Molecular Imaging of Brain Tumors

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Abstract

Non-invasive energy metabolism measurements in brain tumors in vivo are now performed widely as molecular imaging by positron emission tomography. This capability has developed from a large number of basis and clinical science investigations, which have cross- fertilized one another. Apart from precise anatomical localization and quantification, the most intriguing advantage of such imaging is the opportunity to investigate the time course (dynamics) of disease-specific molecular events in the intact organism. Most importantly, molecular imaging represents a key technology in translational research, helping to develop experimental protocols that may later be applied to human patients. Common clinical indications for molecular imaging of primary brain tumors, therefore, contain (1) primary brain tumor diagnosis,

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(2) identification of metabolically most active brain tumor reactions (differentiation of viable tumor tissue from necrosis), and (3) prediction of treatment response by measurement of tumor perfusion or ischemia. The key question remains whether the magnitude of biochemical alterations demonstrated by molecular imaging reveals prognostic value with respect to survival. Molecular imaging may identify early disease and differentiate benign from malignant lesions. Moreover, an early identification of treatment effectiveness could influence patient management by providing objective criteria for evaluation of therapeutic strategies for primary brain tumors. Its novel potential to visualize metabolism and signal transduction to gene expression is used in reporter gene assays to trace the location and temporal level of expression of therapeutic and endogenous genes. Currently, molecular imaging probes are developed to image the function of targets without disturbing them or as a drug in order to modify the target's function. In this new context, the microenvironment of malignant brain tumor and the blood-brain barrier shows increased interest. The objective is transfer gene therapy's experimental knowledge into clinical applications. Molecular imaging closes the gap between in vitro to in vivo integrative biology of disease.

Introduction

Despite increasing experimental research regarding pathophysiological mechanism of primary brain tumors in the recent years, the underlying molecular changes both in the tumor area and its surrounding brain tissue remain only partially understood (Schaller, 2003, 2005; Schaller and Buchfelder, 2006). For this reason, much experimental attention has been directed toward understanding the cellular and molecular mechanism of the glial tumor genesis and the development of noninvasive, high-resolution in vivo imaging technology, especially in vivo molecular imaging (Weissleder and Mahmood, 2001).

Glial Tumor Genesis

A complex series of molecular changes occur in the development of primary brain tumors, which results in (1) dysregulation of the cell cycle (e.g., hypermethylasation of TP53), (2) alterations of apoptosis and cell differentiation (amplification of oncogenes and growth factors and/or their receptors (e.g., MDMD2)), epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), PDGFR (platelet-derived growth factor (PDGF) and its receptor), (3) neovascularization and (4) tumor cell migration and invasion into the brain parenchyma (Weissleder and Mahmood, 2001; Schaller, 2004). For instance, during progression from low-grade astrocytoma (WHO grade II) to anaplastic astrocytoma (WHO grade III) and to glioblastoma multiforme (WHO grade IV) a step-wise accumulation of genetic alterations occurs (Weissleder and Mahmood, 2001; Schaller, 2004): TP53 mutation and PDGF and PDGFR-α overexpression represent early changes during low-grade glioma development. Further progression to anaplastic astrocytoma is associated with pRB alteration and loss of heterozygosis (LOH) of 19q. Finally, malignant progression to glioblastoma multiforme includes LOH 10q and mutations of the PTEN gene (Schaller, 2004). These secondary glioblastomas, which develop from better-differentiated astrocytomas, can be distinguished from de novo glioblastomas on the basis of molecular genetic findings (Schaller, 2004). Amplifications and/or overexpression of the EGFR, p16 deletion, PTEN mutation, pRB alteration, and LOH 10p and 10q are associated with primary glioblastoma.

A clinically most interesting consequence of this research is that molecular alterations have been identified that indicate different therapeutic response of a tumor type to a given drug, which is prognostically relevant. For example, anaplastic oligodendrogliomas with LOH 1p and/or LOH 19q are characteristically sensitive to PCV (procarbazine, lomustine, and vincristine) chemotherapy, and patients' survival is significantly prolonged (DeAngelis et al., 1998).

Molecular Imaging

Intratumoral heterogeneity of brain tumors is not adequately reflected in conventional magnetic resonance imaging (MRI) and evaluation of a contrast enhancing lesion may either under- or over-estimate the presence of active tumor tissue (Levivier et al., 1996). Molecular imaging by positron emission tomography (PET) is therefore performed to gain additional information on metabolic and molecular tumor markers. PET measures and visualizes cellular biochemical processes non-invasively and quantitatively by pattern of in vivo uptake of molecular probes into the brain tissue (Levivier et al., 1996). Because biochemical changes may be related to the growth rate of tumor cells (Levivier et al., 1996) they can be thought as markers of tumor cell proliferation.

In primary brain tumors, molecular imaging allows (1) earlier detection of tumor genesis at pre-disease states, (2) evaluation of the pharmacodynamic and neurotoxicity of chemotherapeutic agents, (3) evaluation of the response to treatment, (4) differentiation between iatrogenic lesions and residual or recurrent tumor tissue (Weissleder and Mahmood, 2001; Jasanoff, 2005).

This chapter reviews the potential of the molecular neuroimaging, particularly PET, in brain tumors, giving an overview regarding the current possibilities and giving additionally link to molecular biological features of brain tumors.

