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# From tumor biology to clinical PET: A review of positron emission tomography (PET) in oncology*

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Cancer cells show increased metabolism of both glucose and amino acids, which can be monitored with $^{18}$F-2-deoxy-2-fluoro-D-glucose (FDG), a glucose analogue, and $^{11}$C-L-methionine (Met), respectively. FDG uptake is higher in fast-growing than in slow-growing tumors. FDG uptake is considered to be a good marker of the grade of malignancy. Several studies have indicated that the degree of FDG uptake in primary lung cancer can be used as a prognostic indicator. Differential diagnosis of lung tumors has been studied extensively with both computed tomography (CT) and positron emission tomography (PET). It has been established that FDG-PET is clinically very useful and that its diagnostic accuracy is higher than that of CT. Detection of lymph node or distant metastases in known cancer patients using a whole-body imaging technique with FDG-PET has become a good indication for PET. FDG uptake may be seen in a variety of tissues due to physiological glucose consumption. Also FDG uptake is not specific for cancer. Various types of active inflammation showed FDG uptake to a certain high level. Understanding of the physiological and benign causes of FDG uptake is important for accurate interpretation of FDG-PET.

In monitoring radio/chemotherapy, changes in FDG uptake correlate with the number of viable cancer cells, whereas Met is a marker of proliferation. Reduction of FDG uptake is a sensitive marker of viable tissue, preceding necrotic extension and volumetric shrinkage. FDG-PET is useful for the detection of recurrence and for monitoring the therapeutic response of tumor tissues in various cancers, including those of the lung, colon, and head and neck. Thus, PET, particularly with FDG, is effective in monitoring cancer cell viability, and is clinically very useful for the diagnosis and detection of recurrence of lung and other cancers.

**Key words:** positron emission tomography, tumor diagnosis, lung cancer, autoradiography, $^{18}$F-fluorodeoxyglucose, $^{11}$C-methionine, radiotherapy monitoring, tumor hypoxia, $^{18}$F-fluoromisonidazole

## INTRODUCTION

POSITRON EMISSION TOMOGRAPHY (PET), especially with $^{18}$F-2-deoxy-2-fluoro-D-glucose (FDG) has been used for the diagnosis of cancers in various organs. Its diagnostic accuracy is generally higher than that of the conventional imaging technique of CT/magnetic resonance imaging (MRI), and PET results may facilitate more appropriate therapeutic planning for cancer patients. Because PET visualizes molecular events in living human tissue, an understanding of the biological characteristics of tumor tissue, and its correlation to tracer uptake, is essential for oncological application of PET. In this review, I focus on the principles of cancer cell metabolism and its correlation with tracer uptake, the distribution of tracers in whole-body imaging and examples of clinical studies, the structure of tumor tissue, effects of therapeutic intervention, and detection of hypoxia. This review is not intended to cover cancers in all organs, but will aid in understanding the physiological and pathological background of tracer uptake and its clinical application. After reading this review and gaining a degree of knowledge about PET in oncology, I recommend to the reader several other

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reviews that have listed clinical data for a wide variety of cancers.[1–8]

## Cancer Cell Metabolism, Proliferation and Tracers

*Glucose metabolism of cancer cells*

An enhanced glycolytic rate in cancer cells was first demonstrated more than 70 years ago. Originally, decreased respiration and an increase in both aerobic and anaerobic glycolysis were considered to be the most important and specific characteristics of cancer cells.[9] Considerable efforts have been devoted to elucidating the role of increased glycolysis in malignant cell proliferation. Studies using Morris hepatoma cell lines revealed that the degree of increased glycolysis and the activity of key enzymes in glycolysis, such as hexokinase, correlated with the rate of tumor growth.[10] But none have conclusively determined whether a high glycolytic rate is essential for cancer cells or is a consequence of other metabolic processes. It was later demonstrated that many, but not all, tumor cells and some proliferating normal cells exhibited high rates of glycolysis, and that increased glycolysis was neither an essential property of proliferating cells nor a distinction between malignancy and benignancy.[11]

More recently, increased glucose transport has been studied in malignant transformed cells.[12] Malignant transformation by oncogenes coincides with high levels of glucose transporter messenger RNA.[13,14] Analyses of resected human cancer tissues have demonstrated increased expression of glucose transporters in brain tumors[15] and various abdominal malignancies.[16]

2-deoxyglucose (2DG) was originally studied as an anticancer drug to inhibit glucose metabolism of tumors,[17,18] but this aim was abandoned due to significant side-effects associated with high pharmacological doses.[19] It has since been used as a $^{14}$C-labeled tracer for glucose metabolism in research.[20] After successful labeling with $^{18}$F to form $^{18}$F-2-deoxy-2-fluoro-D-glucose (FDG, Fig. 1),[21] FDG has been used for tumor imaging.[22–24] FDG is transported into the cell through the glucose transporter protein at the cell membrane, depending on the concentration gradient of glucose from outside to inside the cell. This process does not require energy (ATP). In the cell, FDG is phosphorylated to FDG-6-phosphate by hexokinase and ATP, a rate-limiting step in glycolysis. FDG-6-phosphate is not metabolized further in the glycolytic pathway. The cell membrane is not permeable to intermediate phosphorylated products, so that FDG-6-phosphate remains trapped inside the cell, in a process called "metabolic trapping." The reverse reaction from FDG-6-phosphate to FDG, mediated by glucose-6-phosphatase, is possible only in the liver and epithelia of the renal tubules and small intestine (Fig. 2). Accumulation of FDG by trapping is observed in the brain, myocardium and tumor tissues. FDG uptake reflects accelerated transport of glucose and increased activity of hexokinase.[25] Various

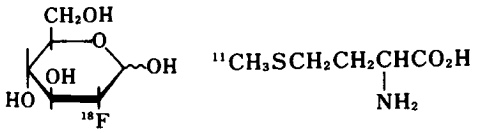



**Fig. 1** Structure of [$^{18}$F]-2-fluoro-2-deoxy-D-glucose (FDG, left) and [$^{11}$C]-L-methionine (Met, right).

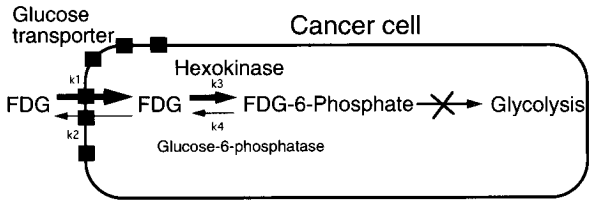



**Fig. 2** Metabolism of FDG. FDG uptake depends on the activity of glucose transporter and hexokinase.

metabolic substrates labeled with $^{11}$C, $^{18}$F, or $^{13}$N have been tested for tumor imaging, and, of these, FDG has become the most important and useful tracer for that indication.

*In vitro* studies of various human cancer cell lines have revealed a strong correlation between FDG uptake and glucose transporter expression, but no correlation between FDG uptake and the level of hexokinase activity[26,27] A strong correlation between glucose transporter expression and FDG uptake has also been reported in breast cancer and in non-small cell lung cancer (NSCLC)[28,29] These findings suggest that glucose transport may be important in characterizing the tumor uptake of FDG. This mechanism in cancer cells appears to differ from that in the brain, where the rate-limiting step for FDG uptake is hexokinase activity.

