnology. Antibodies against K-i67, a cell proliferation antigen, and anti-integrin β1 were from Santa Cruz Biotechnology. The antibodies anti-vascular endothelial growth factor receptor 2 (VEGFR2) and antiheat shock protein (HSP) 27 were from Abcam. The diaminobenzidine (DAB) kit was obtained from Zhongshan Biotechnology Co., Beijing, China.

Cell lines

The human CRC cell lines SW480 and SW620 were from the Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). SW480 cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (10 000 IU/mL), at 5% CO$_2$ in a humidified atmosphere at 37°C. SW620 cells were cultured in L15 medium supplemented with 10% FBS and 1% penicillin/streptomycin (10 000 IU/mL) without CO$_2$ in a humidified atmosphere at 37°C.

Animal tumor model

Eighteen male Balb/C nude mice (6 wk old, 20 g) were obtained from the Animal Laboratory of the Chinese Academy of Sciences. Two tumors per animal were generated by inoculating $5 \times 10^6$ SW480 viable cells into nude mice on the lateral side of the left front leg and the same amount of SW620 cells on the right front leg. Mice were kept under sterile conditions with a standard light/dark cycle and had free access to food and water. Tumor size in the front legs was determined by caliper measurement at least twice a week by the formula $V = 1/2 \times l \times w \times h$ ($l$, length; $w$, width; $h$, height of the tumor). Micro-PET/CT scans of $^{18}$F-FDG and $^{18}$F-FLT uptake were performed for tumors with volumes between 100 and 500 mm$^3$.

X-ray irradiation

Local external beam radiation was applied using a clinical X-ray therapy unit (Precise ELEKDA, 6 MV X-ray, at a dose rate of 388 MU/min). The mice were anesthetized using 1% chloral hydrate (0.45 mg/g body weight) and

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positioned prone on the scanning table. The dual tumors were locally irradiated and the other parts of the mouse body were protected from irradiation with lead shielding. For homogeneous dose distributions, antero-posterior and postero-anterior external beam radiation fields were used. When SW480 and SW620 cells arrived at 50% confluence and when tumor size was about 100-500 mm³, cells and tumors underwent single-dose irradiation at 5 Gy (n = 6 wells or mice), 10 Gy (n = 6) and 20 Gy (n = 6), respectively. After 24 h, tumors underwent micro-PET/CT scanning. All animal experiments were carried out in accordance with the Dutch Law on Animal Experimentation and approved by the institutional committee on animal experimentation of our institution.

**Flow cytometric analysis of cell cycle**
At 24 h after irradiation, SW480 and SW620 cells were washed twice with phosphate-buffered saline (PBS), detached with 0.25% trypsin and fixed with 75% ethanol and stored at 4 °C. Cells were centrifuged to remove 75% ethanol before cell cycle determination, washed twice with PBS and resuspended in 0.5 mL PBS. After cells were stained with propidium iodide in the dark for 10 min, DNA content was measured by flow cytometry (FACS-calibur, Becton Dickinson) to obtain the percentage of cells in each phase.

**PET studies and image analysis**
PET images of tumors in dual-tumor-bearing mice were obtained using the small animal micro-PET/CT (Explore VISTA micro-PET/CT, GE). At 24 h after irradiation, 3 mice in each group underwent 18F-F-FLT PET and 3 mice underwent 18F-FDG PET. The mice were anesthetized and positioned prone in the scanner, and 18F-F-FLT or 18F-FDG was injected via the tail vein at 20 ± 1.84 MBq in 0.25 mL saline. Image data were acquired for 10 min at 1 h after injection. For image reconstruction, list-mode data were sorted into 3-D sinograms, then underwent Fourier rebinning and 2-D ordered-subset expectation maximization reconstruction with 2 iterations and 50 subsets. Image pixel size was 0.385 mm × 0.385 mm × 0.335 mm. For quantitation of tumor uptake of 18F-F-FLT or 18F-FDG, image software was used to analyze the region of interest (ROI) in reconstructed images. Three consecutive coronal slice images containing tumors were selected visually, and ROIs were drawn on the tumor and lung as background, and the ratio of tumor to background (T/NT) uptake was calculated.

