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Neurobiology of Disease

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Neurobiology of Disease 15 (2004) 437–448

Review

Usefulness of positron emission tomography in diagnosis and treatment follow-up of brain tumors

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Received 12 February 2003; revised 11 October 2003; accepted 25 November 2003

Clinical and experimental use of positron emission tomography (PET) is expanding and allows quantitative assessment of brain tumor's pathophysiology and biochemistry. PET therefore provides different biochemical and molecular information about primary brain tumors when compared to histological methods or neuroradiological studies. Common clinical indications for PET contain primary brain tumor diagnosis and identification of the metabolically most active brain tumor reactions (differentiation of viable tumor tissue from necrosis), prediction of treatment response by measurement of tumor perfusion, or ischemia. The interesting key question remains not only whether the magnitude of biochemical alterations demonstrated by PET reveals prognostic value with respect to survival, but also whether it identifies early disease and differentiates benign from malignant lesions. Moreover, an early identification of treatment success or failure by PET could significantly influence patient management by providing more objective decision criteria for evaluation of specific therapeutic strategies. Specially, as PET represents a novel technology for molecular imaging assays of metabolism and signal transduction to gene expression, reporter gene assays are used to trace the location and temporal level of expression of therapeutic and endogenous genes. PET probes and drugs are being developed together as molecular probes to image the function of targets without disturbing them and in mass amounts to modify the target's function as a drug. Molecular imaging by PET helps to close the gap between in vitro to in vivo integrative biology of disease.

Keywords: Positron emission tomography; Brain tumors; (^{18}F)fluorodeoxyglucose; Methionine; Amino acids

Introduction

Despite increasing experimental research about the pathophysiological mechanisms of growth, progression, and invasion of primary brain tumors in the recent years, the underlying molecular changes both in the tumor area and its surrounding brain tissue remains only partially understood (Beaumont and Whittle, 2000; Schaller, 2003; Schaller and Rueegg, 2003). Especially, there is a certain difficulty to transfer the wide range of experimental knowledge into clinical conditions by several lines of evidence. Tumor cell transformation entails major biochemical and molecular changes including modifications of the energy metabolism, e.g., utilization of glucose and other substrates, protein synthesis, and expression of receptors and antigens. Tumor growth also leads to heterogeneity of blood flow owing to focal necrosis, (neo)angiogenesis, and metabolic demands, as well as disruption of transport mechanism of substrates across cell membranes and other physiological boundaries such as the blood-brain barrier (BBB). Positron emission tomography (PET) represents a good modality to solve measure and visualize cellular biochemical processes quantitatively by pattern of in vivo uptake of molecular probes into the brain tissue (Buonocore, 1992). In primary brain tumors, molecular imaging is suited for tissue characterization that can provide additional information assessing response to treatment, differentiating between iatrogenic lesions and residual or recurrent tumor tissue, evaluating the neurotoxicity of treatment, and finally studying the action of chemotherapeutic agents. Since biochemical changes may be related to the growth rate of tumor cells (Del Sole et al., 2001), they can be thought as markers of tumor cell proliferation. Radionuclide-based biochemical imaging with the radionuclide of processes occurring at a cellular level provides additional information that complements findings obtained by anatomical-based imaging methods aimed at depicting structural, vascular, and histological changes. The present short review focuses on the clinical application of biochemical assessment with PET and its possible role in the clinical condition of the treatment of primary brain tumors.

Principles of positron emission tomography

PET represents an analytical imaging technology developed to use compounds labeled with positron emitting radioisotopes as molecular probes to image and measure biochemical processes of mammalian biology in vivo. Molecular probes as used for PET studies are developed to first identify a target process to be studied and to then synthesize a positron-labeled molecule through which an assay can be performed (see Table 1). Because PET cannot provide direct chemical analysis of reaction products within the brain tissue, labeled molecules are used that trace a small number of steps (i.e., one to four) of a biochemical process so that kinetic

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Available online on ScienceDirect (www.sciencedirect.com.)

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doi:10.1016/j.nbd.2003.11.023

Table 1
Positron emission tomography probes used in primary brain tumors

Glucose metabolism
^{18}F-fluorodeoxyglucose (^{18}F-FDG)
Amino acid transport
^{11}C-methylmethionine (^{11}C-MET)
^{18}F-fluoroethylthyrosine (^{18}F-FET)
^{18}F-fluorophenylalanine (^{18}F-FPA)
^{11}C-choline (^{11}CCh)
Cellular proliferation
^{18}F-fluorodeoxyuridine
^{18}F-thymidine
^{18}F-3′-deoxy-3′-fluorothymidine (^{18}F-FLT)
^{18}F-1-(2′-fluoro-2′-deoxy-β-D-ribofuranosyl)thymine (^{18}F-FMAU)

analysis can be used to estimate the concentration of reactants and products over time and, from this, reaction rates.

Although magnetic resonance imaging (MRI) is highly effective in identifying tumor location or size and in detecting even small intracranial lesions, it remains often incapable of differentiating between viable tumor tissue and postoperative morphological changes such as gliosis or tumor-associated brain edema (Celis and Celis, 1985; Christensen, 1990; Mineura et al., 1991). PET may help to overcome this diagnostic dilemma. As spatial resolution and anatomical information of PET imaging technique are sparse, it has to be coregistrated with MRI to differentiate tracer accumulation in suspected brain tissue areas to attain a higher level of accuracy. However, PET alone does provide additional insight into the functional (morphological) aspect of different brain tumor treatment options.

The exact interpretation of brain tumor positron-labeled tracer uptake is mandatory to analyze the biochemical and molecular mechanism that visualize this tracer accumulation over time. This analysis is based upon dynamic PET data acquisition and arterial blood input data, it is achieved by compartment models, and it provides tracer transport and metabolic rate constants (nonlinear fitting) or tracer distribution volume and unidirectional tracer uptake into an irreversible compartment.

Different positron emission tomography probes

Different physiological or biochemical peculiarities of primary brain tumors, distinguishing neoplastic from normal brain tissue, may lead to a better visualization of primary brain tumors in PET imagines: (i) blood volume or tissue perfusion, (ii) BBB disintegration, or (iii) derangement of tumor cell metabolism (Boado et al., 1999; deWolde et al., 1997). Depending on its specific kinetic properties, different radionuclide-based tracers can visualize primary brain tumors by an increased or decreased accumulation relative to normal brain tissue. However, for the exact interpretation of such a tracer uptake within brain tumors, it is mandatory not only to understand the underlying physiological, but also the pathophysiological mechanisms (see Table 2).