*Amino acid metabolism of cancer cells*

In addition to glucose, amino acids are important metabolic substrates for cancer cells. Incorporation of amino acids into the protein fraction has been correlated with tumor growth rate.[30] Increased transport of amino acids by viral transformed malignant cells was demonstrated *in vitro*.[31,32] Many cancer cell lines are dependent on methionine *in vitro* culture media due to increased transmethylation.[33] PET studies have demonstrated that $^{11}$C-L-methionine (Met) is useful for imaging lung cancers,[34,35] breast cancers[36] and malignant melanoma.[37] Met uptake reflects amino acid transport, trans-methylation and protein synthesis in tumor tissue.[38] Met uptake by tumor tissue plateaus early, at about 15–30 min after injection. In the early phase, a significant amount of free Met is pooled inside the tumor tissue. After this early phase, time-related incorporation of the cellular pool of free Met into macromolecules, protein, lipids, RNA and DNA continues until 1 hr or more after injection (Fig. 3).[39] Protein-bound Met also appears in the blood from about 0.5 hr

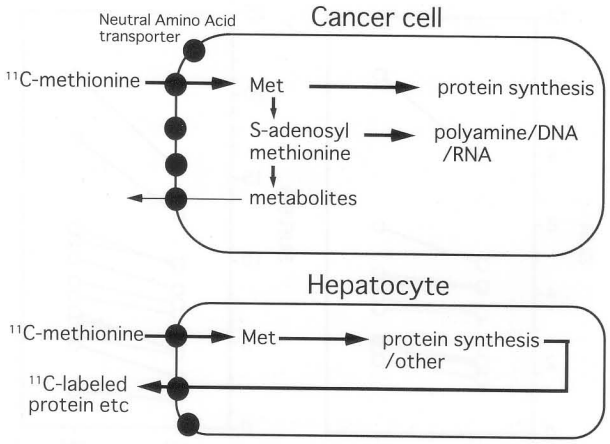



**Fig. 3** Metabolism of $^{11}$C-methionine. It is more complicated than FDG.

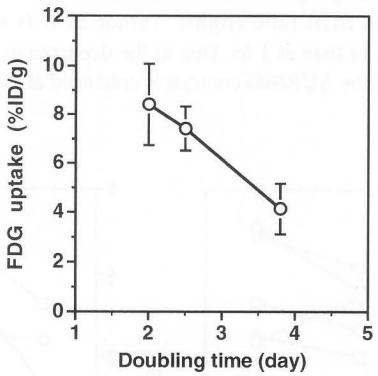



**Fig. 4** Correlation of FDG uptake and tumor growth rates *in vivo*. Results of FDG uptake study using three breast cancer models of mice with different growth rates were plotted. Rapid growing tumor showed higher FDG uptake. Modified from ref. (44).

after injection, and its percentages varies from patient to patient. Subtraction of the protein-bound Met activity from the total blood activity is important to ascertain the true input function for quantitative evaluation of Met uptake by means of a Patlak plot.[40,41]

*Proliferation and FDG or Met*

Both FDG and Met are good markers of tumor viability. Among tumors with different growth rates, FDG and Met uptake is higher in faster-growing than in slow-growing tumors.[39,42] The uptake difference between the faster- and slow-growing tumors is largest with FDG, and therefore FDG uptake is considered to be a good marker of the grade of malignancy (Fig. 4).[39,43,44] In proliferating cancer cells *in vivo*, FDG uptake exhibit cell cycle dependency. Higher uptake is observed in the cells of $G_0/G_1$ and $G_2$ phases than in the S and M phases. Cycling cells may consume glucose during the $G_0/G_1$ phase in preparation for the S (DNA synthesis) phase, and during the $G_2$ (gap 2) phase in preparation for the M phase (mitosis).[45] Among tumors

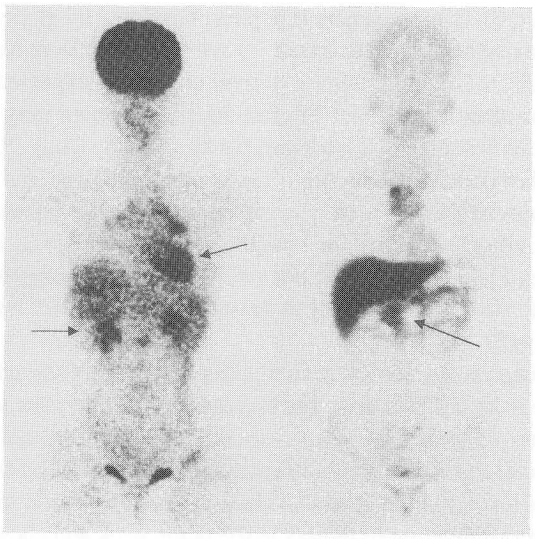


**Fig. 5** Whole-body distribution of FDG (left) and $^{11}$C-methionine (Met, right). In FDG image, high physiological uptake in brain, myocardium (arrow), kidney (arrow), bladder, and moderate uptake by liver were seen. Also mediastinal lymph node metastasis and bone metastasis from breast cancer were noted. In Met image, high uptake by liver and pancreas (arrow) were seen. Note low uptake by brain. Mediastinal tumor (non-invasive thymoma) was seen.

with the same growth rate, FDG uptake may be higher in those exhibiting undifferentiated histology than in well-differentiated tumors.[46]

Met or thymidine uptake has been reported to correlate more directly with proliferation. Cancer cells had the highest uptake of Met or thymidine in the early exponential growth phase, but low uptake in the plateau phase. FDG uptake had a different pattern and was more closely correlated with the number of viable cells.[47,48]

*Thymidine and its analogues*

Thymidine is incorporated into DNA synthesis, and it has been labeled with $^{11}$C for PET imaging. But the short half-life (20 min) and rapid *in vivo* degradation of $^{11}$C have restricted the use and quantitative evaluation of DNA synthesis rate with $^{11}$C-thymidine. [$^{18}$F]-3′-deoxy-3′-fluorothymidine ($^{18}$F-FLT) has been developed with the aim of metabolically trapping the substrate within the cell after phosphorylation by thymidine kinase 1 (TK). Preliminary *in vivo* evaluation of $^{18}$F-FLT with PET gave promising results, both in animal studies and in cancer patients.[49] Further evaluation in a large numbers of patients and a comparison with FDG are necessary.

## Whole-Body Distribution of FDG and Met

*Whole-body distribution*

FDG is transported, phosphorylated, and trapped as FDG-6-phosphate in the brain, heart and tumor tissues. In the

liver, FDG-6-phosphate is dephosphorylated by glucose-6-phosphatase, and excreted.[22] Liver FDG uptake reduces with time, and high tumor to liver contrast is expected at 1 hr after injection.[24] Tumors in the abdomen may be better visualized with a lower background by using FDG rather than Met. Glucose is not excreted in urine, but its analogue FDG is renally excreted, and therefore bladder activity may disturb the imaging of pelvic tumors.[50] The high FDG uptake of the normal brain may also interfere with detection of small metastatic tumors in the brain (Fig. 5).

