GLUT1 Expression in Tissue and ^{18}F-FDG Uptake

For staging of cancer patients, ^{18}F-FDG PET imaging has been applied for about a decade. It is generally accepted that imaging the metabolic activity of tumor tissue provides more sensitive and more specific information about the extent of disease than morphologic or anatomic imaging alone. The metabolic activity offers additional information about cancer biology and can be used to determine tumor aggressiveness and also help to assess response to treatment (*1,2*). In contrast, the rate-limiting step for the cellular accumulation of ^{18}F-FDG in different tissues is still not fully understood. The uptake mechanism and biochemical pathway of ^{18}F-FDG has been extensively studied in vitro and in vivo, and the transport through the cell membrane via glucose transport proteins (GLUTs) and the intracellular phosphorylation by hexokinase (HK) have been identified as key steps for subsequent tissue accumulation. As FDG-6-phosphate is not a suitable substrate for glucose-6-phosphate isomerase, and the enzyme level of glucose-6-phosphatase is generally low in tumors, FDG-6-phosphate accumulates in cells and is visualized by PET. ^{18}F-FDG uptake in tissue, however, is not tumor specific and little is known about the underlying cellular mechanisms of ^{18}F-FDG accumulation in inflammatory tissue. Therefore, the study by Chung et al. on pages 999–1003 in this issue of *The Journal of Nuclear Medicine* (*3*) addresses an important issue of exploring in more detail false-positive and false-negative PET results on a cellular level. ^{18}F-FDG PET imaging of 62 patients with non-small cell lung cancer (NSCLC) was compared with histology and immunohistochemistry of mediastinal lymph nodes obtained from surgery or mediastinoscopy. The GLUT1 expression in metastatic tumors was higher in true-positive lymph nodes than that in false-negative lymph nodes, and metastatic squamous cell carcinoma showed stronger GLUT1 expression than metastatic adenocarcinomas. A higher grade of follicular hyperplasia was found in false-positive compared with true-negative lymph nodes, and the lymphoid follicular cells were strongly positive for the expression of GLUT1. The authors concluded that lymphoid follicular hyperplasia with GLUT1 overexpression may be related to false-positive results in PET imaging, and the weak expression of GLUT1 in adenocarcinomas might be responsible for false-negative results in mediastinal staging.

These conclusions are based on 2 hypotheses: (a) Cellular ^{18}F-FDG uptake is predominantly related to expression of GLUT1, and (b) overexpression of GLUT1 in tissue results in increased ^{18}F-FDG uptake. Therefore, false-negative ^{18}F-FDG PET imaging may be explained by weak GLUT1 expression, and nonmalignant tissue expressing GLUT1 will cause subsequent false-positive ^{18}F-FDG uptake.

Glucose is the major substrate for energy supply of mammalian cells and is a precursor of glycoproteins, triglycerides, glycogen, as well as riboses necessary for RNA and DNA synthesis. Because of its hydrophilic character, specific transport proteins are required for glucose to cross the cell membrane. They are present in all cell types and provide a central pore for the transmembrane passage of glucose. The number of known mammalian glucose transporters (GLUTs) has expanded over the past several years and 2 different families have been identified: sodium glucose cotransporters (SGLTs) and facilitative GLUTs (*4*). SGLTs consist of 14 transmembrane domains, and 6 subtypes (SGLT1–SGLT6) have been identified (*5*). They couple the movement of sodium down a gradient with the transport of glucose up a gradient. Little is known about the expression of SGLTs in tumors; however, SGLT2 has been recently suggested to play a role in glucose uptake of lung cancer metastases (*6*). Thirteen members of the family of facilitative GLUTs (GLUT1–GLUT13) are known (*4*). These various transporters consist of 12 transmembrane domains and are only capable of carrying glucose and other sugars down a gradient. They exhibit different substrate specificities, kinetic properties, and expression in tissue depending on the cellular demand and regulation. GLUT1, which is almost ubiquitously expressed in all cell types, mediates glucose transport into erythrocytes and through the blood–brain barrier, and its expression is also upregulated in many tumors (*5*). GLUT2 is found in the liver, pancreatic β-cells, and other tissues. Its affinity for glucose is lower than that of GLUT1, but it has broader substrate specificity and also transports other sugars such as fructose and glucosamine. GLUT3 has the highest affinity for glucose and is the major form found in neurons. GLUT4 is the insulin-sensitive transporter and is found, for example, in muscle tissue.