2-(^{18}F) fluoro-2-deoxy-D-glucose (FDG)

^{18}F-fluoro-deoxy-glucose (FDG) represents the most common PET tracer used for metabolic studies of primary brain tumors (Coenen et al., 1989; Muzi et al., 2001). The FDG method relies on the particular biochemical behavior of 2-deoxyglucose, which is transported by the same carrier-mediated process as glucose across the BBB and is phosphorylated by hexokinase, but does not undergo further metabolic changes in the cellular glycolytic pathway (see Fig. 1) so that activity-dependent pattern of distribution can be visualized by PET imagines (Ogawa et al., 1991). In normal brain tissue, the phosphorylation of FDG represents the rate-limiting step in the accumulation of the phosphorylated product, FDG-6-P0$_4$, which undergoes very slow degradation during PET studies (Ogawa et al., 1993). In contrast, when glucose is used as a substrate for hexokinase, the metabolism of glucose-6-P0$_4$ continues in complex metabolic pathways through intermediary metabolism (Ogawa et al., 1993). As FDG competes with glucose for hexokinase, FDG-6-P0$_4$ is trapped in cells in proportion to the glucose metabolic rate and its accumulation can be detected by PET studies. This FDG accumulation in a region of interest may be derived by three physiological constants that contain: (i) the glucose transport from blood to brain, (ii) the reverse transport from brain to blood, and (iii) the phosphorylation of glucose (deWolde et al., 1997). However, the metabolic rates of FDG and 2-deoxyglucose are dependent on the native glucose concentration because they are reported to be competitive inhibitors for hexokinase (Bertoni, 1981).

It is well established that primary brain tumors present molecular changes in glucose utilization compared to normal brain tissue (Jager et al., 2001). In vitro, tumor cells reveal a high rate of glucose degradation into lactic acid, even in the presence of oxygen (Voges et al., 1997). Alterations of glucose transport in experimental neoplastic cells have been studied well and can be related both to an increased metabolism and to an increased number of existing glucose transporters (Hatanaka, 1974). Activation of the gene coding for the synthesis of glucose transporter GLUT is generally known as a major early marker of malignant transformation. An overexpression of GLUT-1 (predominantly in low-grade astrocytomas) or GLUT-3 (predominantly in high-grade astrocytomas) has been observed in primary brain tumors (Boado et al., 1994; Nishioka et al., 1992) and may explain the raised level

Table 2
Indication for use of positron emission tomography studies in brain tumors as related to (patho)physiological factors

FDG-PET
1. Ninety percent accurate for tumor grading and prognosis.
2. Can be used for grading and monitoring of progression to a higher degree of malignancy and for differentiating radionecrosis and recurrence. Recurrence may be undetectable due to high glucose consumption in surrounding normal brain tissue.
3. FDG accumulation dependent on (i) glucose transport blood/brain or brain/blood and (ii) phosphorylation of glucose.
L-Amino acids PET
1. Inaccurate for tumor grading and prognosis.
2. Good separation of brain tumor from surrounding normal brain tissue.
3. Amino acid accumulation dependent on (i) increased affinity and (ii) increased amount of carriers (V_{max}).
Thymidine analogs PET
1. Accurate for cell proliferation imaging providing reliable estimation of cellular proliferation by measuring thymidine flux from the blood into DNA tumors.
2. Good estimation of therapeutic efficacy, early detection of recurrence and of malignant transformation.

^{18}F-fluoro-2-deoxy-D-glucose; PET: positron emission tomography.

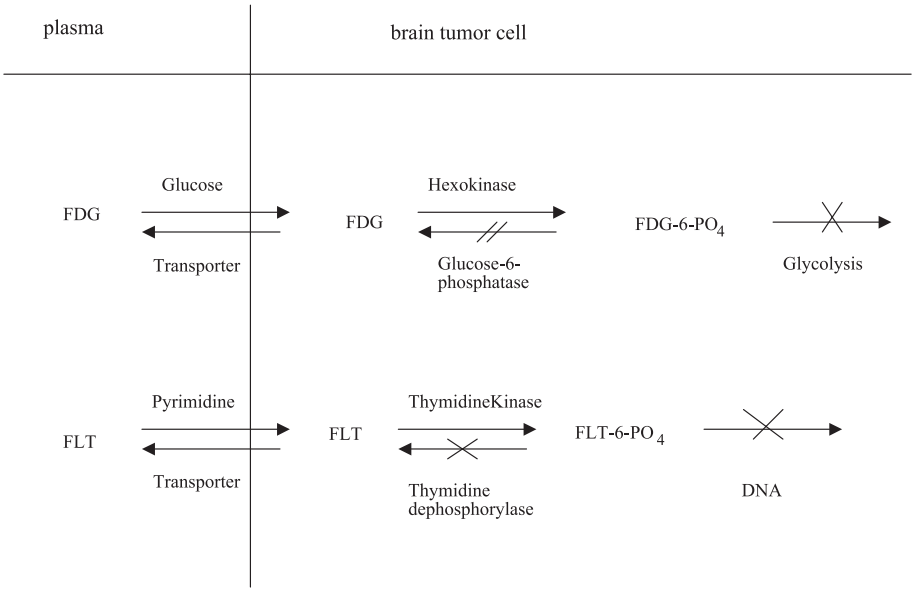

Fig. 1. Tracer kinetic models for FDG and FLT as based on the present pathophysiological knowledge. The basis of the accumulation of FDG and FLT lies on the fact that both tracers are phosphorylated to FDG-6-PO_4 or FLT-6-PO_4 within in the brain tissue but do not undergo further metabolic steps, allowing to visualize activity-dependent pattern of distribution. Legend: FDG: ^{18}F-fluorodeoxyglucose; FLT: ^{18}F-3′-deoxy-3′-fluorothymidine.

of glucose extraction as demonstrated by PET studies (Brooks et al., 1986). In animals' brain tumor models, it can be demonstrated that even in the presence of normal unidirectional glucose influx into tumor cells, the glucose metabolism is doubled in comparison with normal grey matter and that there is an uncoupling between glucose transport and phosphorylation (Herholz et al., 1992; Mies, 1992). The FDG uptake into malignant brain tumor cells is therefore the final pathophysiological consequence of both increased expression of glucose transporter molecules and glycolysis (Nakada et al., 1986). Quantitative assessment of glucose metabolism with PET studies in primary brain tumors is considered to be highly reproducible (Weber et al., 1999) but pretty cumbersome. Although glucose uptake below the normal level of gray matter is seen in low-grade astrocytomas, more malignant grades of astrocytomas and cerebral metastasis typically demonstrate higher glucose consumption (Derlon et al., 1997). The high glucose utilization of normal gray matter allows visualization of contrast between tumor tissue and normal gray matter, complicating the identification and the delineation of tumor tissue by FDG-PET. Therefore, several difficulties in the visual interpretation and lower sensitivity or specificity values for FDG-PET have been reported, especially in low-grade astrocytomas (Rubenstein, 1987).

