2-Deoxy-2-[^{18}F]Fluoro-D-Glucose Uptake and Correlation to Intratumoral Heterogeneity

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Abstract. *The aim of this study was to investigate the pattern of 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG) uptake in relation to the intratumoral histopathological appearance. Materials and Methods: Intratumoral distribution of FDG in nude mice with xenografted tumours originating from an established head and neck squamous cell carcinoma was studied. FDG uptake and the correlation to histopathological appearance was evaluated in four separate quarters of each tumour. Results: Variations in FDG uptake correlating to the presence of tumour cells was demonstrated. Quarters containing more than 50% tumour cells showed a significantly higher FDG uptake (p=0.028) than quarters with more stromal tissue and necrosis. Conclusion: This study shows that the heterogenic FDG uptake within a tumour correlates to histopathological findings and that the variable appearance of tracer uptake on the PET scan depends on distribution of different tissue components in the tumour. This intratumoral heterogeneity calls for caution when evaluating a PET scan where median values of larger areas will be misguiding and thus small areas with high uptake should be regarded as the regions of interest.*

Positron emission tomography (PET) with 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG) provides metabolic images of tumour glucose metabolism in head and neck cancer, as well as in many other tumours. PET imaging using FDG has been shown to be superior to conventional imaging methods in revealing the presence of residual or recurrent disease (1-2). Early detection of residual tumour tissue following curatively intended treatment may allow early initiation of salvage therapy and may improve the chance of survival. No clear advantage of PET imaging has been demonstrated in routine staging of head and neck cancer (3-5).

In the case of an unknown primary tumour with neck node metastasis, it has been claimed that PET can reveal the primary tumour in 10-30% of cases. For examples Menda and Graham reported 27% identification of primary tumours in this situation (4). Conflicting results regarding the true additive effect of FDG-PET in such cases can be attributed to differences in diagnostic work-up and radiological interpretation by the different research groups. Furthermore, several clinical studies have shown FDG-PET to be useful in the prediction of the response to chemo- and radiotherapy early during cytotoxic therapy (6-8). However, the fact that FDG accumulates in inflammatory lesions as well as in tumour tissue reduces the specificity of FDG-PET, leading to false-positive results. The mechanisms governing metabolic changes in a tumour during chemo- and radiotherapy are complex, involving intracellular changes as well as host factors. Attempts are being made in ongoing clinical and experimental studies to elucidate these mechanisms. An important issue is the timing of PET studies during and after therapy, in order to minimize false-positive and false-negative results.

The method of quantification of metabolic activity is also under debate. In many cases visual analysis is sufficient, often providing the same clinical information as the standardized uptake value, SUV, a semiquantitative analysis. Calculations of metabolic rate, MR, a quantitative measurement, require more information and can be laborious, but provide additional important information regarding tumour aggressiveness (9).

An important issue under discussion is the interpretation of the PET scan regarding the volume to be evaluated, *i.e.* the region of interest (ROI). This is of importance for semiquantitative and quantitative calculations. If larger areas in a heterogeneous tumour are chosen as regions of interest

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Key Words: Positron emission tomography, 2-deoxy-2-[^{18}F]fluoro-D-glucose, head and neck, intratumoral heterogeneity, xenograft, squamous cell carcinoma.

for metabolic calculations, the results will be flawed by inclusion of non-vital tumour tissues and non-tumour tissue. Small regions of interest may focus on hotspots, but may suffer from false low calculations due to the partial volume effect.

In this study, we investigated FDG uptake in an untreated xenografted squamous cell carcinoma in relation to histopathological appearance, with reference to intratumoral heterogeneity.

Materials and Methods

Tumour. An established squamous carcinoma cell line, LU-HNxSCC-14, originating from a human head and neck cancer from tongue was used. The cell line expresses wild-type p53 and no cyclin D1 amplification (10). Tumour cells were transferred by subcutaneous inoculation of 2x2x2 mm small tumour sections into one flank of each of 15 nude mice, 5-8 weeks of age as described, elsewhere (11). The tumours were allowed to grow to 10-15 mm in diameter.