The presence of several different transporters in certain tissues and cells indicates that glucose delivery into cells is a process of considerable complexity. Although different types of tumors demonstrate overexpression of

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GLUT1, an inconsistent relationship between ^{18}F-FDG uptake and expression of glucose transport proteins has been found. In 1993, Brown and Wahl reported positive GLUT1 staining in the cell membrane and in the cytoplasm in primary and metastatic breast cancer, but with considerable intratumoral and intertumoral variability (*7*). In a recent study by the same group, GLUT1 expression in breast cancer appeared to be associated with ^{18}F-FDG uptake (*8*). In contrast, Avril et al. did not find a significant correlation between GLUT1 immunohistochemistry and ^{18}F-FDG uptake in breast cancer (*9*). Generally, GLUT1 expression in primary lung tumors is higher than that in normal lung tissue (*10*). Comparing the expression of 5 GLUTs in primary NSCLCs, GLUT1 expression was significantly higher than that of the other GLUTs (*11*). Higashi et al. found a positive correlation between GLUT1 expression and ^{18}F-FDG uptake in NSCLC (*12*). Nevertheless, in 73 patients with stage 1 lung cancer, GLUT1 and GLUT3 transporter expression did not correlate with the level of ^{18}F-FDG uptake (*13*). Kurata et al. suggested that the GLUTs expressed in metastatic lung cancer may be different from those in primary tumors and higher levels of GLUT3 and GLUT5 gene expression were found in liver metastases compared with that of the primary lung tumors (*10*). There was, however, no difference in GLUT1 expression between primary and metastatic tumors. Higher GLUT1 expression was observed in squamous cell carcinomas than that in adenocarcinomas of the lung (*11*), which correlates well with the findings in metastatic lymph nodes reported in the study by Chung et al. (*3*).

To better understand these conflicting findings, it is important to recognize the different methods used and their specific limitations to study the expression of GLUTs in tissue. These include the reverse-transcriptase polymerase chain reaction, Northern blot analysis to detect GLUT messenger RNA (mRNA), and immunohistochemistry of GLUTs using the appropriate antibodies. Some tumor cells, for example, were found to express specific GLUT mRNA, but not the respective protein (*14*). Immunohistochemistry is not a quantitative method per se and the staining intensity may vary depending on the procedure used, and the scoring methods applied for quantification may also introduce a systematic bias. The increased expression of HK in metabolically active tissue, which affects the rate of ^{18}F-FDG uptake, also must be taken into consideration. It has been shown that HK specific activity is higher in tissue homogenates of malignant tumors compared with that in benign tumors or normal tissue (*15*). There are 4 isoenzymes of HK in mammalian tissues. HKI, II, and III have high glucose affinity and are susceptible to product inhibition by glucose-6-phosphate. HKI and HKII are associated with mitochondria, whereas HKIII is localized at the periphery of the nucleus. In cancer, glucose metabolism seems to be primarily regulated by HKII. In human breast cancers, for example, a 13-fold increase in HK specific activity was found compared with that of normal breast tissue (*16*). If phosphorylation is the rate-limiting step for ^{18}F-FDG accumulation in tissue, GLUT1 expression could be subsequently upregulated, explaining partially the conflicting results. Another important aspect is the comparison of findings on a cellular or microscopic level with the PET signal with a resolution in the subcentimeter range. The limitations of quantitatively measuring the ^{18}F-FDG uptake in tumor tissue with static PET acquisition protocols are well known and may also limit imaging–biologic subcellular correlations.