Different transport rates between FDG and glucose across the BBB, in phosphorylation, and in their volumes of distribution within the brain tissue are taken into account by using the principles of competitive substrate kinetics in a term referred as "lumped constant" (LC_{FDG}) (Wienhard, 2002). It represents a ratio of the metabolic rates of FDG and glucose and is assumed to be regionally constant in normal brain (Wienhard, 2002). Spence et al. (1998), examining combined measurements of CMR_{glc} with FDG and (^{11}C)glucose, have demonstrated increased values for LC_{FDG} in human malignant astrocytomas varying widely within different histological subtypes. For this reason, brain tumor's CMR_{glc} rate, as demonstrated by FDG-PET, and a normal brain LC can significantly overestimate the true tumor CMR_{glc} rate by as high as twofold (Spence et al., 1998). These data underline that in primary brain tumors, the FDG method has to be used only with caution and the measured values for CMR_{glc} require careful interpretation (Wienhard, 2002).

Taken together, these data suggest that although some correlation of FDG uptake with histological grading of primary brain tumors can be demonstrated, proliferation rate of tumor tissue cannot be estimated with this tracer.

Labeled amino acids

General conditions such as pH, temperature, and plasma concentration are known to affect the transport rate of amino acids (AA) into brain cells. The main transport route is carrier mediated. Three basic physiological factors can be distinguished in the active transport mechanism (Souba and Pacitti, 1992): (i) AA flux to the brain tissue, which can be formulated as the flow multiplied by the arterial concentration; (ii) the intrinsic activity of the cell membrane AA concentration; and (iii) the rate of intracellular AA metabolism. The visualized activity of the specific carrier by PET is based on both (binding) affinity and the capacity of the transport carrier. The latter can also be expressed as the number of functional transport carriers per cell. Thus, an increased AA transport into the cell is related to (i) an increased affinity (K_m), (ii) an increased amount of carriers (V_{max}), or (iii) a combination of both factors. However, AA transport is generally increased in malignant transformed cells, probably associated with specific biochemical cell surface changes (deWolde et al., 1997). For example, AA transport system A is one of the few identified transport systems that is expressed strongly in transformed or malignant brain tumor cells and appears to be a target of proto-oncogene and oncogene action. In general, however, the process of malignant transformation requires that cells acquire and use different nutrients efficiently for energy, protein synthesis, and cell division (Bode and Kilberg, 1991). Therefore, increased AA transport rate most likely is also a

specific net result of increased AA demand (deWolde et al., 1997). Of the two major biochemical steps in protein metabolism, AA uptake and protein synthesis or increased transport rate of AA may be more pronounced than protein synthesis in malignant transformed brain tumor cells, including even low-grade astrocytoma (deWolde et al., 1997). Several processes contribute to AA transport rather than to protein synthesis, including (i) transamination and transmethylation, (ii) the specific role of methionine in initiation of protein synthesis, and (iii) the use of AA, such as glutamine, for energy metabolism or as precursors of nonproteins.

Nearly all AA have been radiolabeled to study potential PET imaging characteristics because the replacement of a carbon atom by ^{11}C does not chemically change the molecule (deWolde et al., 1997). However, AA differ in case of synthesis, biodistribution, and formation of radiolabeled metabolites in vivo (deWolde et al., 1997). For these reasons, mainly (^{11}C-methyl)-methionine (Herholz et al., 1998) and tyrosine (Salloum et al., 1993; Weckesser et al., 2002) have been studied clinically. More recently, different artificial AA such as *O*-2-(^{18}F)fluoroethyl-L-tyrosine have been developed (Inoue et al., 1998; Kubota et al., 1996; Shotwell et al., 1981; Wester et al., 1999a,b). As the applications of molecular imaging are expanding, radiolabeled AA may gain increased clinical interest. In PET studies of primary brain tumors, the use of radiolabeled AA is established, as its diagnostic accuracy and value seems adequate. For this reason, AA PET may be advantageous in comparison with FDG-PET studies, although tumor specificity is not absolute.

^{11}C-methionine

Brain tumor uptake of methionine was first described more than 20 years ago, but the (patho)physiological mechanisms and biological significance of this phenomenon remains incompletely understood until these days. Methionine and other large neutral AA are transported across the BBB and cell membranes by a particular carrier system that is stereospecific and saturable (deBoer et al., 2003). The additional importance of blood flow in methionine uptake of primary brain tumors was shown by Roelcke et al. (1995), suggesting that at least part of methionine uptake may result from passive diffusion, possibly in brain tumor tissue areas with damaged BBB (Ogawa et al., 1996). In cell uptake studies, methionine transport is usually mediated through the sodium-independent L-transport system in the luminal membrane of endothelial cells, with minor contribution from A-transport system (deBoer et al., 2003). Preclinical studies validating the possible use of methionine in the evaluation of chemo- or radiotherapy of brain tumors generally demonstrate that methionine uptake is reduced more rapidly than FDG but less rapidly and less severely than DNA-RNA tracers such as ^{18}F-fluoro-deoxyuridine (Kubota et al., 1991). Autoradiographic studies confirm methionine uptake predominantly in viable brain tumor cells, with low uptake in macrophages and other cells (Jager et al., 2001). No hypothesis can explain the exact (patho)physiological source of increased methionine uptake. But methionine uptake correlates to cell proliferation in cell culture, Ki-67 expression, and proliferation cell nuclear antigen expression, indicating its role as a marker for active tumor proliferation.