The Microenvironment of Brain Tumors

Pathological characteristics of malignant brain tumors are exemplified by active invasiveness, necrosis, and a specialized form of angiogenesis, known as microvascular hyperplasia. Such pathological features are thought to be due to tissue hypoxia. Therefore, hypoxia is a critical aspect of the surrounding microenvironment of brain tumors, and is generally associated with unfavorable clinical outcomes. Cells that are under hypoxic stress can develop an adaptive response that includes increased rates of glycolysis and angiogenesis or undergo cell death by promoting apoptosis and/or necrosis. The ability of tumor cells to maintain a balance between adaption to hypoxia and cell death is regulated by hypoxia-inducing factors, a family of transcription factors that are essential for the regulation of the expression of a large number of hypoxia-responsive genes. Tumor hypoxia is hypothesized to facilitate metastases, tumor recurrence, invasive potential, resistance to chemotherapy and radiotherapy, which culminate in decreased patient survival. For this reason, effective targeting of hypoxic areas in brain tumors remains a significant therapeutic challenge. New therapeutic options for tumor-targeted drug delivery show promise in treatment of brain tumors that are refractory to traditional therapies. However, the molecular mechanism of targeting to (hypoxic) tumor areas is not well understood. The unique ability, for example, of stem cells, to "home in" on tumor cells and then deliver a desired gene product makes such methods a promising option in brain tumor therapy. Cytolytic viruses and genes coding for anti-tumor cytokines, prodrug-converting enzymes and various neurotrophic factors have been engineered into such options. Novel brain tumor treatment strategies that involved transplantation or infusion of cells that seek out invading tumor cells demand thorough in vivo monitoring. In particular, such treatment methods have attracted great interest because they have demonstrated tropism to tumor cells and even long-distance migration to single tumor cells. However, little is known regarding how these cells exert their beneficial effects in vivo.

Principles of Molecular Imaging in Neurological Sciences

The design of molecular probes for PET studies is usually based on either fundamental biochemical principles or on known pharmacological properties of particular classes of drugs. In reality, it is often difficult to draw a clear distinction between the two, because most modern drugs are designed to interact with predetermined molecular targets that have been identified through pharmacological study as clinically relevant.

Molecular imaging of the central nervous system (CNS) has enabled scientists and researchers to better understand the basic biology of brain function and the way in which various disease processes affect the brain. Unlike other organs, the brain is not easily accessible, and it has a highly selective barrier in that specialized cerebral microvascular endothelium interacts with the cellular milieu of the brain and extracellular matrix to form a neurovascular unit known as the blood-brain barrier (BBB) (Schaller, 2004). BBB dysfunction is a complication of high-grade brain tumors, but it also prevents many therapeutic molecules from entering the CNS (Schaller, 2004). Furthermore, genetic and/or epigenetic abnormalities in the constituents of the BBB may be significant contributing factors in disease etiology.

At present, molecular imaging is able to quantify permeability measurements across the brain endothelium. Malignant primary brain tumors, such as glioblastoma, are characterized by extensive angiogenesis and permeability of the BBB (Schaller, 2004). To date, BBB permeability data have been shown to be useful in preoperative brain tumor grading and potentially also in determining the effectiveness of selective types of therapy in primary brain tumors (Schaller, 2004). For this reason, explorative studies are evaluating new strategies for safe and effective alteration of the BBB permeability to improve local drug delivery into primary brain tumors (Schaller, 2004). As new anti-angiogenesis drugs become available, BBB permeability imaging may also become critical as a surrogate angiogenesis marker to monitor tumor response to these agents.

Characterization of molecular agents at the level of individual cells is crucial if molecular neuroimaging techniques are to be correctly interpreted and trusted as direct measures of physiological or pathophysiological brain function (Schaller, 2004). Determination of delivery methods, subcellular localization patterns, sensor response properties, toxic effects, and in vivo kinetics in simple systems will facilitate application of labeled-agent-based imaging (Jacobs et al., 2003a, b). At the present time, several groups have developed reduced preparations (some are of biological interest in themselves), in which many physiological variables have been minimized, and the effects of contrast agents has been possible with true-cellular or near-cellular resolution (Jacobs et al., 2003a, b). An example of this molecular level characterization is the current possibility to determine P-gp-based drug interactions at the human BBB by PET (Jacobs et al., 2003a, b). From this knowledge to visualize cellular or subcellular functions, the application of molecular neuroimaging techniques has evolved now into the clinical practice of humans.

Before new and potentially more effective treatment strategies, such as gene- and cell-based therapies, can be effectively implemented in the clinical application, certain prerequisites have to be established. First, the exact localization, extent, and metabolic activity of the brain tumors have to be determined to identify the biologically active target tissue for a biological treatment regimen. This necessary prerequisite is usually performed by imaging the expression of up-regulated endogenous genes (Schaller, 2004). Second, neuronal function and functional changes within the surrounding brain tissue have to be assessed in order to save this tissue from therapy-induced damage (Weissleder and Mahmood, 2001). Third, pathognomonic genetic changes leading to disease have to be explored at the molecular level to serve as specific molecular therapeutic targets for patient-tailored therapies (Jacobs et al., 2003a, b). Finally, a concerted noninvasive analysis of both endogenous and exogenous gene expression in animal models as well as the clinical setting is desirable to effectively translate new treatment strategies from experimental into clinical application (Schaller et al., 2007). Non-invasive imaging of endogenous gene expression by means of PET may reveal insight into the molecular basis of pathogenesis, metabolic activity of the glioma, and the extent of treatment response (Schaller, 2004). When exogenous genes are introduced to serve for a therapeutic function, PET imaging techniques may additionally reveal the assessment of the location, magnitude and duration of therapeutic gene expression, and its relation to the therapeutic effect (Schaller et al., 2007). All of these issues can be addressed by multi-modal

molecular imaging techniques and may therefore reveal insight into the molecular basis of pathogenesis and metabolic activity of the brain tumors and the extent of treatment response (Schaller, 2004).