High physiological accumulation of Met is observed in the liver and pancreas due to synthesis of digestive and other enzymes. Moderate uptake may be observed in salivary glands. Generally speaking, Met uptake by normal tissues in the head and chest is low. Therefore, high contrast imaging of tumors in the brain, neck and chest is expected with Met.[35–37,51] Because of the low Met uptake by normal brain, Met-PET is particularly useful in delineating the extent of invasion of brain tumors.[52]

*Peak time of uptake and delayed imaging*

The tracer uptake and clearance of Met are faster than those of FDG. Tumor uptake of Met plateaus at about 15–30 min after injection, while that of FDG plateaus after more than 1 hr has elapsed. PET analysis of breast cancer revealed that the tumor to non-tumor ratio was significantly higher in 3 hr images than in 1.5 hr images.[53] A dynamic FDG-PET study of lung cancer demonstrated the peak of FDG uptake at around 2–2.5 hr.[54] Extrapolation from dynamic FDG-PET studies of lung cancer showed that tumor uptake reached a peak level at around 4–6 hr.[55] In our study, we compared whole-body FDG-PET images at 1 and 2 hr after injection, in patients with lung cancer and malignant lymphoma. We found that, (a) all malignant tumors exhibited a higher FDG uptake at 2 hr than at 1 hr, (b) most normal tissues exhibited a lower FDG uptake at 2 hr than at 1 hr, and (c) consequently, the tumor to background contrast was enhanced at 2 hrs, and the sensitivity was improved (Figs. 6, 7).[56] Delayed imaging may suffer from higher noise due to the radioactivity decay of $^{18}F$, and high sensitivity of the PET scanner detector is therefore extremely important. High sensitivity with 3D data acquisition may be helpful for delayed tumor imaging.

*Effects of blood glucose*

Distribution of FDG is affected by blood glucose levels, because of the competition with glucose as a metabolic substrate. In hyperglycemia (after feeding or in diabetics) FDG uptake by tumor tissue is reduced,[57] but FDG uptake by myocardium and skeletal muscle is increased.[58,59] FDG uptake by the brain is also decreased in hyperglycemia.[57] With glucose loading, a greater reduction in uptake by normal brain than by tumor resulted in enhancement of tumor to normal cortex contrast.[60] But increased FDG

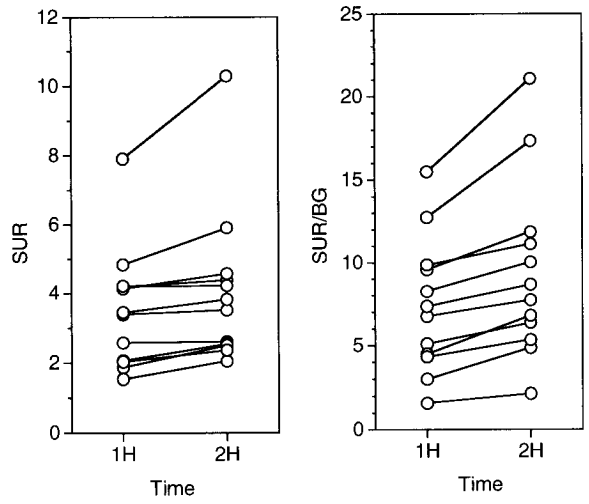



**Fig. 6** FDG uptake of primary lung cancer at 1 hr and 2 hr after injection was plotted as tumor SUR (left) and tumor SUR to background SUR ratio (right). Tumor SUR is significantly higher at 2 hr than at 1 hr. Due to the decrease in background activity at 2 hr, SUR/BG contrast is enhanced at 2 hr. From ref. (56).

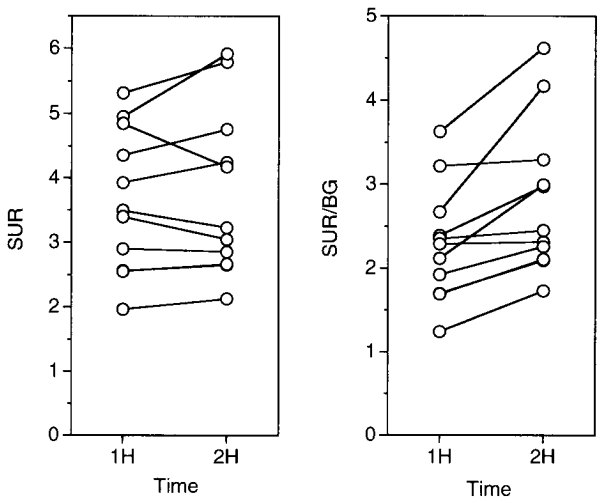



**Fig. 7** FDG uptake of mediastinal lymph node metastasis of lung cancer at 1 hr and 2 hr after injection. Lymph node SUR is not significantly different from 1 hr to 2 hr. Due to the decrease in mediastinal activity at 2 hr, SUR/BG contrast is significantly enhanced at 2 hr. From ref. (56).

uptake by skeletal muscle and decreased tumor uptake resulted in lower tumor to muscle contrast in body tumors in hyperglycemic patients.[61,62] Hyperglycemia could be responsible for false-negative FDG uptake by tumors in the body. Tumor uptake of Met is also affected by amino acid levels and by ingestion of food.[63] A fasting protocol is generally recommended for oncology applications of PET with FDG or Met. If the patient is diabetic, FDG-PET should be re-scheduled after the hyperglycemia has been controlled. But injection of insulin simultaneously with

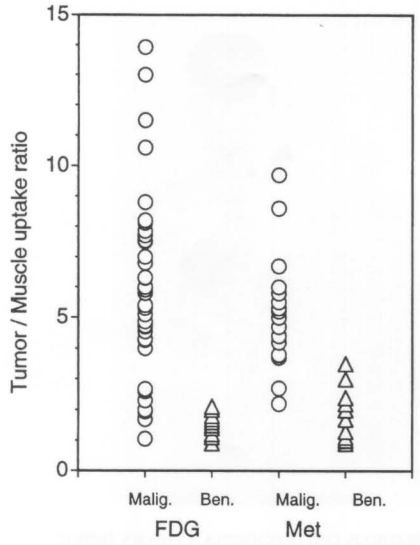


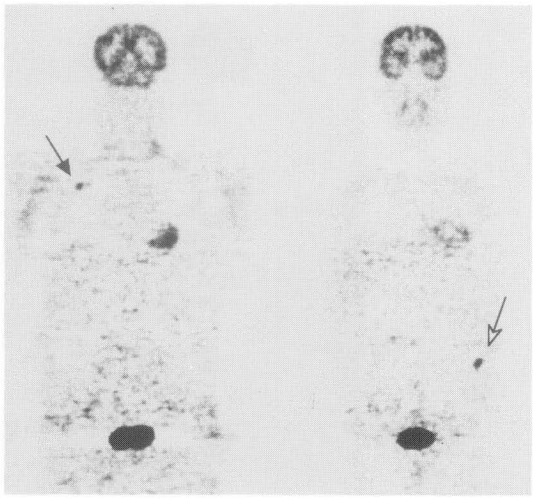

**Fig. 8** Differential diagnosis of solitary pulmonary nodule using FDG or Met and PET. A total of 72 patients were studied prospectively, 46 with FDG and 26 with Met. Note significant differences in the mean uptake of both tracers by malignant and benign lesions, while some overlapping at the border line. Malig.: malignant lesion, Ben.: benign lesion

FDG should be avoided as it leads to increased accumulation of FDG in skeletal muscle, and thus less FDG is available for accumulation in the tumor.[64]

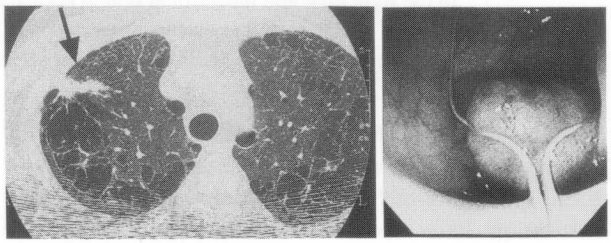
### Clinical Diagnosis of Lung Cancer with PET

The CT scan has played an important role in the diagnosis and staging of lung tumors. Since CT can detect calcification, an important sign of a benign tumor, if the tumor has soft-tissue attenuation without calcification, the differential diagnosis of cancer from a benign lesion is difficult. CT can provide excellent anatomic information, but not metabolic or pathophysiologic information about the lesion.