The increase of carrier-mediated large neutral AA transporters could possibly be due to the beginning of neoplastic angiogenesis because of the up-regulation of vascular endothelial growth factor expression and VEGF receptor type 1 induction have been observed already in low-grade astrocytomas (Plate and Risau, 1995). However, increased uptake in WHO grade 2 astrocytomas seems to be unique to methionine and other large neutral AA (Herholz et al., 1998), whereas contrast enhancement is usually absent and glucose consumption is low, similar to normal white matter (Kubota et al., 1995). In this context, it is to keep in mind that methionine contributes significantly to differentiation of astrocytomas from nontumoral intracranial lesions but does not represent a tumor-specific tracer. However, the methionine uptake does not directly reflect protein synthesis, but it represents cell avidity for AA, which relates to the activity of the transport system and incorporation in proteins.

In clinical application, methionine-PET scanning has been proved to be helpful for brain tumor diagnosis and delineation. Methionine-PET studies avoid many of the problems related to the tumor or nontumor uptake ratio that are encountered with FDG and overcome the difficulty in differentiating brain tumors from other cerebral pathologies that may cause abnormal FDG uptake, that is, infections, radiation necrosis, or brain edema formation (Kubota et al., 1995), especially for determining their tumoral boundaries (Derlon et al., 1997; Ogawa et al., 1991). Although methionine-PET has been reported to offer a reliable diagnostic method for differentiation between recurrent malignant astrocytoma and radiation necrosis or gliosis, it is less sensitive if the brain lesions is hypo- or isometabolic on FDG-PET (Chung et al., 2002; Forsyth et al., 1995).

L-(1-^{11}C)tyrosine

Although the radiosynthesis of L-(l-^{11}C)-tyrosine is difficult, a metabolic model to quantify the protein synthesis rate is described and validated (Daemen et al., 1991b; Luurtsema et al., 1994; Willemsen et al., 1995). Tyrosine is largely incorporated into protein synthesis and generates only a small amount of labeled tissue metabolites on the time scale of ^{11}C PET-studies (Willemsen et al., 1995). On the other hand, plasma metabolites (labeled proteins, labeled CO_2, and acid-soluble metabolites such as ^{11}C-L-dihydroxyphenylalanine) rise to 50% approximately 1 h after injection, requiring arterial sampling and metabolite correction for quantitative determinations of the protein synthesis rate.

L-2-(^{18}F)Fluorotyrosine

Coenen et al. (1989) were among the first to study the metabolism of ^{18}F-labeled tyrosine. Fluoride will not cross the BBB, not be metabolized, and not be incorporated into proteins but will accumulate in bone and might thus interfere with PET studies in organs other than the brain tissue. Additional experiments revealed that the amount of L-2(^{18}F)fluorotyrosine acetylated to tRNA is 1.5–2%, concluding that L-2-(^{18}F)fluorotyrosine is a tracer for measuring the protein synthesis rate (Coenen et al., 1989).

Herholz et al. (1992) studied 15 patients with various primary brain tumors by dynamic PET, demonstrating that uptake of L-2(^{18}F)fluorotyrosine in the tumor tissue was 27% higher than in the surrounding normal brain tissue. Using a three-rate constant, two-tissue compartment model analysis, the authors conclude that this high uptake could be attributed to a twofold increase in the transport rate constant (K_1). Kinetic studies in patients demonstrate that the increased brain tumor accumulation of L-2-(^{18}F)fluorotyrosine is mainly due to increased transport rate rather than increased protein incorporation of L-2-(^{18}F)fluorotyrosine compared with that of L-(methyl-^{3}H)methionine and L-(l-^{14}C)leucine in tumor-bearing mice (Coenen et al., 1989).

L-3-(^{18}F)Fluoro-alpha-methyl tyrosine (FMT)

FMT demonstrates a similar affinity to the neutral AA carrier at the BBB as L-tyrosine but is not metabolized and not incorporated into protein synthesis (Laverman et al., 2002). The main AA transport system involved in FMT uptake appears to be the L-system, as found in studies of FMT kinetics in brain tumor cell lines (Inoue et al., 1999). FMT uptake seems to follow the same uptake route as does the native AA tyrosine, but its major part is not incorporated into protein. This is in contrast to the L-2(^{18}F)fluorotyrosine, which is rapidly incorporated into protein metabolism. Therefore, a kinetic model for tumor uptake of FMT is less complicated than the model for L-2-(^{18}F)fluorotyrosine (Laverman et al., 2002). A clinical study demonstrated the feasibility of using FMT for PET imaging of primary brain tumors (Inoue et al., 1999). Uptake in primary brain tumors is rapid and reaches a maximum at 10 min pi and was higher than uptake of FDG (Inoue et al., 1999). This FMT uptake significantly decreases after additional administration of large neutral AA (Junck et al., 1989) but seems to be independent of the tumor grade (Laverman et al., 2002). Blood clearance is rapid, with a $t_{1/2}$ of about 3 min (Inoue et al., 1999).

O-(2-(^{18}F)Fluoroethyl-L-tyrosine (FET)

Kinetic studies in 10 patients with high-grade brain tumors reveal that a simple two-compartment model is sufficient to analyze FET-kinetics (Weber et al., 2001). The absence of metabolites in the blood up to 60 min pi and a rapid equilibrium of FET between erythrocytes and plasma facilitate quantitative assessment of AA transport system. Schreckenberger et al. (2001) present preliminary results of a comparative study between FET and FDG in patients with metastatic malignant melanoma in supratentorial brain tissue. Although the number of patients was small (n = 7), the tumor uptake of FET and FDG correlated well. In PET studies, metastases showing no FDG uptake were visualized with FET, whereas FET-negative metastases showed FDG uptake, thus indicating that the use of both tracers simultaneously could enhance the sensitivity.

Intensive FET uptake is noted in primary brain tumor areas without contrast enhancement on MRI, demonstrating that the L-system is an important carrier for neutral AA at the BBB. Therefore, BBB disruption appears not to be required for focally increased FET uptake (Daemen et al., 1991a). Thus, FET may be applied, like MET, for delineation of brain tumor infiltration prior to surgical resection and for differential diagnosis of low-grade astrocytomas.

Thymidine analogs

Recently, two thymidine analogs resistant to further metabolism have shown promise as radiolabeled agents in recent PET studies (see Fig. 2). These imaging modalities examine thymidine transport and phosphorylation as an estimate of DNA replication, and from this, cell proliferation.