**Immunohistochemical and Western blotting analysis**
The staining procedure has been described elsewhere. Sections of two kinds of tumors were stained with hematoxylin and eosin and antibodies against anti-integrin β3 (sc-52685), anti-Ki67 (sc-52685), anti-HSP27 (ab2790), and anti-VEGFR2 (ab3968) antibody (1:100). For a negative control, the primary antibody was omitted and replaced with PBS. Specimens were examined under light microscopy. The number of integrin β3, Ki-67-, HSP27-, and VEGFR2-positive and HE-positive cells in adjacent sections was counted in 5 randomly selected fields per section. Western blotting analysis of 150 μg protein from SW480 and SW620 tumors was performed as described earlier. The antibodies were the same as described in the immunocytochemical assay. Integrin β3, Ki-67, HSP27, and VEGFR2 expression was described by gray scale analysis with the Labworks software.

**Statistical analysis**
Data analysis was performed using SPSS v11.5 (SPSS Inc., Chicago, IL.). Percentages of cells in each cell phase after irradiation were compared by one-way ANOVA. Differences in radiotracer uptake before and after irradiation in each mouse were compared by independent-samples t test. Linear regression analysis was used to determine the correlation between radiotracer uptake and radiation dose or cell cycle phase. All data were expressed as mean ± SD. P < 0.05 was considered statistically significant.

**RESULTS**

**Cell cycle analysis**
We examined the effects of irradiation on cell cycle distribution by flow cytometry. At 24 h after irradiation, the proportion of SW480 cells in the G0-G1 phase decreased from 48.33% ± 4.55% at 0 Gy to 26.70% ± 7.09% at 5 Gy, then increased to 87.09% ± 7.43% at 20 Gy; the proportion in the S phase decreased from 33.23% ± 6.09% at 0 Gy to 12.44% ± 4.60% at 20 Gy; and that in the G2-M phase decreased from 18.44% ± 5.67% at 0 Gy to 0.47% ± 0.34% at 20 Gy (Figure 1A). At 0-20 Gy, the proportion of SW620 cells in the G0-G1 phase decreased from 39.37% ± 4.37% to 20.39% ± 5.12%, and that in the S phase increased from 43.57% ± 2.65% to 66.59% ± 7.37%. The proportion in G2-M phase decreased from 17.07% ± 3.09% to 13.02% ± 4.55% (Figure 1B).

**Micro-PET/CT analysis**
Micro-PET/CT scanning results of 18F-F-FLT and 18F-FDG uptake in SW480 and SW620 tumors irradiated with increasing doses are shown in Table 1 and Figure 2. Before irradiation, the T/NT ratio in the ROI for 18F-F-FLT was higher in SW480 (3.65 ± 0.51) than in SW620 tumors (2.22 ± 0.42). At 24 h after irradiation with 20 Gy, the T/NT ratio for 18F-F-FLT uptake was significantly decreased in both SW480 (2.87 ± 0.47, P = 0.008) and SW620 cells (1.76 ± 0.45, P = 0.026) (Figure 2A).

Before irradiation, the T/NT ratio for 18F-FDG in SW480 and SW620 tumors was 2.69 ± 0.98 and 3.09 ± 1.26, respectively (P = 0.524). At 24 h after irradiation at 20 Gy, the T/NT ratio for 18F-FDG uptake was reduced but not significantly (2.40 ± 0.52 and 2.89 ± 0.29, both P > 0.05) (Figure 2B).

**Immunohistochemical and Western blotting analysis**
Integrin β3, HSP27, Ki-67 and VEGFR2 proteins were all overexpressed in SW480 and SW620 tumors (Figure...
SW480 cells showed more intense staining for integrin \(\beta_3\) and HSP27 protein in cytoplasm or nucleus than did SW620 tumors. Integrin \(\beta_3\) protein was also overexpressed in the tumor matrix near vasculature. Staining for VEGFR2 and especially Ki-67 expression was lower in SW480 tumors than in SW620 tumors.