FDG-uptake. On the day of FDG administration, the tumours were measured and the mice were weighed. After 4 h of fasting, during which only sugar-free liquids were allowed, the mice were orally fed 0.2 ml FDG (4 MBq). Forty-five minutes after FDG administration the mice were sacrificed by cervical dislocation and the tumour and brain were removed and weighed. The tumours were divided into four equal parts and each quarter section was weighed, with values ranging from 33 mg to 529 mg with a mean value of 216 mg. The FDG uptake was measured separately in brain and tumour quarters from each mouse in a 3 inch x 3 inch NaI(TI) well counter (in house) (1282 Compu Gamma CS, LKB Wallac, Finland). The FDG uptake in each quarter tumour section was determined from the quotient of radioactivity per unit of weight between brain and quarter tumour sections in each animal.

Histopathological examination. Each quarter section was fixed in formalin and then paraffin-embedded. One or two 5 μm-thick sections were stained with May-Grunwald Giemsa for histopathological examination. The proportion of tumour cells in each slice was estimated. Fifty-nine tumour quarters were examined, as one quarter was damaged. The samples were scored as follows: A=0-50% tumour cells present; B=51-100% tumour cells present. The correlation of FDG uptake with the histopathological findings is illustrated in Figure 1.

Statistics. Data were analyzed with the SPSS analysis software, SPSSWIN (SPSS Inc., Chicago, USA). Differences between FDG uptake and histopathological score were tested with a one-way ANOVA. Groups A and B were tested and found to be normally distributed with the Shapiro-Wilks test. The distributions were illustrated as histograms and box plots.

Results

The stromal tissue was clearly visualized by the May-Grunwald Giemsa staining and the intratumoral heterogeneity was observed in the different quarters, each represented by two slices. The proportion of tumour cells in each quarter varied as did necrotic and cystic areas. Tumour quarters containing predominantly tumour cells and fewer stromal cells showed significantly higher FDG uptake ($p=0.028$) than quarters with more stromal cells and fewer viable tumour cells, as illustrated in Figure 2. Quarters containing larger areas of necrotic cells were also observed to have less FDG uptake than quarters with smaller areas of necrosis. The intratumoral heterogeneity in the different quarters within the same tumour is illustrated in Figure 3. Intratumoral differences in FDG uptake are clearly demonstrated. Differences in FDG uptake can be seen even in small areas of a tumour.

Discussion

The present study demonstrated that intratumoral heterogeneity, as shown by histopathology, corresponds to heterogeneous metabolic activity measured with FDG-PET. The effect was clear, even in small areas of a tumour. These findings support earlier studies, which have shown that FDG uptake represents the fraction of viable cells in a tumour (12-13).

FDG uptake and metabolism within a tumour reflects viability within a tumour, but no clear correlation has been shown to exist between any particular cell or tumour characteristics and FDG metabolism. Host and stromal tissue properties may contribute to metabolic activity and this is an area requiring further investigation. The distribution of nutrients, as well as tracer, to the tumour is dependent on the blood flow which may vary over time due to several reasons such as tumour growth, necrosis and intratumoral pressure. Accumulation time for FDG after administration, around one hour, reflects metabolism in malignant tissue. FDG metabolism reflects the glucose demand of the cells and thus the metabolic activity in a tumour is influenced by the glycolytic status of the tumour cells. Glucose demand is further up-regulated in the malignant transformation by overexpression of glucose transporters such as Glut-1 (14).

Other parameters believed to affect the metabolism in a tumour, such as histological grade and differentiation grade, tumour size and stage, have also been investigated regarding correlation to FDG metabolism. Kitawaga *et al.* (15) found that tumour size and grade correlated to FDG uptake: the larger the tumour and the lower the grade, the higher the FDG uptake. Several additional studies have been conducted to elucidate correlations between FDG uptake and cellular characteristics. Higashi and Clavo found a correlation between large cell volume increase and elevated FDG metabolism temporarily during radiotherapy (16) and Haberkorn *et al.* between larger growth fraction and high FDG avidity (17). A positive but weak relationship was also found between DNA ploidy and FDG uptake, where non-diploid tumours exhibited a higher FDG uptake than diploid tumours (18).