1-(2′-fluor-5-methyldeoxyuracil-beta-D-arabinofuranoside (FMAU) is a thymidine analog with a fluorine substitution in the sugar. It is phosphorylated by thymidine-kinase (TK), incorporated into DNA to a variable extent, stable to degradation, and most of the tracer is cleared unchanged in the urine. Preliminary studies demonstrate its suitability for cell proliferation imaging in primary brain tumors (Jacobs et al., 2001), and further studies are underway to determine its utility in patients.

Another promising compound (^{18}F)3′-deoxy-3′-fluorothymidine (FLT) is taken up by cells and phosphorylated by TK at about 30% of the rate of thymidine. It is resistant to degradation in (animal) blood and is excreted unchanged in the urine. FLT acts as a chain terminator in DNA synthesis so that it is little incorporated into DNA. However, its retention is dependent on the presence of TK, and thus it indirectly reflects cellular proliferation.

Other receptor-bound tracers

The use of radiolabeled tracers, which specifically bind to receptors, has been applied mostly to pituitary adenomas, in particular in the assessment of nonsecreting tumors in the para-

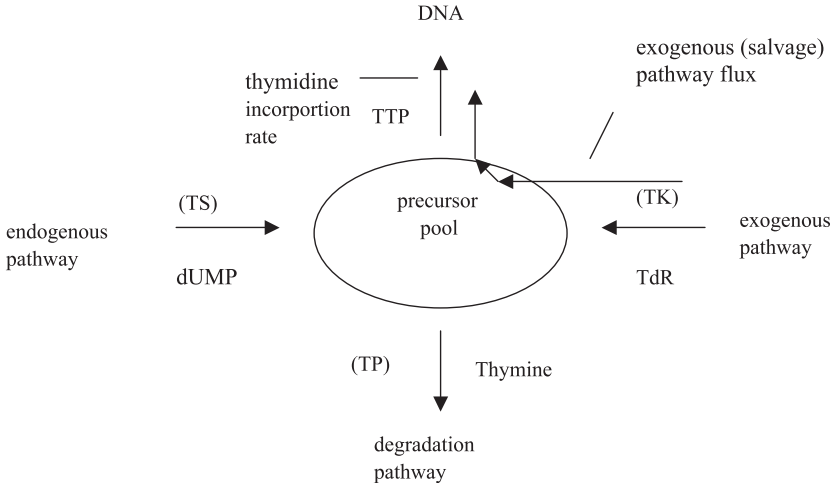

Fig. 2. Four-factor model of thymidine utilization. FLT resists degradation but undergoes glucoronidation; little FLT or FMAU is accumulated in DNA, rather it is retained intracellularly after phosphorylation by cytosolic thymidine kinase 1 (see also Fig. 1). Legend: TS: thymidylate synthase; TP: thymidine phosphorylase; TK: thymidine kinase.

sellar region where radiological differential diagnosis may occasionally be difficult (Schaller, 2002; deHerder et al., 1999; Schaller et al., 1998). The in vivo characterization of the biochemical and functional properties of the hypothalamic tissue may provide useful information about the nature of the pituitary mass. PET studies are used for the assessment of adenomas and other parasellar tumors with ^{11}C-deprenyl, ^{11}C- and ^{18}F-labeled spiperone analogues, ^{123}I-IBZM, and ^{123}I-epiderpride (Bergstrom et al., 1992; Daemen et al., 1991b; Junck et al., 1989; Luciganini et al., 1997; Muhr et al., 1986; Pirker et al., 1996). Some brain tumors demonstrate a high density of benzodiazepine receptors compared with normal tissue. ^{11}C-PK11195 is a ligand that binds with high-affinity to peripheral benzodiazepine receptors and is used to image astrocytomas (Junck et al., 1989; Pappata et al., 1991). Benzodiazepine receptors are increased 20-fold in brain tumors compared with normal brain (Black et al., 1991). As a result, ^{11}C-flumazenil, a benzodiazepine antagonist, can now be used as a tracer to delineate the outer margins of primary brain tumors in PET images.

Amino acid transport across the blood brain barrier

Radiolabeled AA that enter protein synthesis (e.g., tyrosine, and partly methionine) are believed to better reflect the malignant nature and increased proliferation rate of brain tumor cells than do AA that are only transported into the cell (e.g., FMT, FET, ^{11}C-aminoisobytyric acid, and partly methionine). This assumption is based on the concept that increased cellular proliferation requires increased protein synthesis. However, as a consequence, AA transport is increased in malignancy as well, but this increase may be even more pronounced than the increase in protein synthesis. Although difficult to measure experimentally, the size of the intracellular AA pool is probably increased in malignancy. From this pool, AA may enter ribosomal protein synthesis (Shoup et al., 1999). The fraction that enters protein synthesis and the fraction used for other purposes are varying for different AA. The exact extent of the fraction entering protein synthesis may indeed correlate with the proliferation rate, although protein synthesis for cellular maintenance will disturb this correlation. However, PET studies visualize the sum of both fractions, and the total AA signal is therefore also likely to correlate with proliferation rate.

One of the two main AA transporter systems at the BBB represents Na^+-dependent system A, a transporter of small neutral AA, which accepts L-alanine, L-proline, glycine, and alpha-methylaminoisobutyric acid as substrates and is often transinhibited by intracellular substrates of this system (see Fig. 3). It has been suggested that system A is present in the abluminal (brain) side of the BBB because methylaminoisobutyric acid, which is a specific nonmetabolizable substrate for system A, is taken up in an Na^+-dependent manner from the brain side using isolated rat brain capillaries and isolated abluminal membrane vesicles from bovine brain endothelial cells. Several investigations using brain uptake methods have established that system A substrates undergo limited influx transport across the BBB (Stryer, 1995). These investigations suggest that system A plays a role in the efflux transport of small neutral AA at the BBB to maintain their concentration in the brain tissue (Killberg et al., 1981; Shotwell et al., 1983). However, it does not fully explain this hypothesis because the neutral AA are believed to be supplied from the circulating blood (Bode and Kilberg, 1991; Schaller, 1998). Recently, three Na^+-dependent small neutral AA transporter systems have been identified as