Western blotting analysis revealed that HSP27 and integrin \(\beta_3\) expression was higher in SW480 than in SW620 tumors (42.86% ± 5.15% vs 10.10% ± 3.50%, for Hsp27, \(P = 0.002\); and 9.61% ± 3.20% vs 8.43% ± 1.85% for integrin \(\beta_3\), \(P = 0.164\)). The expression of K-i67 and VEGFR2 protein was less pronounced in SW480 than in SW620 tumors (6.5% ± 1.25% and 9.00% ± 2.38% for K-i67, \(P = 0.009\); and 25.33% ± 5.59% and 19.96% ± 4.20% for VEGFR2, \(P < 0.001\)) (Figure 4A and B).

**Linear regression analysis**

We found a significant negative correlation between dose

| Table 1  Ratio of tumor to non-tumor for \(^{18}\)F-fluorothymidine and \(^{18}\)F-fluorodeoxyglucose uptake in SW480 and SW620 tumors in mice (mean ± SD) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Tracer**      | **SW480**       | **SW620**       | **SW480**       | **SW620**       |
|                 | 0 Gy            | 5 Gy            | 10 Gy           | 20 Gy           | 0 Gy            | 5 Gy            | 10 Gy           | 20 Gy           |
| \(^{18}\)F-FLT   | 3.65 ± 0.51     | 3.75 ± 0.71     | 3.04 ± 0.35     | 2.87 ± 0.47*    | 2.22 ± 0.42     | 2.47 ± 0.59     | 2.10 ± 0.55     | 1.76 ± 0.45*    |
| \(^{18}\)F-FDG   | 2.69 ± 0.98     | 2.85 ± 0.47     | 2.62 ± 0.67     | 2.40 ± 0.52     | 3.08 ± 1.26     | 2.92 ± 0.42     | 3.22 ± 0.56     | 2.89 ± 0.29     |

*\(P < 0.05\), \*\(P < 0.01\), 3 mice/group. \(^{18}\)F-FLT: \(^{18}\)F-fluorothymidine; \(^{18}\)F-FDG: \(^{18}\)F-fluorodeoxyglucose.
of radiation and 18F-FLT uptake in SW480 and SW620 tumors \( (r = -0.727, P = 0.004, \text{and } r = -0.664, P = 0.009, \text{respectively}) \). Dose of radiation was positively but not significantly correlated with 18F-FDG uptake in SW480 tumors \( (r = 0.401, P = 0.098) \) and positively but significantly correlated with 18F-FDG uptake in SW620 tumors \( (r = 0.640, P = 0.013) \). Dose of radiation was negatively correlated with proportion of SW480 cells in the G2-M phase \( (r = -0.798, P = 0.001) \) and negatively but not significantly correlated with proportion of SW620 cells in the G2-M phase \( (r = -0.184, P = 0.283) \). Dose of radiation was positively correlated with proportion of SW620 cells in the S phase \( (r = 0.870, P < 0.001) \) and negatively correlated with proportion of SW620 cells in the G0-G1 phase \( (r = -0.673, P = 0.008) \). The T/NT ratio for 18F-FLT uptake was positively correlated with integrin β3 and HP27 expression \( (r = 0.813, P = 0.004) \), but not with Ki-67 or VEGFR2 expression. Similarly, the T/NT ratio for 18F-FDG uptake was not significantly correlated with integrin β3, HP27, Ki-67 and or VEGFR2 expression.