Molecular Imaging Strategies

The biochemistry of life involves a complex series of interdependent interactions that comprise homeostasis. These interactions can be envisaged as the interaction of the genome and the "functional" molecules it encodes through bioenergetically driven chemical interactions (Paulmurugan et al., 2004). Such so-called "split reporter system" can be used to efficiently screen small molecule drugs that modulate protein-protein interactions, and also to assess drugs in living animals or even humans. Both represent essential steps in the preclinical evaluation of candidate pharmaceutical agents targeting protein-protein interactions, including signaling pathways in brain tumor cells (Paulmurugan et al., 2004). Such simplified models are comprised of three key interactive groups of molecules:

- Those related to the genome, including nucleosides and nucleotides, nucleic acids, aptamers, and oligonucleotides.
- Those arising from expression of the genome, namely, the proteins, including enzymes, receptors, structural elements, and antibodies.
- Those providing energy to drive these systems, specially glucose, acetate, and fatty acids, and compounds that reflect oxygen-dependent processes.

There is great interdependency among these groups, involving highly complex pathways that are integral components of the cell and the organism (Paulmurugan et al., 2004). Image-based study of these components with true substrates (e.g., (^{11}C) glucose) may be difficult because the accumulated radioactivity in tissue will reflect the totality of their biochemical reactions. Because PET radiopharmaceuticals can be prepared at highly specific activities it is possible to use PET to study virtually all processes at sub-physiological concentrations, thereby avoiding biochemical perturbations of the processes under investigation (Paulmurugan et al., 2004). But, full validation of false substrates and natural substrates alike is necessary to ensure that images and parametric image data can be used in support of a clinical diagnosis (Blasberg and Tjuvajev, 2003). The study of these small molecule-mediated protein-protein interactions is important in understanding abnormal signal transduction pathways in a variety of neurological disorders including primary brain tumors, and well as in optimizing the process of drug development and validation (Blasberg and Tjuvajev, 2003).

The three most widely used molecular imaging strategies are (1) direct, (2) indirect, and (3) surrogate. Direct molecular imaging generally involves direct probe-target interactions, whereby the resultant image of probe localization and image intensity is directly related to its interaction with the target epitope or enzyme (Schaller, 2004; Blasberg and Tjuvajev, 2003). This strategy is based on imaging the target directly, for example, the receptor, usually with a target-specific probe. Indirect molecular imaging is more complex in that it may involve multiple components (Levivier et al., 1996; Schaller, 2004). Indirect evaluation may be achieved by using specific substrate probes for a target enzyme. One type of indirect imaging that is now being widely used, is reporter imaging, which involves a reporter gene and a reporter probe (Blasberg and Tjuvajev, 2003). These components must be complementary; the reporter gene product is frequently an enzyme that converts a reporter probe to a metabolite that is selectively trapped within transduced cells (Weissleder and Mahmood, 2001; Blasberg and Tjuvajev, 2003). Alternatively, the reporter gene product can be a receptor or transporter that irreversibly traps the probe in transduced cells during the period of image acquisition. Indirect molecular imaging is currently being more widely used than direct molecular imaging, particularly in preclinical animal studies. Surrogate or biomarker molecular imaging strategies reflect downstream affects of one or more endogenous molecular-genetic processes. This latter approach is particularly attractive for potential translation

into clinical studies in the near term because it uses established radiopharmaceuticals and clinical imaging protocols already in use in clinical medicine (or soon to be implemented) (Blasberg and Tjuvajev, 2003). Molecular imaging could provide information on in vivo distribution of biological markers in response to targeted therapy and could improve for example the selection of patients before therapies.

In brain tumors, tumor cell growth and proliferation with the consecutive recurrence attract the greatest interest both for diagnosis and therapy. For this reason, the molecular imaging is also focused on these pathophysiological changes. Clearly, metabolism, genome, and protein expression inseparably manifest in the tumor phenotype, underpinning all biochemically based diagnostics and therapeutics for brain tumors and representing, therefore, potential targets for molecular imaging (Weissleder and Mahmood, 2001).

Principles of Positron Emission Tomography

The general principles of nuclear medicine imaging also apply to molecular imaging. These include knowledge of the normal distribution of a tumor tracer that determines in what regions of the body the method may be successful. For example, high background uptake in an organ may interfere with tumor visualization in that organ. Another main factor is the level of uptake. Visualization depends on the detecting system (PET camera, gamma camera), but also strongly on the amount of tracer that is present at or in the target. In theory, a sub-millimeter lesion can be detected as long as tracer uptake is high enough. On the other hand, a large lesion may be missed when the uptake level is too low. In contrast with radiological methods, it is, therefore, in principle not possible to determine a detection threshold, although in daily practice this threshold is generally around 0.5–1 cm for the best methods.

