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Experimental radiobiology

Effect of increase of radiation dose on local control relates to pre-treatment FDG uptake in FaDu tumours in nude mice

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Abstract

***Objectives*: To investigate whether heterogeneity in [^{18}F]2-fluoro-2-deoxy-D-glucose (FDG) uptake in a single tumour line, i.e. in tumours with identical genetic background, relates to radiation response.**

***Materials and methods*: Sixty-two human FaDu head and neck squamous cell carcinomas in nude mice with a diameter of 7 mm entered the study. FDG-PET scanning was performed without anaesthesia on an animal PET scanner immediately prior to irradiation in order to determine maximum standardized uptake values (SUV_{max}). Single dose irradiations of 25 or 35 Gy were applied under normal blood flow conditions using 200 kV X-rays (0.5 mm Cu, ~1.2 Gy min^{-1}). The mice were observed for 120 days after irradiation, experimental endpoint was local tumour control evaluated using the Kaplan–Meier method.**

***Results*: Analyzing all 62 animals, tumour control probability after irradiation with 25 Gy was significantly lower than after irradiation with 35 Gy (29% vs. 57%, log rank $p = 0.016$). Pre-treatment SUV_{max} values ranged from 0.72 to 3.47, the median SUV_{max} value was 1.59. In tumours with FDG uptake less than the median SUV_{max}, local control was 37% after 25 Gy vs. 47% after 35 Gy ($p = 0.37$). In contrast, substantial differences in local tumour control were found in tumours with FDG uptake above the median SUV_{max} (24% vs. 71%, $p = 0.006$). Multivariate Cox analysis revealed a significant decrease of hazard of recurrence with increasing dose and SUV_{max}.**

***Conclusions*: An increase of radiation dose had a greater effect on local control in FaDu tumours with higher FDG uptake than in tumours with lower FDG uptake. This supports the hypothesis that pre-treatment FDG-PET may provide useful information for heterogeneous radiation dose prescription in subvolumes of tumours of individual patients. As only one tumour model was studied and single doses were applied, confirmatory investigations using further tumour models and fractionated radiotherapy are warranted.**
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Keywords: [^{18}F]FDG; Positron emission tomography; Irradiation; Squamous cell carcinoma; Human tumour xenografts; Local tumour control

In recent years radiotracer-based molecular imaging with positron emission tomography (PET), mainly using the glucose analogue [^{18}F]2-fluoro-2-deoxy-D-glucose (FDG), has been shown to improve accuracy in the diagnosis and staging of various tumours [1,5,6,9,10,13,21]. Furthermore it has proven beneficial for both therapy monitoring and differentiating between residual or recurrent tumour and non-specific post-therapeutic changes [15–17]. The integration of FDG PET/CT fusion imaging into radiation treatment planning by taking into account the metabolic and biologic characteristics of the tumour demonstrated to have significant impact on the determination of irradiation treatment volumes [7,8,12,19,24]. It is well recognized that in different patients tumours of the same histology may show a varying avidity for FDG. This *inter*-tumoural heterogeneity has been demonstrated in several studies to correlate with outcome of radiotherapy [14,18,23]. In addition, a tumour in an individual patient may show subvolumes with lower and higher FDG uptake (Fig. 1) [6,25], i.e. *intra*-tumoural heterogeneity. It is currently unknown whether these subvolumes in individual tumours differ in their radiation response.

The present study investigates FDG uptake prior to irradiation in FaDu human squamous cell carcinoma in nude mice using animal PET. The tumours were irradiated with single doses at two different dose levels and followed up for local

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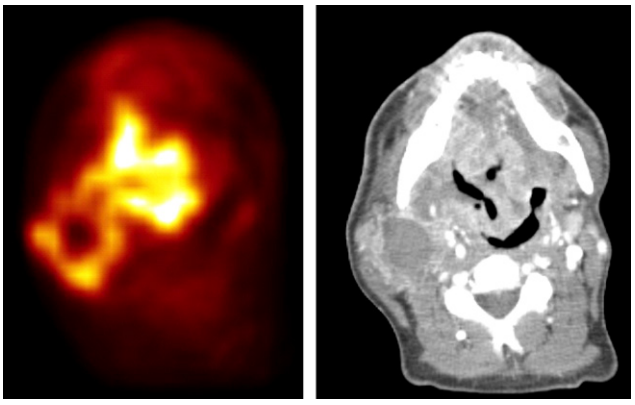

Fig. 1. FDG PET (left) and CT (right) of a patient with tonsil carcinoma right and bicervical lymph node metastasis before start of irradiation therapy showing intratumoural heterogeneity of FDG uptake.

tumour control. The results obtained in this preclinical model are expected to shed some light on the question whether heterogeneity in FDG-uptake in a single tumour line, i.e. in tumours with identical genetic background, may relate to radiation response.

