The impact of gene expression on ^{18}F-FDG kinetics; a new chapter for diagnostic nuclear medicine

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Abstract

Nuclear medicine procedures are the methods of choice for the assessment of the tracer kinetics in a volume over time. Fluorine-18 fluoro-deoxyglucose (^{18}F-FDG) is primarily a marker of tumor viability and the kinetics of ^{18}F-FDG reflects major biological factors like angiogenesis and proliferation. The correct interpretation of ^{18}F-FDG tracer kinetics demands the knowledge about the association of quantitative positron emission tomography (PET) data and gene expression. The use of gene arrays is helpful to obtain expression data for a large number of genes from tissue samples. However, limited data are available about quantitative ^{18}F-FDG data and gene array results. Studies in primary liver cancer patients revealed that the ^{18}F-FDG uptake was associated with genes related to tumor cell adhesion and tumor invasion. We noted in patients with giant cell tumors a correlation of the ^{18}F-FDG uptake, as measured by the standardized uptake value (SUV) and the cell division cycle 2 (cdc2) gene expression. The effect of therapeutic interventions is dependent on the agent used for treatment. In gastrointestinal stromal tumors the change in ^{18}F-FDG uptake is most likely due to an antiproliferative effect. However, this may be different in other tumor types and for other treatment protocols, therefore dedicated studies of the ^{18}F-FDG kinetics and gene expression are needed. Based on the recent data available in colorectal tumors and gene expression, we were able to demonstrate that at least two key genes of the angiogenesis, vascular endothelial growth factor (VEGF-A) and angiopoietin-2, have a major impact on the tracer kinetics. Furthermore, regression functions for the ^{18}F-FDG kinetics and gene expression data facilitate the calculation of parametric images of the gene expression, reflecting the spatial distribution of angiogenesis in a colorectal tumor. Currently the development of information management systems for the prediction of clinical relevant information in individual patients is in progress to retrieve the optimum on information from individual ^{18}F-FDG patient examinations to support individualization of treatment management.

Keywords: PET -18F-FDG – Angiogenesis – Gene expression – Gene array

Introduction

Positron emission tomography (PET) has found widespread use in the last two decades. Besides the progress in the performance of PET cameras, the availability of a tracer like fluorine-18-fluoro-deoxyglucose (^{18}F-FDG) has supported the application of PET for clinical purpose, especially in oncological patients. Gambhir et al. (2001) performed a meta-analysis of the literature concerning PET with ^{18}F-FDG [1]. The overall sensitivity was high in most of these studies while the specificity was variable, primarily due to the accumulation of ^{18}F-FDG in some benign diseases, in particular in inflammatory processes [2, 3]. However, to improve the specificity of PET examinations other tracers may be used, which provide different information as compared to ^{18}F-FDG. Another option is to quantify the ^{18}F-FDG kinetics most accurately in order to assess differences in tracer kinetics with regard to histology [2, 4].

The kinetics of ^{18}F-FDG is primarily dependent on glucose transporters and hexokinases, because it is an analogue of glucose. ^{18}F-FDG is transported into the cells like glucose via the facilitated glucose transporters. Currently 14 facilitated glucose transporters are known, which are abbreviated with SLC2A1 to SLC2A14 [5]. Furthermore, for SLC2A3 four different pseudogenes are identified. This network of glucose transporters, guarantees that glucose is always available for the energy metabolism of the cells. The fast availability of glucose for the energy metabolism of the cells, is generally essential for all cells therefore nature has developed a nearly failure-proof system to get glucose into the cells. In contrast to glucose, the sodium dependent glucose transporters are not transporting ^{18}F-FDG. The intracellular ^{18}F-FDG is phosphorylated but then trapped in the majority of tumors due to a low dephosp horylation rate. Four different hexokinases have been identified [5].

Changes in glucose transporters and hexokinases expression had been reported in several diseases. However, it is known from experimental studies that the kinetics of glucose metabolism is modulated by several other factors, which have an impact on glucose transporters and/or hexokinases. Studies in two human small cell lung cancer cell lines by Petersen et al. (2001) gave evidence for a dependency of SLC2A1 and SLC2A3 on the vascular endothelial growth factor, which is a key element of tumor angiogenesis [6]. Others focused on the impact of hypoxia on glucose metabolism [7]. Airley and Mobasheri (2007), evaluated the impact of hypoxia on angiogenesis and glucose metabolism and noted a dependency of angiogenesis and glucose metabolism on hypoxia [7]. Recently we investigated the impact of angiogenesis related genes on the ^{18}F-FDG kinetics in patients with colorectal tumors [8]. The ^{18}F-FDG kinetics was quantified with a two-tissue compartment model and significant correlations were obtained for the compartment parameters and the gene expression