Imaging of brain tumors with (^{18}F)-FDG was the first oncologic application of PET (Blasberg and Tjuvajev, 2003). (^{18}F)-FDG is actively transported across the BBB into the cell, where it is phosphorylated. PET, therefore, allows the quantitative localization of expression of endogenous or exogenous genes coding for enzymes or receptors by measuring the accumulation or binding of the respective enzyme substrates or receptor binding compounds (Tjuvajev et al., 2002). Depending on the radiotracer, various molecular processes can be visualized in primary brain tumors by PET, most of them related to an increased cell proliferation with astrocytoma. Radiolabeled 2-deoxy-2-(^{18}F)fluoro-D-glucose (FDG) (41), methyl-(11C)-L-methionine ((^{11}C)-MET) and 3′-deoxy-3′-(^{18}F)fluoro-L-thymidine ((^{18}-F)-thymidine) are taken up by proliferating gliomas depending on their tumor grades as a reflection of increased activity of membrane transporters for glucose ((^{18}F)-FDG), amino acids ((^{11}C)-MET), and nucleosides ((^{18}F)-thymidine) as well as increased expression of cellular hexokinase FDG and thymidine kinase ((^{18}F)-thymidine) genes, which specifically phosphorylate FDG and ^{18}F-thymidine, respectively (Schaller et al., 2007; Paulmurugan et al., 2004; Blasberg and Tjuvajev, 2003; Tjuvajev et al., 2002).

Positron Emission Tomography Imaging and Its Relationship to Brain Tumors in Experimental Research

On the Way to Gene Therapy: The Reporter Gene Concept

Imaging reporter gene is a new method to noninvasively and repetitively visualize the location, duration, and magnitude of transgene expression in living animals. Because the radionuclide approach has sufficient sensitivity to measure gene expression in vivo, the approach of using imaging reporter genes with high-resolution PET scanning in brain tumors has been increasingly investigated during the last years (Schaller, 2004; Schaller et al., 2007). Reporter gene imaging is an indirect approach of visualizing the expression of exogenous and endogenous genes, as well as specific intracellular protein-protein interactions (Schaller et al., 2007). Reporter gene imaging can

also be used for monitoring brain tumors cells or neural stem cells (Schaller et al., 2007).

An enormous gap exists between rapidly increasing theoretical knowledge regarding pathophysiological and genetic mechanisms of brain tumors and current lack of its clinical application (Schaller et al., 2007). Therefore, there is a need for animal models to shed more light on the complexity of varying molecular genetic aspects and to have a critical impact on the safety and efficacy of specific treatment modalities, such as gene therapy, in its clinical application (Schaller et al., 2007). In the effort to transfer genes into animals, a safe vector and expression system has to be developed to achieve efficient target and regulated alteration of specific (therapeutic) gene expression (Schaller et al., 2007). In the recent years, animal models of various glial cell lines have been established using different retrovirus or adenovirus mediated reporter genes (Tjuvajev et al., 2002) and radiolabeled tracers as reporter probes (Tjuvajev et al., 2002). Reporter genes can be used (1) to image vector targeting and the level of suicide gene (HSV1-tk) expression (Tjuvajev et al., 2002), (2) to image the regulation of endogenous genes and signal-transduction pathways (Tjuvajev et al., 2002), and (3) to monitor and quantitatively assess the expression of a second transgene that is cis-linked to the reporter gene by an internal ribosome entry site sequence (Schaller et al., 2006). By providing in vivo biochemical and physiological data in quantitative and tomographical terms, PET tracer methods can serve as an interface lining in vitro with in vivo findings of brain tumors. From the scientific point of view, three main research areas appear to be of current interest to study biological relevant mechanisms of primary brain tumor non-invasively by PET; (1) measuring biologically relevant tumor cell mechanisms (e.g., proliferation, angiogenesis, hypoxia, and apoptosis); (2) localizing of magnitude and duration of gene expression; and (3) verifying treatment efficacy on tumor cell metabolism (Tjuvajev et al., 2002). In the context of gene therapy, it is assumed that specific biochemical mechanisms of brain tumor cells, which can be measured with PET, determine individual treatment response (Schaller et al., 2006). Consequently, different radiolabeled probes for non-invasive imaging reporter gene expression have been developed: the quantification of herpes simples virus type 1 thymidine kinase (HSV-1-tk) and cellular thymidine kinase (ctk) gene expression relies on determination of accumulation rates of specific marker substrates such as 2′-fluoro-2′-deoxy-1β-D-arabinofuranosyl-5-(^{124}I)iodo-uracil (^{124}I) FIAU) or 9-(4-(^{18}F)fluoro-3-(hydroxymethyl) butyl)guanine ((^{18}F)FHBG) for HSV-1-tk and 3′-deoxy-3′(^{18}F)fluoro-L-thymidine (^{18}F)FLT for ctk expression, respectively (DeAngelis et al., 1998; Schaller et al., 2007; Tjuvajev et al., 2002). For example, glial RG2 cell cultures, accumulation rate of (^{14}C)FIAU normalized to that of (^{3}H) TdR was proportional to HSV-1-tk mRNA expression (Tjuvajev et al., 2002).