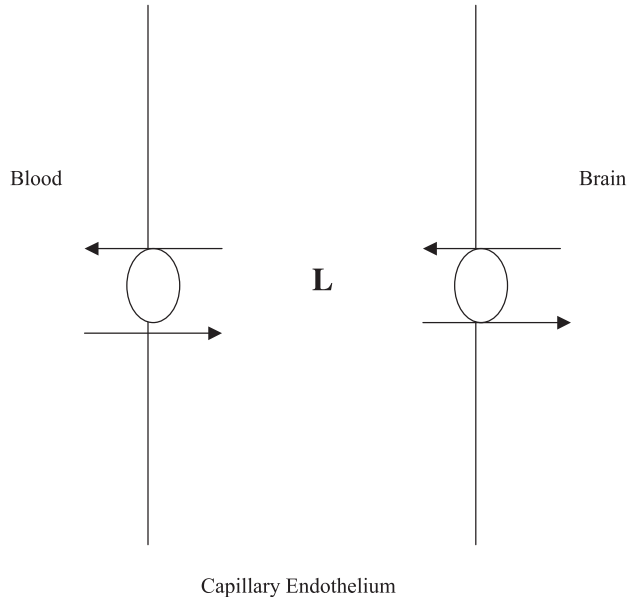

Fig. 3. Schematic illustration of large amino acid transport system at the brain capillary endothelium and their localization to the capillary luminal (plasma) or abluminal (brain) membrane. Legend: L: Amino acid transport system L, mediating high-affinity, sodium-independent uptake of amino acids with "large neutral" side chains, including L-tyrosine and L-methionine.

system A isoforms, namely, GlnT/ATA1/SAT1, ATA2/SAT2/SA1, and ATA3 (Christensen, 1990; Oxender and Christensen, 1963; Shotwell et al., 1981; Shoup et al., 1999). Moreover, Alfieri et al. (2001) suggest that ATA2 expression and its AA transport activity in porcine endothelial cells are under osmotic regulation. To understand the regulation and physiological and/or pathophysiological functions of system A at the BBB, it is important to better identify isoforms of system A and clarify the transport functions of system A at the BBB under pathological conditions (Christensen, 1990; Oxender and Christensen, 1963; Shotwell et al., 1981; Souba and Pacitti, 1992). The osmoregulation in the brain tissue may play a role in detoxification in the brain to project it against adverse effects such as brain edema or brain tissue damage (Jager et al., 2001).

The large neutral AA are transported by the Na^+-independent L (leucine preferring) AA transport system (LAT) that is expressed at the BBB and is mainly dependent on the concentrations of the AA outside and inside the cell. The direction of the transport is determined by the concentration gradient at the cell membrane. Nevertheless, in vitro studies have shown that the L-system may accumulate AA intracellularly against a concentration gradient: an AA with affinity to the A- and L-transporter system, such as methionine, may enter the cell by the active, energy-dependent A-system and exit in exchange for tyrosine using the L-system. Large AA are transported via this system comprising a heterodimer of the 4F2hc heavy chain and the large LAT1 light chain, which is similar to other AA transporters (Alfieri et al., 2001). From cloning experiments and full-length cDNA, it can be derived that the bovine LAT1 composes 505 AA and has a predictive molecular mass of 55 kDa (Boado et al., 1999). Using Northern blotting experiments, it is estimated that LAT1 is profoundly unregulated in brain capillaries (Boado et al., 1999). The amount of LAT1 mRNA in bovine brain-capillary-endothelial cells is very high compared to

other organ tissues (Boado et al., 1999). However, the K_m of the L-system at the BBB is much smaller (10–100 γM) than of those in peripheral tissues (1–10 mM) (Pardrige, 1983; Pardrige, 1998). In addition, the K_m of LAT1 at BBB is similar to the plasma concentration of circulating large AA, which means that this transporter is saturated under normal conditions, making the brain tissue vulnerable to pathological effects of hyperaminoacidemia (Pardrige, 1986). cDNAs of rat and human LAT2 have also been cloned, and it is suggested that LAT2 is expressed at the BBB (Segawa et al., 1999). The K_m of the LAT2 transporter for leucine is shown (Sato et al., 1999) and approximately 10 times greater than the K_m of leucine by the LAT1 transporter (Pardrige, 1983). Recently, it could be demonstrated that LAT1 is the predominant functional active LAT isoform at the microvascular endothelium of rat brain tissue (Killian and Chikahle, 2001; Killberg et al., 1981).

Although each system is specifically designed for the transport of particular AA, most systems are able to take up nonmetabolizable or synthetic AA analogues. System A recognizes *N*-alkylated AA and reveal specificity for 2-aminoisobutryric acid and its *N*-methylated derivative 2-methylaminosiobutyric acid. System L is reactive with the nonmetabolizable analogue 2-aminoabicyclo-(2.2.1)-hetane-2-carboxylic acid.

Positron emission tomography imaging and its relationship to brain tumors

Brain tumor detection

Generally, a high sensitivity is reported for primary brain tumor detection with PET (see Table 2). Initial FDG-PET studies can identify elevated FDG uptake in primary brain tumors (Derlon et al., 1997) with good correlation of the grade of malignancy (see Table 3). Thus, low-grade astrocytomas are not easily identified or appear as hypometabolic areas surrounded by normal high FDG uptake within the cerebral cortex hindering a clear definition of exact tumor extension. Many clinical studies have demonstrated that methionine-PET imaging is highly accurate in defining of tumor boundaries both in primary or recurrent brain tumors, regardless of their histological grading (Patronas et al., 1985). For example, Ogawa et al. (1993) demonstrate an excellent 97% sensitivity for methionine-PET in 32 patients with high-grade astrocytomas but only a 61% sensitivity in low-grade astrocytomas. Mosskin et al. (1986) present a patient-based sensitivity of 84% using stereotactic biopsies from primary brain tumor and normal brain tissue areas, indicating that tumor specificity of methionine contains a certain rate of false-positive results. In a large series of astrocytomas, 95% of 37 lesions are clearly visualized in methionine-PET studies, whereas FDG shows 41% as hypermetabolic, of which mostly are high-grade astrocytomas; and 49% as hypometabolic lesions, while 10% are difficult to distinguish from surrounding normal brain tissue (Kaschten et al., 1998). The reported advantage of methionine over FDG in delineating astrocytomas is probably not relevant in CNS lymphoma, where FDG uptake is much higher in tumor than normal brain tissue (Roelcke and Leenders, 1999).

Experience with tyrosine as radiolabeled ligand for PET studies in primary brain tumors is more limited. Pruim et al. (1995) using tyrosine PET imagines for both primary and recurrent brain tumors (including metastases and cerebral lymphomas) find 91% of 22 tumors positive for uptake. Wienhard et al. (1991) could demonstrate increased uptake and transport rates of 2(^{18}F)-fluorotyrosine in primary brain tumors (n = 15). Such an uptake appears more related to AA transport than to protein synthesis.