data of VEGF-A and angiopoietin-2 [8]. Correlation coefficients of r=0.75 and r=0.76 were obtained for these genes respectively, which demonstrates, that about 56%-58 % of the variance of the data is explained by an impact of angiogenesis on the ^{18}F-FDG kinetics in these tumors. According to experimental data obtained in non small cells lung cancer (NSCLC) [6], we also noted a dependency of K1 on the vascular endothelium growth factor (VEGF-A) expression in colorectal tumors [8]. Therefore, angiogenesis plays a key role regarding the ^{18}F-FDG uptake in colorectal tumors and the knowledge of the dependencies to the ^{18}F-FDG kinetics can help to predict the amount of angiogenesis in these tumors.
Overall, the current literature data give evidence for an interaction between several biological processes like angiogenesis, hypoxia and the metabolism of glucose and thus also of ^{18}F-FDG. Therefore, more detailed knowledge of ^{18}F-FDG kinetics and its dependency on gene expression may help to achieve an improved interpretation of ^{18}F-FDG uptake in tumors. The ^{18}F-FDG kinetics can be accurately quantified using dynamic PET data and the application of a two-tissue compartment model on volumes of interests (VOI) or even on a voxel base, to achieve parametric images. In order to assess the dependency of ^{18}F-FDG kinetics on a large number of genes the use of gene array examinations of tumor and reference tissues is required. The correlative analysis of both, PET and gene expression data, demands the use of dedicated software, because most of the gene array analysis software does not support the correlative assessment of multiple large data sets. We developed dedicated software, Gene-PET, which facilitates the associative and correlative analysis of two large matrices, e.g. gene array data and PET data, or clinical data, etc [9]. The software is currently in use at our center for correlative PET and gene array studies as well as for the development of new radiopharmaceuticals, based on the identification of target structures and in silico modeling methods.

Only a limited number of authors have evaluated the dependencies of ^{18}F-FDG uptake and gene expression in different tumor types. A literature search for "^{18}F-FDG, gene expression, array" revealed seven major publications. Lee et al. (2004) evaluated the ^{18}F-FDG uptake in ten patients with hepatocellular carcinomas [10]. The authors compared the PET data with those obtained by gene array analysis using an array providing quantitative data of about 10108 genes. Cluster analysis revealed that eleven genes of 991 enhanced expressed genes, were associated with an increased ^{18}F-FDG uptake. Among these, in particular genes related to tumor cell adhesion and tumor invasion, were correlated with an enhanced ^{18}F-FDG metabolism [10]. This correlation is important for the interpretation of the ^{18}F-FDG uptake values in terms of tumor invasiveness. Based on the results from Lee et al., (2001) a high ^{18}F-FDG uptake is associated with a higher likelihood of tumor invasiveness and tumor spread. Therefore, the quantification of the ^{18}F-FDG uptake may be helpful for the individualization of treatment management in these patients.

Tumors with different histologies can also have different ^{18}F-FDG kinetics. However, treatment may also modulate the ^{18}F-FDG uptake. The effect of treatment with imatinib on gene expression and ^{18}F-FDG-PET results was evaluated by Trent et al. (2006) [11]. The authors examined eleven patients with gastrointestinal stromal tumors (GIST) prior and after treatment with imatinib, using PET and ^{18}F-FDG. Furthermore, tumor specimen, were obtained by biopsy and/or surgery and analyzed using gene array technology. Additionally, experimental studies were performed with imatinib sensitive and resistant cell lines. Interestingly, the authors noted an inverse correlation of ^{18}F-FDG uptake and the expression of the insulin-like growth factor binding protein-3 (IGFBP-3). Patients with response to treatment and decreased ^{18}F-FDG uptake following therapy had enhanced expression of IGFBP-3 [11]. It is known from other studies that IGFBP-3 induces apoptosis directly [12]. Apoptosis is a complex mechanism, initially associated with a stop of cell proliferation. The impact of IGFBP-3 on the cell cycle may explain the association with low ^{18}F-FDG uptake values. We noted in patients with giant cell tumors a significant correlation of the ^{18}F-FDG uptake, as measured by the standardized uptake value (SUV) and the cell division cycle 2 (cdc2) gene expression [13]. The cdc2 gene encodes a protein kinase that interacts with cyclins and controls the cell cycle. It has a major impact on the G1 to S and G2 to M transition. Thus, the therapeutically induced enhanced IGFBP-3 expression may modulate the ^{18}F-FDG kinetics via the cdc2 cell cycle mechanism.