Experimental Attempts of the Reporter Gene Concept

Strategies for non-invasive and quantitative imaging of gene expression in vivo have been developed over the past decade. Non-invasive assessment of the dynamics of gene regulation is of interest for the detection of endogenous disease-specific biological alterations (e.g., signal transduction) and for monitoring the induction and regulation of therapeutic genes (e.g., gene therapy) (Schaller et al., 2006). Several radiolabeled probes have been developed and used to image viral or cellular TK expression in experimental animal brain tumors; these probes can be conveniently separated into pyrimidine nucleoside, acycloguanosine, and thymidine nucleoside derivates (Tjuvajev et al., 2002). A major problem with comparing different radiolabeled markers in experimental studies lies in the fact that until present times, there was (1) no paired comparison between transduced and wild-type glioma cells in the same animal tissue and (2) no comparison to a standard tracer in the same animal tissue to test the probes efficacy (Schaller et al., 2006).

To develop efficient and safe gene therapy approaches, the herpes simplex virus type 1 thymidine kinase gene (HSV-1-tk) has been shown

to function as a marker gene for the direct noninvasive in vivo localization of thymidine kinase (TK) expression by PET using radiolabeled nucleoside analogues as specific TK substrates (Schaller et al., 2006). Moreover, the gene encoding dopamine type 2 receptor (d2r) could be used as a PET marker gene using specific radiolabeled receptor binding compounds (Jacobs et al., 2001; Schaller et al., 2006). The sensitivity of (^{18}F) FHBG in visualizing cells expressing TK-GFP gene has not yet been determined in animal models of glioma cell lines. Our in vivo studies revealed a much higher accumulation of (^{18}F) FHBG with time in transduced cell lines compared with that in non-transduced control cell lines, consistent with monophosphorylation of the tracer by TK-GFP (Jacobs et al., 2001). Cellular accumulation of (^{18}F)FHBG may be unrelated to cell growth rate in wild-type cells (doubling time, 24) compared with that in transduced cells (doubling time 48). Thus, higher uptake in transduced cells supports the hypothesis that the tracer is selectively phosphorylated by TK-GFP only and not by native thymidine kinase suggesting a significant time-dependent difference in the distribution and magnitude of (^{18}F) FHBG (Jacobs et al., 2001). Moreover, (^{18}F) FHBG does not penetrate the intact BBB and can serve only as a marker substrate for HSV-1-TK expression in the brain when the BBB is disrupted (Jacobs et al., 2001). In addition, the PET images provide important spatial information that identifies the viable portion of transduced tissue at a given "imaging time window" (Jacobs et al., 2001). Therefore, it should be possible to infer the biodistribution of a therapeutic gene's expression, delivered by an appropriate TK-vector and regulated by an appropriate promoter in an animal model of F-98 glioma cell lines by imaging with (^{18}F)FHBG, respectively.

Positron Emission Tomography Imaging and Its Relationship to Brain Tumors in Clinical Medicine

Techniques for human brain imaging have undergone rapid developments in recent years. Technological progress has enabled the assessment of many physiological parameters in vivo that are highly relevant for primary brain tumor grading, tissue characterization, definition of the extent and infiltration of tumors, and planning and monitoring of therapy.

Table 2.1 Indication for use of positron emission tomography studies in brain tumors related to (patho) physiological factors

^{18}F-FDG-PET
1. Ninety percent accurate for tumor grading and prognosis
2. Can be used for grading and monitoring for 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 tissues.
3. ^{18}F-FDG accumulation dependent on
(i) glucose transport blood/brain or brain/blood
(ii) phosphorylation of glucose
L-Amino acids PET
1. Only partly accurate for tumor grading and prognosis
2. Good separation of brain tumor from surrounding normal brain tissue
3. Monitoring for progression to a higher degree of malignancy
4. Differentiating stable disease from tumor regrowth
5. 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 of tumors
2. Good estimation of therapeutic efficacy, early detection of recurrence and of malignant transformation

Adapted with Permission from Jacobs et al. (2003a, b)
Legend: *FDG* (18F)fluoro-2-deoxy-D-glucose, *PET* positron emission tomography

Brain Tumor Detection

Generally, a high sensitivity is reported for primary brain tumor detection with PET (Table 2.1). Initial FDG-PET studies could identify elevated FDG uptake in primary brain tumors (Jacobs et al., 2001) with good correlation of the grade of malignancy (Table 2.2). 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. However, amino

Table 2.2 ^{18}F-FDG positron emission tomography with a gold standard of biopsy or radiographic follow-up

	FDG-PET diagnosis		FDG-PET diagnosis in brain metastasis patients only		FDG-PET diagnosis in primary tumor patients only		Magnetic resonance imaging diagnosis	
Gold Standard	Tumor (%)	Necrosis (%)	Tumor (%)	Necrosis (%)	Tumor (%)	Necrosis (%)	Tumor (%)	Necrosis (%)
Tumor	47	16	42	17	75	13		
Necrosis	7	3	8	33	0	13	36	9
Sensitivity	75	0	71	0	86	0	94	0
Specificity	81	0	80	0	100	0	50	0