**DISCUSSION**

Therapy monitoring plays a major role in the evaluation of therapeutic approaches\[16-18\]. 18F-FDG-PET is clinically used for the diagnosis, staging and re-staging of a wide variety of tumors. However, the technique contains several shortcomings in reflecting changes in tumors after treatment, especially radiotherapy\[19-22\]. Several research groups have suggested that 18F-FLT, as a cell proliferation tracer, is a more cancer-specific tracer than 18F-FDG\[23-27\], but some results are contradictory\[28\]. In the present study, we investigated 18F-FLT-PET as a potential tool for monitoring the early response to irradiation in a mouse model of dual-tumor-bearing CRC. In clinical practice, we have often found primary lesions and metastatic lymph nodes together in the same patient. A model of dual tumors created with CRC SW480 and SW620 cells is similar to clinical practice, so we compared the uptake of 18F-FLT and 18F-FDG in response to irradiation in the two kinds of CRC tumors.
In a micro-PET study, we found a higher uptake of \(^{18}\text{F-FLT}\) than \(^{18}\text{F-FDG}\) in SW480 and SW620 tumors. After irradiation for 24 h, the uptake of \(^{18}\text{F-FLT}\) in SW480 or SW620 tumors increased at a low dose (5 Gy), then reduced gradually with increasing radiation dose. A statistical difference was found in both tumor groups although \(^{18}\text{F-FLT}\) uptake reduced more significantly in SW480. Whereas the \(^{18}\text{F-FDG}\) uptake was increased at a low dose (5 Gy) and reduced slightly at a high dose (20 Gy) without a significant difference. Liang et al.\(^{[5]}\) did not find a dose-dependent decrease in uptake of \(^{18}\text{F-FLT}\) in tumors. However, in the current study, with a dose greater than 5 Gy, the T/NT ratio for \(^{18}\text{F-FLT}\) uptake was dose-dependently decreased in both SW480 and SW620 tumors. A significantly negative correlation was found between dose of radiation and \(^{18}\text{F-FLT}\) uptake in SW480 and SW620 tumors. No correlation was found between dose of radiation and \(^{18}\text{F-FDG}\) uptake. So \(^{18}\text{F-FLT}\) uptake can be more sensitive and accurate than \(^{18}\text{F-FDG}\) to monitor the response to irradiation after 24 h.

Recent studies have shown that a decrease in cellular proliferation rate is one of the early events in response to tumor treatment. In the present study, after 24 h irradiation, cell cycle redistribution was found, and proliferation inhibition of the two kinds of CRC cells occurred in a dose-dependent manner, and the response to beam dose was different. G0-M phase decrease and G0-G1 phase arrest were found earlier in SW480 than that in SW620 cells. We also found a decrease in the proportion of SW480 and SW620 cells in the S phase with the increasing radiation dose, and the dose of irradiation was negatively correlated with proportion of G0-M phase in both kinds of cells. T/NT ratio for \(^{18}\text{F-FLT}\) was negatively correlated with proportion of SW480 or SW620 cells in the G0-M phase. The proportion of cells decreased in the G0-G1 arrest and S phases may not be important for the \(^{18}\text{F-FLT}\) uptake decrease in SW480 and SW620 tumors.

A previous study with another tumor cell line also showed that tumor uptake of \(^{18}\text{F-FDG}\) was decreased at 24 h after irradiation\(^{[18]}\). The mechanism of the increased \(^{18}\text{F-FDG}\) uptake after 24 h irradiation at a low dose (5 Gy) has remained unclear. It may be due to the G2-M or S phase arrest enhancing metabolism after irradiation in a short time. After 24 h irradiation, the two kinds of tumors were excised immediately. No necrosis formation occurred in the tumors possibly due to the short time (24 h) after radiotherapy.

In our dual-tumor-bearing animal study, the reason for the difference in \(^{18}\text{F-FLT}\) and \(^{18}\text{F-FDG}\) uptake in response to irradiation is not clear. The different response may be related to different biological characteristics, such as tumor marker expression. A large number of different proteins are expressed in SW480 and SW620 cell lines. In this study, we selected 4 tumor biomarkers, integrin \(\beta_3\), Ki67, HSP27 and VEGFR2, known as tumor invasion, proliferation, apoptosis, angiogenesis markers, respectively\(^{[10-12]}\). VEGFR2 or Ki-67 expression was stronger in SW620 than in SW480 cells. In contrast, HSP27 and integrin \(\beta_3\) expression was more intense in SW480 cells. The T/NT ratio for \(^{18}\text{F-FLT}\) was significantly correlated with HSP27 and integrin \(\beta_3\) expression. Tumor biomarkers play a major role in indicating tumor characteristics, exploring possible mechanisms, and in evaluating and suggesting new therapeutic approaches. The results suggest that other factors, besides proliferation, may influence the response to irradiation.