To predict the nature of non-calcified lung tumors, we performed a prospective study of 70 patients by means of FDG or Met and PET. A pathological diagnosis was obtained at biopsy or surgery. Lesions with a no-malignancy result from biopsy, or lesions responded to antibiotic treatment were clinically diagnosed as abscesses, and lesions that were followed up for more than one year without a change in size or nature were also clinically diagnosed as benign. The tumor to muscle radioactivity ratio was used for evaluation of tumor uptake of tracers. Tumors less than 1 cm in diameter were difficult to evaluate accurately due to the limitations of PET resolution. Compared to the final diagnosis, Met studies exhibited a sensitivity of 90%, specificity of 67% and accuracy of 83%. FDG studies exhibited a sensitivity of 89%, specificity of 92% and accuracy of 90% (Fig. 8).[65] This study suggested that PET, especially with FDG, might be



a



b

**Fig. 9** a: A nodule was found in the right apex of lung. Cytological examination of sputum nor bronchofiberscope examination did not provide specific diagnosis. Whole-body FDG-PET (coronal slices) showed focal intense FDG uptake corresponding to the nodule (black arrow), and an unexpected high uptake in the left lower abdomen (white arrow). Surgery demonstrated lung squamous cell carcinoma. Endoscopy showed a large colon polyp. It was resected and revealed adenoma of borderline malignancy. b: CT scan of the lung nodule, and endoscopic photography of colon polyp.

clinically very useful for differential diagnosis of lung tumors (Fig. 9). The original version of this report on 46 patients presented at the Society of Nuclear Medicine meeting in 1989, was the first report of actual clinical use of PET for diagnostic oncology. Subsequently a large number of clinical studies from the US and Europe were reported and all agreed that PET was superior to CT for this purpose.[66]

After the introduction of the whole-body imaging technique with FDG-PET, detection of lymph node or distant metastases in known cancer patients has become another common application of PET (Fig. 10).[67] With CT/MRI, the diagnosis of lymph node metastasis has been based on a size criterion (>1 cm in diameter), but small lymph nodes could still have metastases and large lymph nodes could be benign due to inflammatory reactions. The diagnosis of metastasis by using metabolic criteria with PET may be superior to the conventional imaging tech-

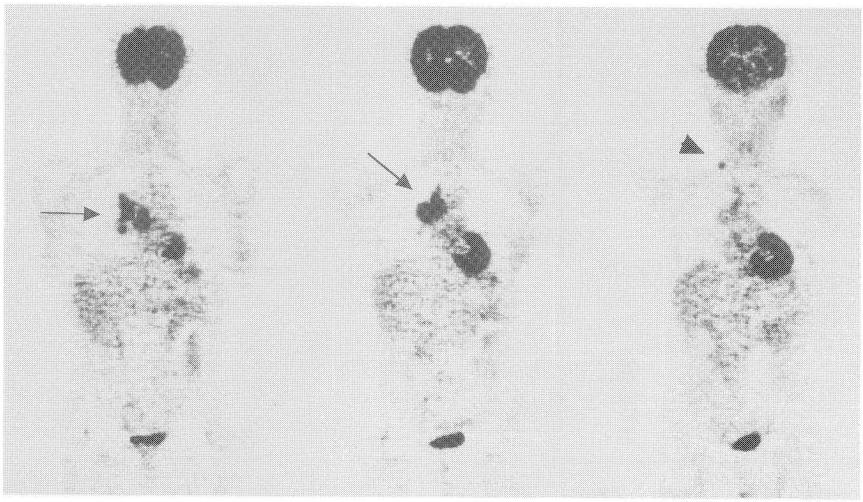
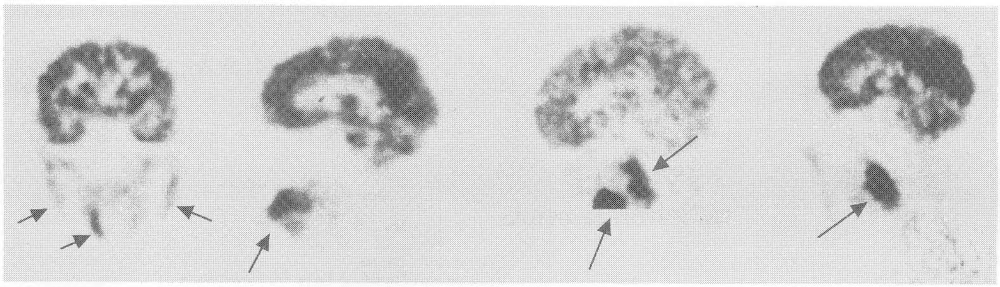


**Fig. 10** Whole-body FDG PET image (coronal slices) of lung squamous cell carcinoma. Primary tumor in the right lung and mediastinal lymph node metastasis (long arrows) shown by PET were consistent with CT findings. PET showed unexpected high uptake lesion (short arrow) in right neck confirmed as lymph node metastasis. Radiotherapy was selected and PET helped radiotherapy planning.

niques of CT scan or MRI. Our results of whole-body PET for the detection of metastases yielded a sensitivity of 93%, specificity of 63% and accuracy of 88%.[68] Other institutions have reported even better results with over 90% accuracy.[66,69] Whole-body PET enables very sensitive detection of whole-body spread of disease with a single examination, that may change the strategy of patient therapy, and may be cost-effective. Recently, FDG-PET for the diagnosis of lung cancer was approved for medical insurance reimbursement by the Health Care Financing Administration (HCFA) in the USA (Medicare), and by medical insurance funds in Germany and the UK, but is unfortunately still pending in Japan at this moment (October 2001).

In a recent report on stage diagnosis of lung cancer from Guy's Hospital in London, whole-body FDG-PET studies of 97 patients scheduled for operation were compared for clinical stage as assessed by CT and follow-up, and with the result of operation or biopsy.[70] The sensitivity and specificity for diagnosis of N2 or N3 mediastinal lymph nodes were CT: 20% and 89.9%, and PET: 70.6% and 97% (sensitivity and specificity, respectively). Changes in the staging of disease by PET occurred in 26.8%, the N factors were changed in 13.4% and changes in the M factor (that is, discovery of unexpected metastasis) occurred in 16.5% of patients. There were changes in treatment policies in 37% of patients based on the PET findings. Conversely, PET failed to detect 7 of 10 brain metastases. The researchers concluded that the degree (SUV) of tumor FDG accumulation detected by PET was the best prognostic factor with the exception of the operative stage. A report from Duke University, USA, compared FDG-PET and conventional imaging with CT, bone scintigraphy, brain CT or MRI for staging of 100 patients with NSCLC with reference to the pathological stage.[71] PET staging was accurate in 83% of patients, compared with an accuracy of 65% for conventional methods ($p < 0.005$). Staging of mediastinal lymph nodes was correct by PET in 85% and by CT in 58% ($p < 0.001$). Nine patients had metastases detected by PET that were not found by conventional imaging, and 10 patients suspected of metastases by conventional imaging were correctly diagnosed as no-metastasis by PET. The authors concluded that whole-body FDG-PET was more accurate than conventional imaging (chest CT, bone scan, brain CT or MRI) in staging lung cancer.