Adapted with Permission from Jacobs et al. (2003a, b)
Legend: *FDG* (18F)-fluoro-2-deoxy-D-glucose, *PET* positron emission tomography

acid transport is generally increased in malignant transformation (Schaller, 2004; Jacobs et al., 2001). In animal models, upregulation of the amino acid transporter in the supporting vasculature of brain tumor tissue has been shown responsible for increased facilitation of amino acid transport into the tumor cell (Jacobs et al., 2001). Factors involved in this active transport have been reviewed: flux of the amino acid to the tissue, the intrinsic activity of the amino acid transporter, and the rate of intracellular amino acid metabolism (Jacobs et al., 2001). Many clinical studies have demonstrated that (^{11}C)-MET-PET imaging, (amino acid tracer studies) is highly accurate in defining of tumor boundaries both in primary or recurrent brain tumors, regardless of their histological grading (Jacobs et al., 2001). For example, it is demonstrated an excellent 97 % sensitivity for (^{11}C)-MET-PET in 32 patients with high-grade astrocytomas but only a 61 % sensitivity in low-grade astrocytomas have been demonstrated (Jacobs et al., 2001). Other present a patient-based sensitivity of 84 % using stereotactic biopsies from primary brain tumors and normal brain tissue areas, indicating that tumor specificity of (^{11}C)-MET contains a certain rate of false-positive results (Jacobs et al., 2001). In another large series of astrocytomas, 95 % of 37 lesions are clearly visualized in (^{11}C)-MET-PET studies, whereas (^{18}F)-FDG shows 41 % as hypermetabolic, of which most are high-graded astrocytomas; and 49 % as hypometabolic lesions, while 10 % are difficult to distinguish from surrounding normal brain tissue (Jacobs et al., 2001). The reported advantage of (^{11}C)-MET over (^{18}F)-FDG in delineating astrocytomas is probably not relevant in CNS lymphoma, where (^{18}F)-FDG uptake is much higher in tumor than normal brain tissue (Jacobs et al., 2001).

Experience with (^{18}F)-tyrosine as radiolabeled liganding for PET studies in primary brain tumors is more limited. (^{18}F)-tyrosine PET imaging for both primary and recurrent brain tumors (including metastases and cerebral lymphomas) found 91 % of 22 tumors positive for uptake (cited in Jacobs et al., 2001 and Giese et al., 1998). Others could demonstrate increased uptake and transport rates of (^{18}F)-tyrosine in primary brain tumors (n = 15) (cited in Jacobs et al., 2001). Such an uptake appears more related to amino acid transport than to protein synthesis.

Molecular Signaling Pathways, Changes in Vascular Permeability and Angiogenic Potential of Brain Tumors

Within the brain, dissemination of glioma cells follows myelinated fiber tracts and extracellular matrix containing structures such as the basement membranes of blood vessels (Giese et al., 1998; Schaller et al., 2008). These patterns represent the two major routes of invasion frequently observed in clinical disease. For this reason, much of the interest in angiogenesis and hypoxia has led to investigating diagnostic imaging methodologies and developing efficacious agents against angiogenesis in primary brain tumors. In many ways, because of the cytostatic effects of these agents on tumor growth and tumor-associated endothelial cells, the effects of therapy are not immediately evident. Hence, finding clinically applicable

imaging tools and pathologic surrogate markers is an important step in translating glioma biology to therapeutics. There is a variety of strategies in the approach to experimental therapeutics that target the hypoxia-inducible factor pathway, the endogenous antiangiogenic and proangiogenic factors and their receptors, adhesion molecules, matrix proteases and cytokines, and the existing vasculature.

While PET imaging provides more information regarding the metabolic and molecular events of primary brain tumor activity, limited resolution prevents anatomical surgical planning and hinders the understanding of its vascular status. Currently, Dynamic Contrast-Enhanced (DCE)-MRI, and general contrast MRI are, therefore, the best techniques to assess the (micro)vascular status of malignant primary brain tumors (Giese et al., 1998; Schaller et al., 2008). However, promising studies may open some new indications for PET in primary brain tumors. This reveals the complexity of tumor vasculature and heterogeneity that may aid in therapeutic management especially in non-enhancing high-grade gliomas (Giese et al., 1998; Schaller et al., 2008).

Many low-grade gliomas respond to chemotherapy. Cerebral blood flow (CBF) and microvessel density may be critical for drug delivery. Low-grade gliomas are heterogenous tumors with regard to the distribution of amino acid uptake and CBF (Giese et al., 1998; Schaller et al., 2008). In the tumor center, both are coupled, whereas in the tumor periphery, where tumor infiltration of surrounding brain occurs, CBF may be low irrespective of increased (^{18}F)-FET uptake (Giese et al., 1998; Schaller et al., 2008).

However, blood volume and blood flow are independent of different biomarkers of brain tumor perfusion. Therefore, both should be measured when characterizing the efficacy of antiangiogenic therapies (Giese et al., 1998; Schaller et al., 2008). A promising approach for the diagnosis of primary brain tumor is targeting extracellular structures that are involved in angiogenic processes, such as the extra domain B (ED-B) of fibronectin (^{76}Br)-L19-SIP (ED-B fibronectin-binding human antibody derivate (L19-SIP)) specifically accumulates at the target site, enabling detailed PET of tumor neovasculature (Jacobs et al., 2001; Schaller et al., 2008).