Although our study population is small, differences were found in the \(^{18}\text{F-FLT}\) and \(^{18}\text{F-FDG}\) uptake response in CRC to radiotherapy on PET. \(^{18}\text{F-FLT}\) PET may be a useful noninvasive imaging modality to assess early response to irradiation in both primary and metastatic CRC lesions. \(^{18}\text{F-FLT}\)-PET is also quick and effective in monitoring the response to irradiation in primary tumors. The capacity of \(^{18}\text{F-FLT}\) to reveal response to irradiation within 24 h may be useful for individualizing therapy. Additional studies are being carried out to investigate other radiation doses and the mechanism among different tumor cells.

In conclusion, compared with \(^{18}\text{F-FDG-PET}\), \(^{18}\text{F-FLT-PET}\) might be better in monitoring the response to 24 h irradiation in both primary and metastatic CRC lesions with increasing radiation doses.

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**COMMENTS**

**Background**

Colorectal cancer is one of the most frequently encountered malignancies in China and is associated with a high mortality rate. Irradiation therapy has long been used for curative or palliative management in colorectal cancer (CRC). When irradiation was performed, primary CRC and metastatic lymph node metastatic lesions often appear in the same patient. As it takes weeks to months to detect the change, it is difficult to evaluate the early responses to therapy via morphological means. Noninvasive methods for monitoring early responses to radiotherapy would be of great value in individualized treatment. So there are some questions to answer: Which \(^{18}\text{F-fluorodeoxyglucose (F-FDG)}\) or \(^{18}\text{F-fluorothymidine (F-FLT)}\) could depict the difference earlier, and which is more suitable for doing so? This study was designed to investigate whether \(^{18}\text{F-FLT-PET}\) could be used to reflect the early effect of irradiation in both CRC primary and lymph node metastatic lesions as compared with \(^{18}\text{F-FDG-PET}\).

**Research frontiers**

Recently, many studies claimed that PET has particular promise as a biomarker for anticancer therapies, can be used longitudinally and provides information on the patient or tumor. Early identification of cancer patients who are responding or resistant to radiotherapy may lead to individualized therapeutic approaches and improved clinical outcomes. \(^{18}\text{F-FLT-PET}\) may be a promising imaging modality for monitoring the early effects of radiation therapy.

**Innovations and breakthroughs**

\(^{18}\text{F-FLT PET}\) may be used as a useful noninvasive imaging modality to monitor early response to irradiation for different CRCs. This is the first study to report in a new angle that \(^{18}\text{F-FDG}\) is more helpful than \(^{18}\text{F-FDG}\) in reflecting the early effects of irradiation in CRC primary lesions or lymph metastatic lesions.
Early responses to irradiation in tumor-bearing mice

**Applications**

SW480 and SW620 tumors, either primary or from metastatic lymph nodes have different responses to irradiation at early phase; $^{18}$-FLT response to irradiation is more sensitive than $^{18}$-FDG. Evaluation of the response to irradiation would be helpful for individualizing treatment and improving outcomes of CRC patients in clinical practice.

**Terminology**

$^{18}$-FDG is the most widely used PET tracer, but it has several shortcomings in reflecting changes in tumors after treatment, especially radiotherapy. $^{18}$-FLT is a pyrimidine analogue and believed to be an agent for imaging cellular proliferation via the salvage pathway of DNA synthesis, which is closely associated with cellular proliferation. The 4 kinds of tumor biomarkers, HSP27, Integrin, VEGFR2 and K67, are related to tumor differentiation, invasion, angiogenesis and proliferation respectively.

**Peer review**

The authors investigated whether $^{18}$-FLT-PET or $^{18}$-FDG-PET could be used to reflect the early effects of irradiation in CRC primary lesions or lymph metastatic lesions. The results revealed that $^{18}$-FLT-PET might be better in monitoring the response to 24 h irradiation in both primary and metastatic CRC lesions with increasing radiation doses. The results are interesting and helpful for individualized treatment in clinical practice.

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