Materials and methods

Animals and tumour model

The experiments were performed using 7–14-week-old female NMRI (nu/nu) mice from the specific pathogen-free breeding facility of the Experimental Centre of the Medical Faculty Carl Gustav Carus, University of Technology, Dresden. The animal facilities and experiments were approved in accordance with institutional guidelines and the German animal welfare regulations. The animals were maintained and kept as described elsewhere [28]. To immunosuppress the nude mice further, they were whole-body irradiated 2 days before tumour transplantation with 4 Gy (200 kV X-rays, 0.5 mm Cu filter, $\sim$1 Gy min^{-1}).

FaDu is an established human hypopharyngeal squamous cell carcinoma line, kept in high passage by the American Type Culture Collection (Rockville, MD, USA). FaDu grows as an undifferentiated carcinoma in nude mice. For the experiments, source tumours were cut into small pieces and transplanted subcutaneously into the right hind-leg of anaesthetized mice [27]. Quality assurance included the monitoring of the tumours by histology, LDH-iso-enzyme pattern, volume doubling time (VDT) and microsatellite analysis.

Experimental design, PET data acquisition and imaging studies

At 7 mm tumour diameter, a baseline FDG-PET scan prior to radiotherapy was performed. Tumours were randomized into two dose groups of either 25 or 35 Gy single dose irradiation.

The non-anaesthetized animals were immobilized in a prone position, using plastic tubes, fixed on a lucite plate, the tumour-bearing leg positioned outside the tube. PET studies were performed with a microPET (Concord microPET® P4, Siemens Medical Solutions Inc., TN, USA). For the purpose of attenuation correction, a ^{57}Co transmission scan of 10 min was done before the tracer injection. 4–11 MBq FDG was injected within 15 s in a 300 μL volume into the tail vein of the animals. Data acquisition 30–60 min p.i. was evaluated. PET images were reconstructed iteratively by a 3D-ordered-subset expectation maximization algorithm (3D OSEM/MAP). No additional efforts were undertaken for the correction of partial-volume effects and recovery. 3D tumour regions of interest (ROI) were determined for the subsequent data analysis. The maximum standardized uptake value (SUV_{max}) was used to minimize the partial volume effect [11], and calculated for each PET measurement as the ratio of maximal FDG uptake in a tumour to the injected FDG activity normalized to body weight using Rover software (ROI Visualization, Evaluation and Image Registration, ABX Radeberg, Germany).

Local tumour irradiation was performed under ambient conditions without anaesthesia to air-breathing animals (200 kV X-rays, 0.5 mm Cu, dose rate $\sim$1.2 Gy min^{-1}, 45 cm focus-field distance). Up to five animals were irradiated simultaneously in specially designed jigs. For treatment the mice were immobilized in a plastic tube fixed on a lucite plate. The tumour bearing leg was held positioned in the irradiation field by a foot holder distal to the tumour.

Follow-up, determination of tumour volumes and local tumour control

The present study includes data obtained from a total of 62 animals. Tumour diameters were measured twice per week using a digital calliper. Tumour volumes were determined by the formula of a rotational ellipsoid: $\pi/6 \times a \times b^2$, where a is the longest and b is the perpendicular shorter tumour axis. Conversion of tumour volumes to tumour mass (mg) was performed by a calibration curve based on excision weights [22]. The animals were sacrificed when the recurrent tumour either reached the mean diameter of 12–15 mm or when the animal appeared to suffer.

Local tumour control was evaluated at day 120 after the end of irradiation. It has been previously shown that this observation period is sufficient to detect virtually all regrowing FaDu tumours [3]. Animals censored before day 20 ($n = 1$) were excluded from the analysis. Local recurrences were scored when the volume increased for at least three consecutive measurements after passing a nadir. For the statistical analysis, tumours were divided into two groups below or above median SUV_{max}. Kaplan–Meier estimates of local tumour control of the two groups were compared using a log-rank test. Multivariate analysis of time to local failure was performed using the Cox Proportional Hazards model with two continuous covariates: dose and SUV_{max}. All calculations and analyses were performed using the STATA/SE 8.0 software (STATA Corporation, College Station, TX, USA). Medians were compared using the Mann–Whitney-*U*-Test. *p*-values of less than 0.05 were considered as statistically significant and *p*-values between 0.05 and 0.1 as a statistical trend.