Table 3
FDG-PET diagnoses compared with a gold standard of biopsy or radiographic follow-up (adapted from Coenen et al., 1989)

Gold standard	FDG-PET diagnosis		FDG-PET diagnosis in brain metastasis patients only		FDG-PET diagnosis in primary tumor patients only	
	Tumor (%)	Necrosis (%)	Tumor (%)	Necrosis (%)	Tumor (%)	Necrosis (%)
Tumor	47	16	42	17	75	13
Necrosis	7	3	8	33	0	13
Sensitivity	75		71		86	
Specificity	81		80		100	

Abbreviation: FDG = ^{18}F-fluorodeoxyglucose; PET = positron emission tomography.

Brain tumor grading

A PET imaging with FDG is considered useful in the diagnostic workup of suspected primary brain tumors or metastases, as it may identify focal hypermetabolic changed brain areas. Different molecular imaging studies have related the grade of malignancy of astrocytomas to the rate of FDG uptake in PET and can show that while low-grade astrocytomas display low FDG uptake, anaplastic astrocytomas and glioblastoma multiform have markedly elevated uptake (deWolde et al., 1997). FDG-PET imagines have also been proposed as a useful tool to assess the tumor grade in oligodendrogliomas and gangliogliomas (Derlon et al., 1989).

Nearly all PET studies on tumor detection also addressed the feasibility of tumor characterization and grading, comparing uptake both between benign and malignant processes and between various grades of malignancy. This clinically useful aspect is supported by in vitro studies, in which methionine uptake was shown to correlate with proliferation markers (Kole et al., 1999; Wienhard et al., 1991). Sato et al. (1999) demonstrate a clear positive correlation between proliferating cell nuclear antigen index and methionine uptake, indicating that methionine is taken up more rapidly and accumulates in highly proliferative tissue. Somewhat surprising is that this relationship is not confirmed for tyrosine uptake (n = 20) (deWolde et al., 1997). Different methionine accumulations in vivo have shown an uptake in low-grade astrocytomas being near background uptake but a high uptake in oligodendrogliomas (Derlon et al., 1989). Derlon et al. (1989) suggest that this difference could be clinically useful. In this context, good and possibly clinical useful differentiation (without overlap) between skull base meningiomas and benign neuromas can be demonstrated by Nyberg et al. (1997). The largest study was performed by Herholz et al. (1992), finding 79% accuracy in distinguishing astrocytomas from nonneoplastic lesions in 196 patients with a suspected primary brain tumor.

Brain tumor delineation

Many studies have demonstrated that the margins of brain tumors, as assessed by methionine uptake, are frequently wider than the anatomic boundaries, as demonstrated by MRI (Bergstrom

et al., 1983; Cont, 1995; Kaschten et al., 1998; Mineura et al., 1991; Mosskin et al., 1986). This fact is explained by the lack of contrast enhancement in MRI in intratumoral areas with an intact BBB. This phenomenon may be even more pronounced in low-grade tumors and in diffuse gliomatosis (Mineura et al., 1991). In comparison with FDG-PET, such a better tumor delineation is reported both for methionine and FET (Kaschten et al., 1998). Methionine or FDG scanning is combined with activation studies using radiolabeled water ($H_2^{15}O$) to depict tumor extension in relation to functional brain areas (Duncan et al., 1997), with the aim to achieve a subsequent more aggressive surgical resections with a reduced risk of neurological impairment.

Biopsy localization

Stereotactic biopsies of localizations that are based on either methionine or FDG-PET seem to be more successful to find accurate brain tumor tissue than are biopsy trajectories based on CT only (see Table 3). Especially strong uptake reduction of MET in necrotic parts or especially high uptake in anaplastic parts of the tumor tissue may influence the surgical planning and subsequent results of brain tumor biopsies. In comparison with FDG, methionine is advantageous in offering better detection of nonanaplastic tumor zones and brain tissue with infiltrating neoplastic cells (Goldman et al., 1997). Planning of biopsy trajectories is suggested to be improved by tyrosine, particularly in low-grade astrocytomas (Go et al., 1994).

Differential diagnosis

MRI usually establishes the differential diagnosis between "normal" brain tissue and malignant or nonmalignant lesions. However, this is not the case in AIDS with neurological manifestations characteristic of intracerebral space-occupying lesions, so that FDG-PET has been used to differentiate between toxoplasmosis and lymphoma (Costa et al., 1995): high uptake of FDG is strongly suggestive of a malignant lymphoma presenting as an extremely metabolically active tumor, while a relatively hypometabolic lesion can be demonstrated in toxoplasmosis. The problem of specificity, however, may limit the usefulness of FDG-PET as a routine method, as inflammatory lesions can also accumulate FDG (Wurker et al., 1996).

Evaluation of therapy

In the early postoperative period, FDG-PET can be used to differentiate residual tumor tissue from postoperative surgical effects (Hanson et al., 1990; Kim et al., 1992). It seems clear that a decline in tumor tissue uptake of FDG weeks or months after therapy is suggestive of a good response to treatment, indicating either a reduced number of viable cells or reduced metabolism of damaged cells (Haberkorn et al., 1993).

After intensive irradiation or chemotherapy for malignant brain tumors, MRI is not able to distinguish tumor progression from radiation damage or necrosis. Some PET methods appear promising as relatively specific indices of therapeutic response. FDG uptake suggest the presence of viable brain tumor tissue (at least when high tumor uptake of FDG was noted before therapy), while absence of FDG uptake suggests that necrosis may be present (DiChiro et al., 1988; Ishikawa et al., 1993). An increase in brain tumor metabolism compared to studies before therapy predicts