One major important biological process in most of the tumors is angiogenesis. Several approaches had been investigated to assess angiogenesis with nuclear medicine procedures [14]. The ^{18}F-FDG kinetics is also closely associated with angiogenesis in tumors like in colorectal carcinomas. We were able to demonstrate that the angiogenesis-related gene expression contributes to more than 50% of the variance of the kinetic ^{18}F-FDG data in colorectal tumors [8]. The parametric data of the ^{18}F-FDG kinetics may therefore be used together with dedicated regression functions for VEGF-A and angiopoietin-2 to predict the expression of these genes on a VOI base. Expanding this approach to a voxel base, it is even possible to calculate parametric images of the predicted VEGF-A and angiopoietin-2 expression in these tumors. This type of parametric imaging, based on the correlation of ^{18}F-FDG kinetics and gene expression data, is new and currently in development at our center (Fig. 1). Interestingly, the parametric image of K1 (Fig. 1, middle) is overall comparable to the parametric image of VEGF-A (Fig. 1, lower), because K1 is dependent on angiogenesis. The distribution of the maximum SUV in the ^{18}F-FDG uptake image (Fig, 1, upper) however is different, because the uptake in colorectal tumors is dependent on k3. Generally, information management of 4D PET data sets is one of the major tasks in future. This comprises the processing of dynamic PET data and the feature extraction from these 4D data sets using compartment and non-compartment models. The results from the feature extraction methods can be used to predict important biological parameters like angiogenesis, provided that we know the correlation between the PET data and the expression of angiogenesis relat-

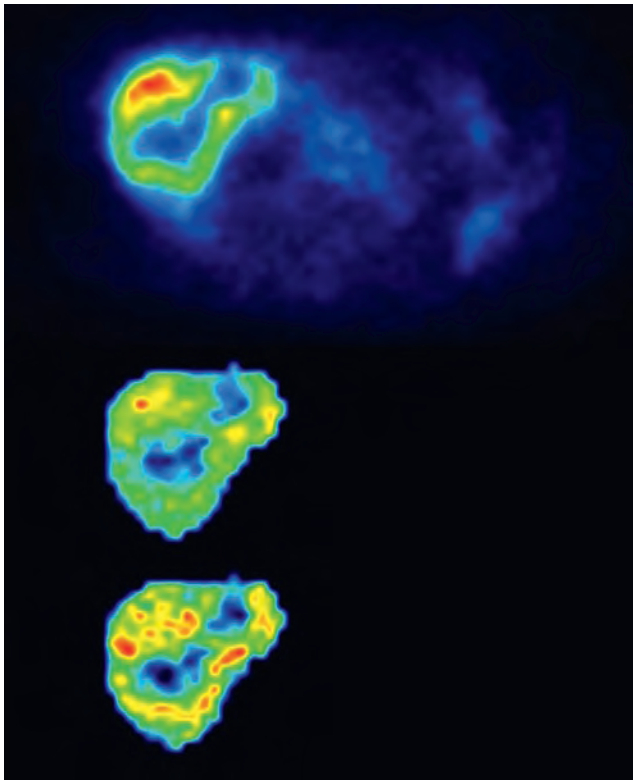

Figure 1. Upper: ^{18}F-FDG uptake image (SUV image) of a colorectal tumor. Middle: parametric image of K1 (transport of ^{18}F-FDG into the cells). Lower: Prediction of VEGF-A expression in the colorectal tumor. The calculation is based on parametric data obtained from a dynamic PET examination.

ed genes in tumors. Thus, we can obtain individual predictive data in patients, which can be used to optimize treatment. For example, prediction of angiogenesis may be used to decide about an anti-angiogenetic treatment in individual patients.

Correlative studies of PET and gene expression are basic tools to combine quantitative PET data and biological parameters. If the knowledge about the dependencies and correlations between tracer kinetics and biological factors is expanded, we can provide more detailed information about a tumor individually. Thus, the treatment management of oncological patients can be optimized and individualized. Currently most of the PET examinations are performed just as static studies, sometimes as double time point examinations. Interestingly, the approach is very different e.g. in magnetic resonance imaging, with the increasing use of dynamic contrast material studies and the calculation of parametric images. We should remember that nuclear medicine actually is based on the dynamics of tracer distribution and dynamic examinations should find more use in future to provide improved, detailed information. Besides the standard acquisition of a dynamic study for one hour, shortened acquisition protocols combining a dynamic study for only 10-20 min with late static images may be used alternatively for routine patient examinations [15]. Even parametric images can be calculated from a shortened acquisition protocol. Thus, nuclear medicine procedures may find more attention in the future for the diagnostics and treatment management of oncological patients.

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