FDG-PET for evaluation of lung cancer is the most extensively studied and most widely accepted clinical application of PET in oncology. Only a few studies have reported the use of $^{11}$C-methionine (Met) for lung cancer.[35,65] Miyazawa et al.[72] studied Met-PET of 24 patients with NSCLC and analyzed resected tumor tissue by DNA flow cytometry. The Met uptake rate in tumors correlated well with proliferation indices (S phase fraction, S + G2/M phase fraction). The authors concluded that the tumor uptake rate of Met reflected the tumor growth rate in NSCLC. Yasukawa et al.[73] compared Met-PET and CT for the diagnosis of lymph node metastases in 41 patients with lung cancer. The diagnostic indices of Met-PET vs. CT were, sensitivity (86.1%, 52.8%), specificity (91.1%, 84.4%), and accuracy (89.7%, 75.4%) (Met-PET, CT, respectively). They concluded that Met-PET was superior to CT for diagnosis of lymph node metastasis of lung cancer. Met-PET appears to be as useful as FDG-PET for lung cancer, but the short half-life of $^{11}$C (20 min) is a major drawback for the widespread use of Met. Met uptake is more selective for cancer cells than is FDG,[39] and its normal brain uptake is low, so that Met-PET appears to have an advantage over FDG-PET for imaging brain tumors.[52] Further studies with Met and $^{18}$F-



a-1 a-2 b c

**Fig. 11** Physiological functional uptake (a, b) and pathological uptake (c) of FDG. Chewing gum task of normal volunteer showed increased FDG uptake of anterior tongue (a-2 saggital slice, arrow), masseter muscle and medial pterygoid muscle (a-1 coronal slice, arrows) (modified from ref. 86, courtesy of Dr. Rikimaru). Four years old boy of developmental delay was studied with FDG PET which showed very high FDG uptake in the base of tongue and posterior wall of pharynx (b, arrows). PET operator noticed him constant sucking of his thumb after injection and during scanning. FDG uptake is consistent with functioning muscle for finger sucking. Oropharyngeal cancer after radiotherapy showed increased FDG uptake of base of tongue (c, arrow), confirmed as recurrence by biopsy.

labeled amino acid analogues are necessary to establish the role of non-FDG PET in clinical oncology PET.[74–77]

## FDG-PET, Proliferation and Survival

Ahuja et al.[78] reported that the degree of FDG uptake in primary lung cancer correlated with survival. On hundred and fifty-five NSCLC patients were studied with FDG-PET. The stage at presentation, cell type, tumor size and survival data were recorded. A standardized uptake ratio (SUR) was calculated for FDG uptake by the primary lesion and was correlated with clinical information to determine prognostic significance. Multivariate analysis demonstrated that an SUR >10 provided prognostic information independent of the clinical stage and lesion size.[78]

Higashi et al.[79] demonstrated that FDG uptake correlated with cell proliferation rather than with the cellular density of NSCLC. They studied 31 patients with NSCLC, and tumor FDG uptake was evaluated with SUV. Cell proliferation as assayed by the PCNA labeling index was evaluated by the immunohistochemistry of tumor tissues resected at thoracotomy. Cellular density was also evaluated. FDG uptake correlated significantly with the PCNA labeling index, but only weakly with cellular density. Bronchoalveolar carcinoma (BAC), which is a well-differentiated tumor known to have an FDG uptake as low as benign lesions,[80] had a significantly lower SUV and PCNA labeling index than tumors of other histology, but no significant differences in cellular density were evident between BAC and tumors of other histology. This suggested that FDG accumulation in tumor tissue would be a useful index of proliferation, of the degree of differentiation, and of patient survival.

A similar study has been reported in breast cancer by Oshida et al.[81] who examined FDG uptake and various known prognostic indicators by multivariate analysis, in 70 patients with breast cancer, to assess their contribution to overall and relapse-free survival. FDG uptake evaluated with DAR was an independent prognostic factor for relapse-free survival of breast cancer patients. Other independent prognostic indicators included the number of positive lymph nodes and histologic grade.

In head and neck cancer, Minn et al.[82] conducted a univariate analysis and reported that FDG uptake was clearly correlated with survival, but in their multivariate analysis, the only independent predictors of survival were the mitotic count and stage of disease.

## Non-Pathological Functional Uptake of FDG

Whole-body glucose metabolism can be monitored with FDG. Therefore, high FDG accumulation may be observed in various tissues, such as muscle, due to physiological requirements. Accurate knowledge of such physiological accumulations is necessary to diagnose tumors in whole-body FDG-PET. FDG uptake by the limb muscles due to running[83] and other exercises that consume glucose as the energy source, can easily be recognized. Confusion may arise in the distinction between normal shoulder and neck muscle uptake and lymph node metastases. Movement of the shoulder or neck after FDG injection and tension associated with anxiety may be causes of increased FDG uptake. Muscle uptake may be longitudinal and symmetrical. It was reported that administration of diazepam prevented muscle uptake.[84] Speech during the phase of FDG uptake increases FDG activity in the laryngeal muscles[85] and tongue movement or sucking may increase FDG uptake in pharyngeal muscles. Chewing may increase FDG uptake in the muscles of mastication (Fig. 11).[86] Eye movement will induce FDG uptake in ocular muscles that then appear like two "V" letters. Non-pathological FDG uptake by the palatine tonsil was

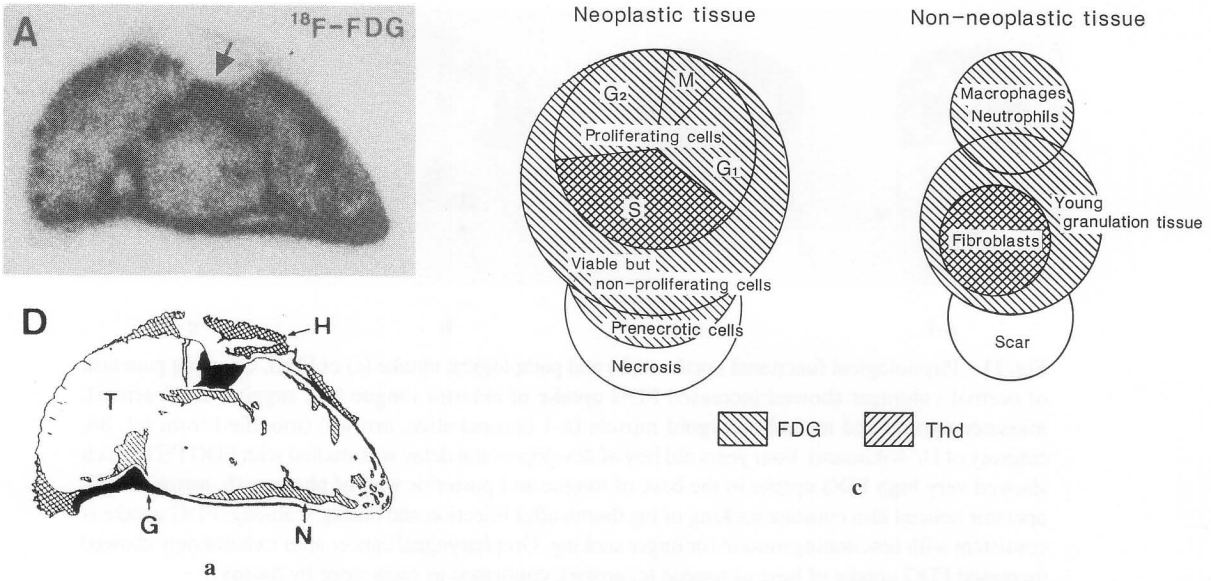



**Fig. 12** FDG distribution within a mouse FM3A tumor by autoradiography (a). Illustration of histology was bottom (ref. 97). Highest uptake (arrow) was seen in the granulation tissue. Separate microscopic ARG study showed that, about 24% of FDG in this tumor was from macrophages and granulation issue, 78% from cancer cell (b). Distribution model of FDG in neoplastic cells and non-neoplastic tissues in tumor (c) (ref. 100).

observed in about 50% of patients.[87]

Inflammatory bowel disease is another cause of FDG uptake,[88] but the normal colon and small intestine also often exhibit increased FDG uptake. This can be partially explained by smooth muscle in the colon, but details of this mechanism are unknown. FDG distribution in liver and kidney was described in the previous section. Several reviews of functional and non-malignant FDG uptake have been published previously.[89–93]

### Tumor Structure and Distribution of FDG and Methionine

False-positive FDG or methionine uptake has been reported in active inflammation such as abscesses,[94] tuberculosis,[65,95] aspergillosis,[65] sarcoidosis and other lesions.[90,96] Because of this limitation, the accuracy of PET cannot reach 100%. In addition, early post-operative scarring and the early inflammatory reaction after radiotherapy exhibited increased FDG uptake.