Hypoxia is a critical event in tumor progression and angiogenesis. Proteins important for tumor angiogenesis and invasion have been detected in hypoxic brain foci. It was shown that HIF-1 alpha, VEGF-A, and VEGFR2 (Flk-1) protein and mRNA expression levels were significantly higher and MMP-9 was significantly upregulated in brain tumor tissues compared to normal brain (Herholz et al., 2007; Schaller et al., 2008). Together, these results suggest the critical role of hypoxia in tumor angiogenesis and invasion (Schaller et al., 2008). However, underexpression of VEGF-A does not result in complete inhibition of angiogenesis. Moreover, these tumors have a different perfusion phenotype, suggesting that angiogenesis is mediated by an alternative pathway (Herholz et al., 2007). Current results indicate that VEGF-D is an alternative mediator of this angiogenesis (Herholz et al., 2007; Schaller et al., 2008). Nevertheless, (^{18}F)-FMISO PET provides a noninvasive assessment of hypoxia in glioma and is prognostic for treatment outcomes in the majority of patients: (1) an uptake is observed in all high-grade gliomas but not in low grade gliomas and (2) a significant relationship is found between (^{18}F)-FDG or (^{18}F)-FMISO uptake and expression of VEGF-R1 and Ki67 expression (Schaller et al., 2008).

Brain Tumor Grading

PET imaging with (^{18}F)-FDG is considered useful in the diagnostic workup of suspected primary brain tumors or metastases, as it may identify focal hypermetabolic brain areas. Different molecular imaging studies have related the grade of malignancy of astrocytomas to the rate of (^{18}F)-FDG uptake in PET. It was shown that low-grade astrocytomas had low and glioblastoma multiforme had elevated uptake (Schaller et al., 2008). (^{18}F) FDG-PET imagines are also a useful tool to assess the tumor grade in oligodendrogliomas and gangliogliomas (Schaller et al., 2008).

Nearly all PET studies on tumor detection also address the feasibility of tumor characterization

and grading. The uptake is compared between benign and malignant processes and between various grades of malignancy. This clinically useful aspect is supported by in vitro proliferation markers (Schaller et al., 2008). Positive correlation was demonstrated between proliferation cell nuclear antigen index and (^{11}C)-MET uptake, indicating that (^{11}C)-MET is taken up more rapidly and accumulated in highly proliferative tissue (Schaller et al., 2008). Somewhat surprising is that this relationship was not confirmed for (^{18}F)-tyrosine uptake (n=20) (Schaller et al., 2008). Different (^{11}C)-MET accumulations in vivo have shown an uptake corresponding to the background uptake in low-grade astrocytomas, but a high uptake in oligodendrogliomas (cited in Schaller et al., 2008). It has been suggested that this difference could be clinically useful (Schaller et al., 2008). In this context, differentiation between skull base meningiomas and benign neuromas was demonstrated by Nyberg et al. with good specificity (Schaller et al., 2008). The largest study performed by Herholz et al., (2007), found 79 % accuracy in distinguishing astrocytomas from nonneoplastic lesions in 196 patients with a suspected primary brain tumor. Therefore, transport across the BBB is not the rate-limiting step for (^{18}F)-FDG, whereas transport across the BBB does appear to be the rate liming step for amino acid tracers such as (^{11}C)-MET. Transport of the (^{18}F)-amino acid analog (^{3}O)-methyl-6-(^{18}F)-fluoro-L-DOPA via sodium-independent, high-capacity amino acid transport systems has been demonstrated in tumor cell lines (Schaller et al., 2008).

Cerebral A_1 adenosine receptor (A_1ARs) represents a potential indicator of the cerebral response of glioma invasion. With molecular imaging, A_1AR signal intensity was increased in a zone surrounding experimental tumor in a rat glioma model (Schaller et al., 2008). The results of the first-8-cyclopentyl-3-(3-(^{18}F)fluoropropyl)-1-propylxanthine ((^{18}F)-CPFPX)-PET study on a patient with recurrent glioblastoma multiform confirmed the finding of animal models (Schaller et al., 2008). Molecular imaging with (^{18}F)-CPFPX-PET may open novel possibilities for experimental and clinical insights into the cerebral response to tumor invasion.

Brain Tumor Delineation

Many studies have demonstrated that the margins of primary brain tumors, as assessed by (^{11}C)-MET-uptake, are frequently wider than the anatomic boundaries, as demonstrated by MRI (Giese et al., 1998; Schaller et al., 2008). This is explained by the lack of contrast enhancement in MRI in intratumoral areas with an intact BBB. In low-grade tumors and in diffuse gliomatosis, this phenomenon may be even more pronounced (Schaller et al., 2008). In comparison with (^{18}F)-FDG-PET, a better tumor delineation has been reported both for (^{11}C)-MET and (^{18}F)-tyrosine-PET (4). (^{11}C)-MET of (^{18}F)-FDG scanning is combined with activation studies using radiolabeled with ($H_2{}^{15}O$) to depict tumor extension in relation to functional brain areas (Schaller et al., 2008), with the aim to permit a more aggressive surgical resection with a reduced risk of neurological impairment.

(^{18}F)-thymidine PET is useful for evaluating the histological grade and cellular proliferation of brain tumors, as well as for the detection and delineation of brain tumors that show decreased or similar uptake compared with normal gray matter of (^{18}F)-FEG-PET (Schaller et al., 2008). (^{18}F)-thymidine PET, however, does not appear sufficiently useful for differentiating tumors from non-tumor lesions.

Biopsy Localization

Stereotactic biopsies should be taken from the most malignant part of the tumor, which can be identified by changes in microvascular structure and metabolic activity (Schaller et al., 2008). But primary brain tumors are histologically heterogeneous. Accurate grading and diagnosis are especially important for directing the therapeutic approach and providing the prognosis in patients with nonresectable tumors. Stereotactic biopsies of localizations that are based on either methionine or (^{18}F)-FDG-PET seem to be more successful to find accurate brain tumor tissue than are biopsy trajectories based on CT only (Table 2.2) (Herholz et al., 2007; Schaller et al., 2008).