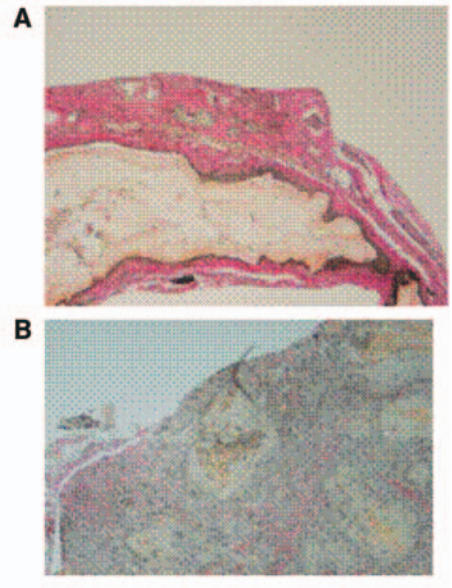

Figure 1. *Example of the correlation between the histopathological appearance with May-Grunwald Giemsa staining and FDG uptake in slices from different tumour quarters. Upper image, A, shows the histopathological appearance with a larger necrotic area, with fewer tumour cells and FDG uptake 0.1 in relation to brain FDG uptake, while the lower image, B, shows more tumour cells and a higher FDG ratio of 0.5.*

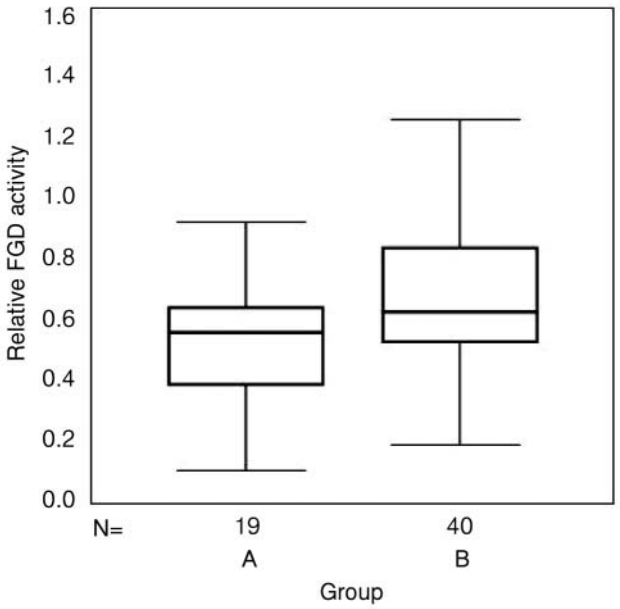

Figure 2. *Boxplot diagram of the FDG uptake in each quarter tumour section (N=number of tumour quarter sections). The samples scored: group A=0-50% tumour cells present and group B=51-100% tumour cells present.*