In order to clarify the mechanisms of FDG uptake by inflammation and tumor tissues, we have performed autoradiographic studies. Tumor tissue is comprised of cancer cells and non-neoplastic tissues (stroma). Stroma includes macrophages, neutrophils and lymphocytes that infiltrate from capillaries due to the host-tumor immune reaction, and also includes granulation tissue comprised of fibroblasts, collagen fibers, and capillaries. Capillary

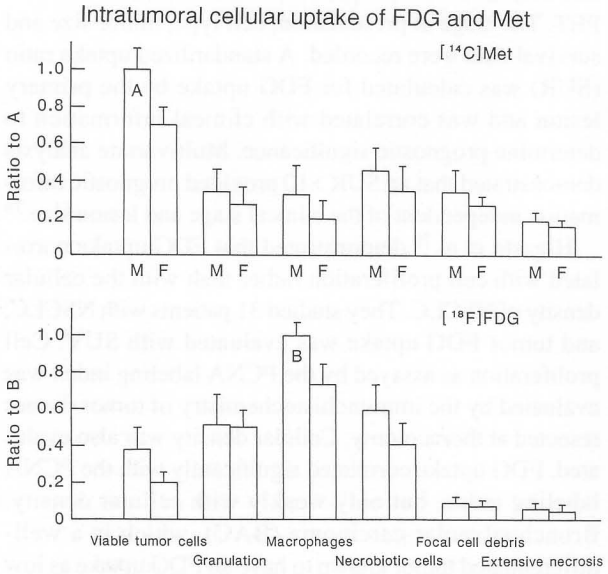



**Fig. 13** Comparison of intratumoral cellular uptake of FDG and methionine. Two types of mouse tumor autoradiography were analyzed, M; hepatoma MH134 and F; breast cancer FM3A. Tracer uptake was expressed as the ratio to the highest uptake component within the tissue. Reconstructed from ref. (39).

growth is enhanced by angiogenic factors in tumor tissue. All these tissues consume glucose, at various levels. In addition to cancer cells, high FDG uptake is seen in

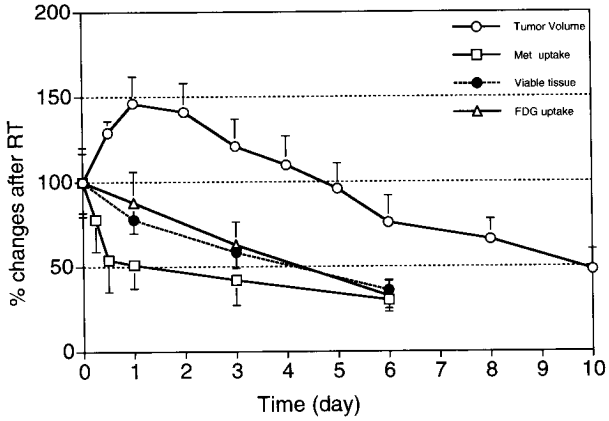



**Fig. 14** Tumor volume (%), viable tissue by histology (%), methionine uptake and FDG uptake by tumor were compared after 20 Gy of radiotherapy of *in vivo* rat tumor model AH109A. Please note rapid reduction of methionine uptake, followed by reductions of FDG uptake and viable tissue, and delayed volume reduction. Reconstructed from ref. (101, 102).

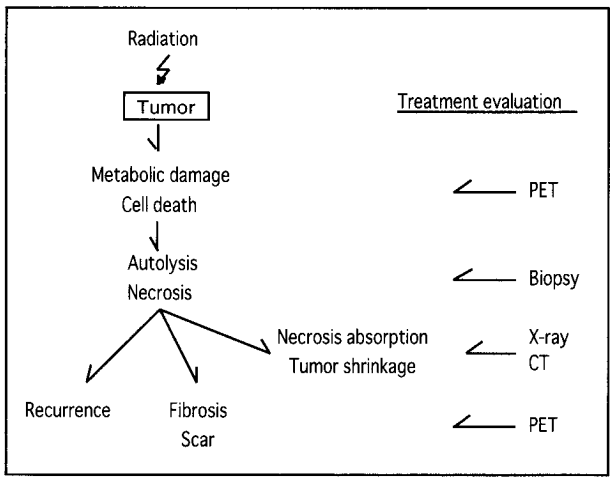



**Fig. 15** Possible role of PET in radiotherapy evaluation of tumor.

activated macrophages and young granulation tissue (Fig. 12).[97] The latter two may cause false-positive FDG uptake in inflammation, sarcoidosis and early post-operative scarring. FDG uptake was also studied in an experimental model of inflammation, and silver grains were observed by FDG in infiltrating immune cells and in granulation tissue by autoradiography.[98] Immune-deficient nude mice are not an appropriate model to study this problem, as nude mice have no active immune system, and therefore both their lymphocytes and macrophages exhibit very low glucose metabolism.[99] Studies with a syngeneic animal tumor model and an inflammation model, and PET-pathology correlation, have all demonstrated this phenomenon.

Compared to FDG, Met distribution in tumor tissue is more specific for viable cancer cells. Granulation tissue and macrophages exhibited uptake of Met at levels lower than those of FDG. Tumor uptake of Met is apparently predominantly by viable cancer cells (Fig. 13).[39]

### Why PET for Treatment Evaluation?

Tumor size measurement by X-ray or CT has been the standard method of the treatment evaluation of the cancer. But tumors containing non-active tissues, such as fibrosis, necrosis and injured cells about to die, produce a diagnostic dilemma in that the residual mass after treatment does not always equate with residual disease. Monitoring the viability of the tumor and evaluation of the residual mass by means of PET would therefore be beneficial for cancer patients.

### Tumor Uptake Response to Radiotherapy

Tumor tissue response to therapy and its correlation to tracer uptake must be evaluated carefully. We have compared tumor volume, amount of viable tumor tissue, and tracer uptake, after experimental radiotherapy in a rat tumor model.[100–103] $^{3}$H-Thymidine and $^{14}$C-Methionine uptake exhibited a rapid and sensitive response to irradiation, preceding both volumetric shrinkage and necrotic extension. FDG uptake almost paralleled the necrotic extension that preceded volumetric shrinkage (Fig. 14). FDG exhibited a wide range of uptake changes and a steady response to irradiation. FDG uptake was linearly correlated with the percentage of viable tissue *in vivo*. Thymidine, a marker of proliferation, underestimated the amount of viable tissue. Results with Met more closely resembled those obtained with thymidine than with FDG.[47,104]

Radiotherapy (and also chemotherapy) results in injury to DNA, RNA, protein and membranes of cancer cells, so that altered metabolism and cell death are reflected in a reduction in Met or Thd uptake, which precedes the autolysis of cells observed as necrosis. The reduction of viable tumor tissue is reflected by the decrease in FDG uptake. No visible reduction of tumor volume is evident until a large part of the necrotic tissue has been removed. PET with FDG enables functional evaluation of tumor viability to assess the therapeutic effects on tumor tissue, earlier than morphologic evaluation of tumor volume reduction by CT scan. Long after the therapy, if necrosis is replaced by fibrosis, differential diagnosis of recurrent viable cancer cells from fibrosis (scar) may be another useful indication for PET (Fig. 15).[105] Our studies and the above considerations on applications for PET, have dealt with FDG uptake changes at least one day or later after radiotherapy. These observations differ from the acute reaction described in the next paragraph.