Chung et al. (*3*) found high expression of GLUT1 in lymphoid follicular cells and a higher grade of follicular hyperplasia in false-positive compared with that of true-negative lymph nodes. Lymph nodes generally react to inflammatory processes in the area they drain, and any infectious agent—including bacterial, viral, protozoan, and parasitic pathogens—can cause follicular hyperplasia. The offending organism is brought into the lymph node by lymphatic drainage and is phagocytized and degraded within macrophages in the lymph nodes, initiating an immune reaction. Microscopically, the follicles are significantly enlarged and the germinal centers are particularly prominent, where multiple mitotic figures and active proliferation of cells may be seen. Does the increased GLUT1 expression of lymphoid follicular cells sufficiently explain false-positive ^{18}F-FDG PET imaging in lymph nodes? First, lymphoid follicles are present in all lymph nodes and any kind of immunostimulation results in an increase in the number and size of lymphoid follicles. Second, the size of lymphoid follicles is generally $<$1 mm, although the number of follicles can exceed 100 per lymph node. On the other hand, reactive lymph nodes with lymphoid follicular hyperplasia are very common—for example, in cervical or inguinal lymph nodes without causing a significant number of false-positive PET results. A limitation in the study of Chung et al. is the association of specific lymph nodes obtained during surgery with areas of increased ^{18}F-FDG uptake in PET imaging. The exact anatomic localization of ^{18}F-FDG–positive mediastinal lymph nodes is difficult to define, and combined PET/CT imaging may help match specific lymph nodes in future studies. Ideally, a small amount of ^{18}F-FDG injected before surgery would allow assay of the ^{18}F-FDG uptake in all harvested lymph nodes, which is frequently in the range between 20 and 50 per patient during lung cancer surgery. It would also allow direct comparison of the grade of follicular hyperplasia with ^{18}F-FDG uptake. Since only GLUT1 located in the cellular membrane can contribute to the ^{18}F-FDG influx into cells, quantitative Western blot analysis of membrane preparations combined with immunofluorescence labeling identifying cytoplasmic versus membrane-localized GLUTs would allow further elucidation of the role of GLUT1 in lymphoid follicular cells. The crucial question, however, is

whether tissue is ^{18}F-FDG avid because it is found to (over)express GLUT1. Various tissues in the body present with high levels of GLUTs without demonstrating increased ^{18}F-FDG uptake. Prostate cancer has increased expression of GLUT1 and GLUT12 but is frequently negative in ^{18}F-FDG PET imaging (*17*). It is important to consider that even when GLUT1 is expressed in cells, it is not necessarily located only at the cell surface, and, hence, some may not be active in transmembrane transport. Hypoxia, for example, has been shown to result in translocation of GLUT1 and GLUT4 to the plasma membrane as well as activation of preexisting GLUT1 in the plasma membrane (*18*). Although tumors frequently overexpress GLUT1, the cellular uptake of ^{18}F-FDG is not exclusive to GLUT1 and, thus, positive GLUT1 staining might not be directly related to increased ^{18}F-FDG uptake in PET imaging.

What do we learn from this study? There is no conclusive data available to completely explain the cellular mechanisms and regulations of glucose metabolism and related ^{18}F-FDG uptake in cancer and normal tissue. There are even reasons to believe that it might not be possible with current technology to exactly determine the molecular, cellular, tissue-, and organ-related variables that define the ^{18}F-FDG uptake in various tissues. Taking into account the inter- and intratumor variability in cellular density, macroscopic and microscopic blood supply, fraction of hypoxic tissue, and myriad enzyme systems involved not only in glucose and ^{18}F-FDG uptake, but also in ^{18}F-FDG accumulation, the resulting ^{18}F-FDG signal is more likely to be determined by a complex interaction of all of these factors, rather than by one rate-limiting step. The one specific factor that is determining the ^{18}F-FDG accumulation in tumor and nontumor tissue just might not exist.

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