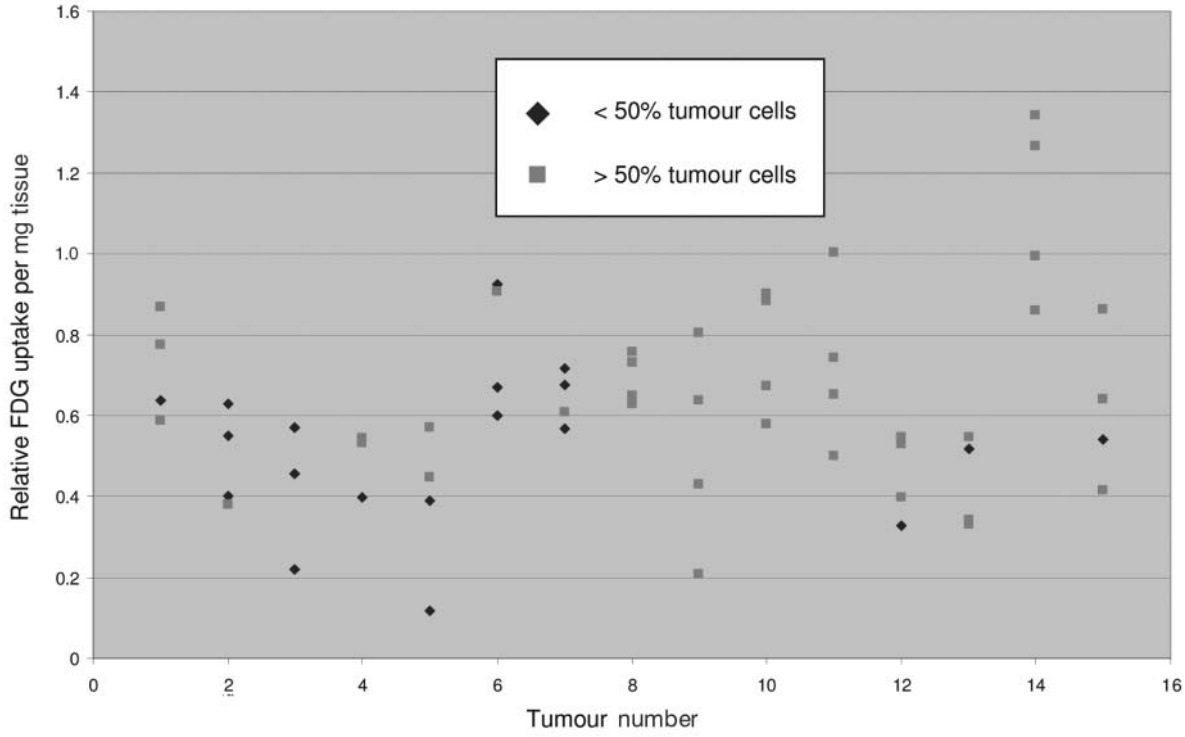

Figure 3. *The intratumoral heterogeneity illustrated by the relative FDG uptake per mg tissue in the quarter sections of the same tumour (15 tumours and 59 quarters were investigated).*

Cytotoxic treatment leads not only to complex cellular responses involving intracellular tumour changes, but also to changes in the surrounding stromal cells and incoming inflammatory cells such as monocytes, lymphocytes and granulocytes. These reactive cells are FDG avid (19-21). During therapy, the large numbers of inflammatory response cells take up FDG and thus result in a non-malignant hypermetabolism, *i.e.* false-positive activity, in the PET scan. In a leukemia tumour model xenografted onto nude mice, Spaepen *et al.* showed that this false-positive contribution to tumour metabolism was visible on days 1-10 after cytotoxic therapy, then declined such that on day 15 the FDG metabolism represented the viable tumour fraction (22). This suggests that early prediction of the response to treatment is possible with FDG-PET. In other words, tumour response can be predicted by the metabolic changes, which occur before radiological or palpable tumour regression can be established. Several clinical studies have proven that this is the case, in head and neck cancers as well as in other tumours (23-25).

Areas exhibiting high FDG uptake, so called hotspots, are explained by a high metabolic activity due to such factors as increased glucose transport, glycolytic status, hypoxia, proliferation rate, grade of differentiation and stromal influence on the metabolic situation. These characteristics represent aggressive behaviour which must be controlled in order to achieve tumour control.

The heterogeneity in tracer uptake and metabolism correlated to histopathological appearance found in this study should be considered when an ROI in the PET scan is defined. This is the case when a semi-quantative or quantative analysis of the metabolic activity is carried out. SUV, as a simple method, is frequently used but cautions must be issued. If larger regions are chosen, tissue not representing tumour tissue, such as necrotic tumour and stromal tissue, will be included, and a mean value of the regional metabolism will not reflect the true tumour behaviour. Small areas with metabolic hotspots are the areas of interest and only these should be included when analyzing FDG metabolism in a malignant tumour.

Acknowledgements

This work was supported by the Foundations of the University Hospital of Lund, the Swedish Cancer Society (1304-B05-19XBC, 475-B02-01XAB and 4839-B05-03PCC), the King Gustaf V Jubilee Fund (05-44161) and governmental funding of clinical research within the Health Care region of Scania, R&D funding, Laryngologfonden, Nilsson's Cancer Foundation.

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Received March 2, 2007
Revised May 4, 2007
Accepted May 10, 2007