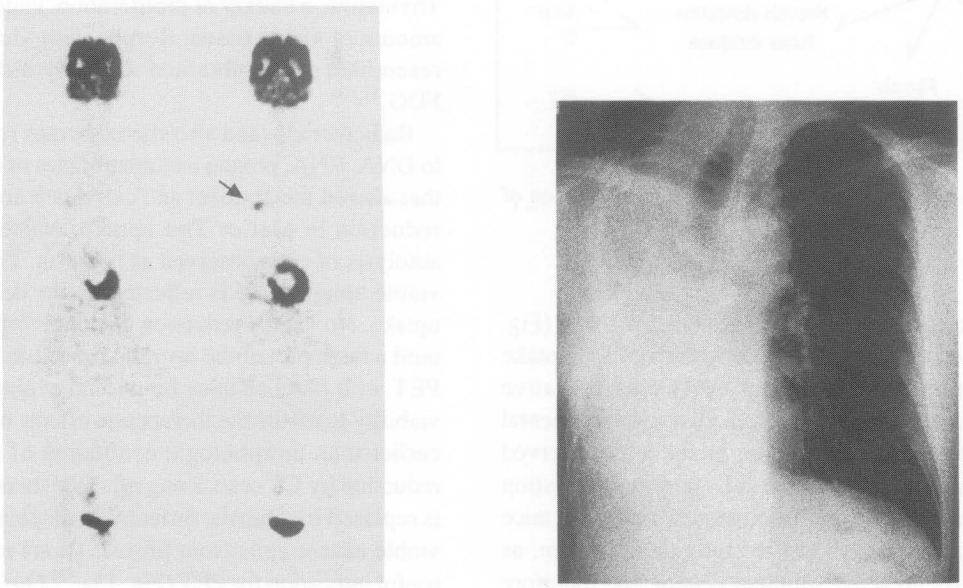
The acute reaction of FDG uptake by cancer cells to radiotherapy or chemotherapy is more complicated.

Fujibayashi et al.[106] reported that FDG uptake by cultured human cancer cells increased from 1 hr after 30 Gy of radiotherapy and peaked at 3 hr, after which time it decreased. Both glucose transporter-1 mRNA expression and hexokinase activity were significantly increased. After the inhibition experiments, the authors concluded that the transient increase in glucose metabolism occurred via a process at the level of gene expression. The same group reported a FDG-PET study of brain tumor patients before and 4 hr after a 24–32 Gy single dose of stereotactic radiosurgery.[107] The influx constants, Ki, of FDG in irradiated tumors exhibited a 30 ± 14% increase after radiotherapy. In metastatic tumors, the percentage volume decrease was positively correlated with the percentage change in Ki ratio. The authors suggested that hyper-acute changes in glucose metabolism could predict a therapeutic response. In a study on human tumor xenografts, rapid increase in FDG uptake after radiotherapy was observed only in the most radiosensitive tumor line and was accompanied by apoptosis in the tissue.[108] A similar transient increase in FDG uptake by cancer cells was reported soon after experimental chemotherapy by Slossman et al.[109] These early reactions of glucose metabolism to therapy may be related to cellular stress responses. This could involve a phenomenon similar to the mechanism of heat shock proteins, but this has not been well studied to date.

### Treatment Evaluation with Met-PET

In a long-term follow-up study of lung cancer, changes in Met uptake were superior to changes in tumor volume detected by CT scan in assessing the effects of radiotherapy, and detecting recurrence.[110] We have compared the early treatment response of Met uptake and tumor volume in order to predict the final outcome of the treatment results in 19 lung cancer patients. In the Met uptake data, the early-recurrence group was clearly distinguishable from the no-recurrence group. But the late-recurrence group was indistinguishable from the no-recurrence group, probably because the number of residual viable cancer cells at the end of radiotherapy was not large enough to detect with PET. When the reduction in Met uptake was combined with the reduction in tumor volume, the differentiation of each group became easier than when a single parameter was used. When a residual mass is visible on CT, PET appears to be useful in evaluating tumor viability.[111]

Effects of radiotherapy on head and neck cancer were studied with Met-PET by Nuutinen et al.[112] Met uptake by tumors exhibited a significant decrease during the first 2–3 weeks of radiotherapy. The rate of decrease in Met uptake was comparable in relapsing patients and those who remained locally-controlled, so that the use of Met-PET for prediction of response to radiotherapy appears to be limited. Effects of chemotherapy or hormonal therapy on breast cancer metastases were also evaluated with Met-



a b

**Fig. 16** FDG-PET for the detection of lung cancer recurrence (a). Large cell carcinoma in the right upper lobe of lung was resected by right pneumonectomy and 6th rib resection. One and half year later, elevation of CEA to 449 suggested recurrence. However, chest x-ray (b), CT scan of chest, abdomen and brain cannot identify the focus of recurrence. Coronal images of FDG-PET showed high FDG uptake in right supraclavicular lymph node (arrow). Biopsy confirmed recurrence.

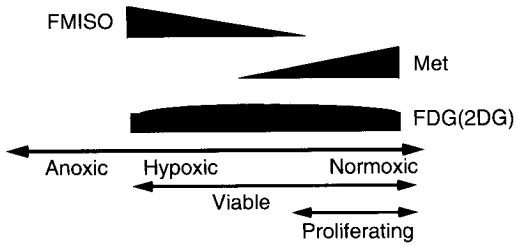



**Fig. 17** Distribution model of FDG, methionine and fluoromisonidazole along the level of oxygen in tumor tissue. From ref. (126).

PET by Huovinen et al.[113] Met uptake by tumors decreased when clinical objective regression of the tumor was later attained, and increased in patients where progressive disease was observed during treatment. They concluded that changes in amino acid metabolism as detected by Met-PET precede the clinical response, and may be of clinical value in predicting the treatment response. Met uptake by a variety of tumors appears to decrease earlier than the regression of tumor volume or other clinical indicators, but it may remain difficult to predict whether the tumor will finally relapse.