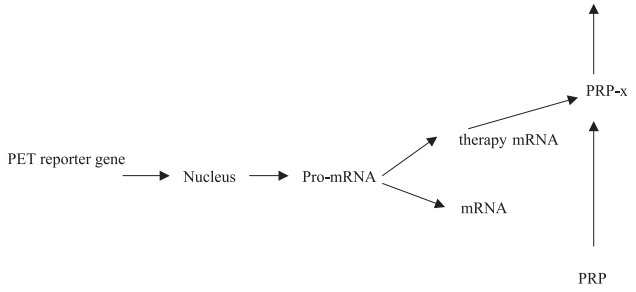

Fig. 4. Schematic illustration of PET reporter gene and PET reporter probe for imaging gene expression. The PET reporter gene complex is transfected into target cells by a vector. Inside the transfected cell, the PET reporter gene is transcript to PET reporter mRNA (therapy mRNA) and then translated on the ribosomes to a protein (enzyme). After administration of a radiolabeled probe and its transport into the cell, the probe is phosphorylated by the PET reporter gene product. The phosphorylated radiolabeled probe does not readily cross the cell membrane and is trapped within the cell. Thus, the magnitude of probe accumulation in the cell (i.e., the level of radioactivity) reflects the level of PET reporter enzyme activity and the level of PET reporter gene expression. Legend: PRP: PET reporter probe.

longer survival (deWitte et al., 1994), resulting from predominant killing of low energy-consuming cells or stimulation of quiescent cells, either tumor or normal, to become more active metabolically. Within a certain volume of a specific tissue, the ratio and density of normal cells to tumor cells improves, leading to increased regional metabolism.

Changes in proliferation pattern can be assessed by monitoring thymidine uptake (reflecting DNA synthesis) (see Fig. 4) or methionine uptake (reflecting new protein synthesis). A novel strategy directly images apoptosis based on detection of the associated increased phosphatidylserine expression. Agents that are trapped when reduced can be used to assess tumor hypoxia, a cause of failure of chemotherapy or radiotherapy treatments. Defining these brain tumors regions likely to be refractory to noninvasive treatments could allow more selective targeting of cell kill therapies or surgical excision.

Detection of recurrent or residual viable brain tumor tissue can be troublesome in brain tumors treated by surgery or irradiation. In vitro evidence is somewhat conflicting, but it can be demonstrated that methionine-PET is suitable for follow-up of the treatment effects (Chao et al., 2001; Holzer et al., 1993; Schifter et al., 1993; Lilja et al., 1989; Segawa et al., 1999; Wurker et al., 1996). For example, Wurker et al. (1996) demonstrate a dose-dependent reduction in uptake in low-grade astrocytomas up to 1 year after brachytherapy, whereas FDG uptake is unchanged. Sonoda et al. (1998) find no methionine uptake in six of seven cases of radionecrosis that are difficult to assess using MRI or CT. Remarkably, the protein synthesis rate, determined by using tyrosine-PET, remains unchanged in 80% of patients after radiotherapy (Heesters et al., 1998). Four hours after irradiation, the increase in tumor FDG uptake compared to the preirradiation study is significantly correlated with a decrease in tumor size, as subsequently assessed with MRI. For malignant astrocytomas, this relationship has not been assessed yet. Voges et al. (1997) report on a series of 46 patients who underwent serial methionine and FDG-PET studies following interstitial brachytherapy: methionine is superior to FDG in delineating residual of recurrent

tumor tissue. This finding confirms earlier data on the comparison of FDG and AA in visualization of untreated low- and high-grade astrocytomas.

Several PET studies try to establish a relationship between metabolic response and prognosis after initiation of chemotherapy in patients with glioblastoma multiform. The change of FDG uptake induced by chemotherapy can be correlated with survival. Both positive or inverse correlations can be found between metabolic responses and survival, making these data inconclusive so far. In a more recent study, methionine is found to be superior to FDG in monitoring the treatment effects in low-grade astrocytomas (Wurker et al., 1996).

Prognosis

FDG-PET is used both to predict the survival of untreated patients and to confirm suspected recurrence of high-grade astrocytomas. All these studies reveal that FDG may differentiate recurrence from other therapy-related changes, demonstrating that tumoral FDG uptake lower than adjacent cortical tissue is associated with a longer survival time than observed in tumor FDG uptake higher than in the adjacent cortex (DiChiro et al., 1982). Later, a relationship between glucose metabolism as assessed by FDG uptake and risk of malignant evolution in low-grade astrocytomas is demonstrated. One may conclude that the presence of areas of increased FDG uptake in a histologically proven low-grade astrocytoma predicts an adverse clinical course: patients with hypermetabolic tumors demonstrate a median survival of 7 months after FDG-PET compared with 33 months for those with hypometabolic tumor. PET can be used to separate high-grade astrocytomas into subgroups with good prognosis (hypometabolic 78% 1 year survival) (Alavi et al., 1988). Residual or recurrent high-grade astrocytomas showing high glucose utilization present with a mean survival period of 5 months; whereas in those tumors showing lower utilization, mean survival is 19 months (DiChiro, 1987; Patronas et al., 1985).

Experience with other tracers than FDG is limited, but in a quantitative evaluation of methionine uptake with low-grade astrocytomas, those patients with a low tumor uptake in the baseline study demonstrate a significantly better prognosis than those with a high uptake (Muutinen et al., 2000). The prognostic information by AA PET studies is provided by the presence, but not by the intensity, of uptake. The current data suggest caution in relating high AA uptake values to poor prognosis despite the capability of AA imaging to help determine the presence and extent of astrocytomas.

Future

New opportunities in the area of diagnostic imaging are being created by the development of methods for the assessment of location, magnitude, and duration of gene expression (Jacobs et al., 2001) (see Fig. 4). These methods can be based on the use of antisense oligodeoxynucleotides targeted towards the mRNA of a gene of choice or on the use of a reporter gene to assess the expression of a gene. Although these imaging methods are still in their beginning, they may soon become relevant for application in patients (Jacobs et al., 2001), for example, by PET studies of the delivery of a suicide gene, that is, a gene that when delivered to cells, renders them sensitive to a prodrug.

Conclusion

The amount of information to be gained from applying the increasing number of radionuclides in a variety of pathological situations is potentially very great. Biochemical or molecular imaging with radionuclides of processes that occur at a cellular level provides information that complements findings obtained by anatomical imaging aimed at depicting structural, vascular, and histological changes. For brain tumor diagnosis, radiolabeled AA demonstrate adequate sensitivity and specificity. For tumor grading, the role of the current data is still controversial because conflicting reports exist. In addition, thresholds frequently are defined retrospectively—a methodologically suboptimal choice—and the true clinical impact is therefore unclear. Reasonable evidence exists that radiolabeled amino acids have supplemental value in the evaluation of treatment and the detection of recurrence of brain tumors. PET represents a unique methodology for examining the biochemical features of brain tumors and can therefore function as a translational bridge between in vitro biologic discovery and clinical medicine.

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