### Treatment Evaluation with FDG-PET

Excellent results have been reported in the detection of lung cancer recurrence after various treatments.[114,115] FDG-PET is superior to MRI and CT for differentiation of recurrence from post-operative scarring in colon cancer.[116] Nevertheless, several false-positive studies have been reported after radiotherapy in lung, colon, and head and neck cancer, where cancer recurrence was denied after biopsy or clinical follow-up despite the increased FDG uptake. FDG uptake by normal chest wall within the radiation field has also been reported, which peaked at 6 months after radiotherapy.[117] Attention must be paid to this increased FDG uptake by post-radiotherapy inflammatory tissue. An autoradiography study demonstrated that the macrophage layer and granulation tissue at the rim of necrosis after fractionated radiotherapy exhibited high FDG uptake despite the absence of viable cancer cells. High FDG uptake was also observed in the muscle within the radiation field adjacent to the tumor.[118]

Bury et al.[119] reported FDG-PET studies of 129 patients in detecting residual or recurrent NSCLC after surgery, or radiotherapy and/or chemotherapy. PET revealed increased FDG uptake in all cases (n = 60) of persistent or recurrent tumor, whereas CT was nonspecific in 17 cases. There were 5 false-positives with PET and 3 with CT. In detecting residual or recurrent NSCLC, PET and CT showed sensitivities of 100% and 71%, specificities of 92% and 95%, and accuracies of 96% and 84%, respectively. PET correctly identified response to therapy in 96% of patients. PET appeared to be more accurate than conventional imaging in distinguishing residual or recurrent tumor from fibrotic scar in patients undergoing treatment for NSCLC (Fig. 16).

Detection of recurrence in patients with previously treated head and neck cancer is difficult. CT/MRI findings are often equivocal or inconclusive because of distortion of anatomic structures and the presence of diffuse soft-tissue swelling caused by previous treatment. Contrast enhancement can be detected with CT/MRI in both recurrent tumors and post-surgical or post-radiation changes. Anzai et al.[120] reported a comparison of FDG-PET and CT/MRI to detect recurrence of head and neck cancer in 12 patients. Sensitivity (77%, 25%) and specificity (100%, 75%; PET and CT/MRI, respectively) were assessed. FDG-PET exhibited significantly better diagnostic accuracy than CT/MRI. Fischbein[121] reported similar results in 35 patients with squamous cell carcinoma of the head and neck, and sensitivity and specificity for residual/recurrent disease at the primary site were 100% and 64% respectively, and for nodal diseases 93% and 77%. Kao et al.[122] studied 36 nasopharyngeal carcinomas (NPC) with FDG-PET and CT, 4 months after radiotherapy. The diagnostic results for FDG vs. CT were sensitivities of 100% and 72%, specificities of 96% and 88%, and accuracies of 97% and 83%, respectively. FDG-PET was superior to CT for detection of recurrent or persistent NPC. In our preliminary study to detect recurrence of head and neck cancer after radio-chemo-therapy, FDG-PET was compared with CT/MRI. Twenty-nine lesions in 26 patients were examined and compared to the results of biopsy, surgery, or clinical follow-up. Sensitivity (78%, 67%; PET and CT/MRI, respectively), specificity (70%, 20%), and accuracy (72%, 34%) were assessed. There were two false-negative, and 6 false-positive results with FDG-PET, the latter including 3 cases of post-radiotherapy inflammation. In this study, FDG-PET exhibited significant superiority to CT/MRI in the detection of recurrent head and neck cancer. In particular, the specificity of PET (70%) was vastly superior to that of CT/MRI (20%) (see Fig. 11c).[123]

### Detection of Hypoxic Tumor Tissue

Prediction of the sensitivity or resistance of a tumor to radio- or chemo-therapy would be of great benefit to the patient and physician. The imbalance between cancer cell growth and capillary growth produces heterogeneous perfusion patterns in tumor tissue, which eventually lead to regional hypoxia or tissue necrosis. The presence of hypoxic cells in a tumor is very relevant to radioresistance, and can also contribute to chemoresistance. Non-invasive assessment of tumor hypoxia has been studied with several radio-tracers to predict radioresistance of tumors.

Misonidazole and its derivatives are metabolically trapped in cells that are alive but hypoxic, and are used as

markers of hypoxic tissues. $^{18}$F-fluoromisonidazole (FMISO) has been developed and studied for imaging hypoxic tissue.[124,125] We studied FMISO uptake in a rat tumor model of hypoxia and in controls, and investigated the correlation between intra-tumoral distributions of FMISO, $^{14}$C-2-deoxyglucose (2DG) and $^{14}$C-methionine (Met). Double-tracer autoradiography of the tumor demonstrated that the areas of high FMISO uptake had low uptake of Met, whereas low FMISO uptake areas had high Met uptake. FMISO showed high grain density in the tumor rim surrounding the necrotic area. 2DG showed a more uniform distribution over the entire area of viable cells. The mean uptake of FMISO by hypoxic, radioresistant tumors was significantly higher than by control tumors ($p < 0.05$), and both 2DG and Met uptake by the control tumors was higher than by hypoxic tumors. When individual tumors were examined, FMISO uptake was inversely correlated with that of Met ($r = -0.507$, $p < 0.02$), but 2DG exhibited almost uniform uptake with no significant correlation with FMISO. We concluded that there was a large overlap in the distribution of FMISO and 2DG within the tumor, but only a small overlap in the distribution of FMISO and Met (Fig. 17).[126] In our previous study we used micro-autoradiography to demonstrate that uptake of FDG was increased in prenecrotic (hypoxic) cells at the peripheral rim of necrosis.[45] But in double-tracer autoradiography at the macro level, increased FDG uptake at the periphery of necrosis was not clear. The combination of FMISO and other tracers in a PET study would possibly be more helpful than a single-tracer study in predicting the response of tumor tissues to radiotherapy.

The influence of hypoxia on FDG, methionine and leucine accumulation in cultured human cancer cells was reported by Clavo et al.[127] and Minn et al.[128] $^{3}$H-FDG accumulation is increased in hypoxic cancer cells, in part due to increased membrane expression of the GLUT1 glucose transporter. Hypoxia was associated with decreased cellular uptake of thymidine. The decrease in acid-precipitable $^{3}$H-leucine in hypoxic conditions may indicate a decline in protein synthesis, whereas the unchanged $^{3}$H-methionine uptake probably reflects unaltered amino acid transport and slow trans-methylation.[129] Recently, the mechanism of increased FDG uptake by hypoxic cells was further studied by Burgman et al.[130] A two-fold increase in $^{3}$H-FDG uptake was reported under hypoxic conditions, but no changes in the cellular levels of glucose transporter proteins or hexokinase were observed. That study, with reducing or oxidizing agents, suggested that hypoxia-induced modification (reduction) of the cysteine residues in GLUT may be the mechanism underlying the hypoxia-induced increase in $^{3}$H-FDG uptake. Enhancement of transmembrane $^{3}$H-FDG transport without translocation of GLUTs to the plasma membrane may result either from increased affinity of GLUTs or activation of dormant GLUTs that pre-exist within the plasma membrane. This is inconsistent with the findings of Clavo et al.[127] whose study suggested increased membrane expression of the GLUT1.

The finding of Clavo et al.[127] were consistent with those of Waki et al.[26] in that GLUT activity, and not hexokinase activity, was rate-limiting for $^{3}$H-FDG uptake in cancer cells.

$^{18}$F-fluoroerythronitroimidazole (FETNIM) has been reported as an agent for detecting tumor hypoxia by imaging. The tumor to blood distribution ratio of FETNIM at 4 hr after injection was significantly higher than that of FMISO. Autoradiographs indicated that both agents could help differentiate hypoxic from necrotic regions in tumors. FETNIM is reported to be easier to prepare, less costly, and more hydrophilic than FMISO.[131] PET studies using FETNIM in cancer patients have been performed and will be reported from the University of Turku.

## CONCLUSION

PET with FDG can effectively monitor molecular events involved in glucose transport and phosphorylation *in vivo*, parameters that usually reflect the viability of cancer cells in tumor tissue. FDG-PET is clinically very useful for differential diagnosis, detection of metastasis in the whole-body, and for detection of recurrence of lung and other cancers. Further investigations are required to clarify the usefulness of PET in predicting sensitivity or resistance of tumors to chemo/radio therapy.

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