Especially strong uptake reduction of (^{11}C)-MET in necrotic parts or high uptake in anaplastic parts of the tumor tissue may influence the surgical planning and subsequent results of brain tumor biopsies. Methionine is better in detection of nonanaplastic tumor zones and brain tissue with infiltrating neoplastic cells than (^{18}F)-FDG (Schaller et al., 2008). Planning of biopsy trajectories may be improved by tyrosine, particularly in low-grade astrocytomas (Schaller et al., 2008).

Positron Emission Tomography Guided Treatment

Because PET activity reflects tumor metabolic activity, using PET to guide treatment seems to be a logical approach. Studies using PET to delineated tumor volumes for radiation therapy have been reported. In a study of 27 patients with glioblastoma, does escalation using an (^{18}F)-FDG PET-defined volume was investigated (Schaller et al., 2008), demonstrating that (^{18}F)-FDG PET uptake was the only parameter significant for predicting survival and time to tumor progression. However, in a subsequent report of 40 patients, such radiation dose escalation based on (^{18}F)-FEG PET volume did not result in improved survival or time to tumor progression, compared with historical controls (Schaller et al., 2008).

Differential Diagnosis

MRI usually establishes the differential diagnosis between "normal" brain tissue and malignant or nonmalignant lesions. However, AIDS related lesions are difficult to distinguish. Here (^{18}F)-FDG-PET has been used to differentiate between toxoplasmosis and lymphoma (Schaller et al., 2008): High grade uptake of (^{18}F)-FDG is strongly suggestive of a malignant lymphoma presenting as an extremely metabolically active tumor, while a toxoplasmosis presents as hypometabolic lesion (Table 2.3). The problem of specificity, however, may limit the usefulness of (^{18}F)-FDG-PET as a routine method, as inflammatory lesions are able to also accumulate (^{18}F)-FDG (Schaller et al., 2008).

Positron Emission Tomography in Pediatric Brain Tumors

Less is known regarding PET for pediatric brain tumors. Approximately, 10 years ago, clinical studies suggested that PET technology might gain widespread clinical application in pediatric brain tumors. The first (^{18}F)-FDG-PET studies of isolated case showed a relationship between (^{18}F)-FDG uptake and the degree of tumor malignancy (Schaller et al., 2008), the response to chemotherapy and highlighted the heterogeneity of (^{18}F)-FDG uptake in pediatric brain tumors. Still, the literature remains scant and indication for PET application in pediatric brain tumors is not clearly defined (Schaller et al., 2008). A recent study on (^{18}F)-FDG and (^{11}C)-MET-PET in 27 untreated primary pediatric brain tumors found that both (^{18}F)-FDG and (^{11}C)-MET uptake is associated with malignancy grade and may give valuable additional information on clinical aggressiveness (Schaller et al., 2008).

Positron Emission Tomography in Experimental Brain Tumors Models

Molecular imaging studies in experimental brain tumor models over the past 10 years aimed toward (1) the development of new radiotracers for cellular proliferation and protein synthesis, (2) characterization of these tracers with respect to their ability to detect responses to radio- and chemotherapy at a relatively early stage, (3) strategies for imaging transcriptional regulation and migration of tumor cells, and (4) imaging the expression of exogenous genes carrying a marker or therapeutic function and introduced into experimental gliomas for the purpose of developing improved gene therapeutic vectors. These experimental strategies have been previously reviewed in detail (Schaller et al., 2008).

New developments aim toward (1) the detection of tumor cell migration in vivo (Schaller et al., 2008), (2) the establishment of in vivo assays for direct imaging of tumor-specific signal transduction pathways (e.g. p53-, E2F-1 and HIF-1-α regulated pathways (Schaller et al., 2008)),

Table 2.3 Overview of brain tumors and its imaging by positron emission tomography

Tumor entities (% of all primary brain tumors)	Positron emission tomography study	
	FDG[a]	Methionine[b]
1. Gliomas		
Pilocytic astrocytoma WHO I° (<3 %)	Variable, focally increased	Up to 2-fold
Astrocytoma WHO II° (<5 %)	Decreased	1- to 2–fold
Anaplastic astrocytoma WHO III° (<5 %)	Variable	2- to 3-fold
Glioblastoma multiforme WHO IV° (<20–25 %)	Increased	>2.5-fold
Oligodendroglioma WHO II°/III° (<5 %)	Decreased/increased	>2.5-fold
Oligoastrocytoma WHO II°/III° (<5 %)	Decreased/increased	2- to 3-fold
Ependymomas (2–3 %)	Decreased	1.3- to 2.7 fold
2. Neuronal and glioneuronal tumors		
Dysembryoplastic neuroepithelial tumor (<1 %)	Decreased benzodiazepine receptor	Density as possible reason for epileptogenicity
Dysplastic gangliocytoma (<1 %)	Increased	Increased
Ganglioglioma (<1 %)	Variable, depending on WHO grade	NA
Central neurocytoma (<1 %)	Increased, depending on proliferative activity	Increased
3. Tumor of the pineal gland (<1 %)		
Pineoblastoma	Increased	NA
4. Embryogenic tumors		
Medulloblastoma (20–25 % <15 y.o.: 1 % > 20 y.o.)	Strongly increased	Increased
Primitive neuroectodermal tumors (PNET)	Decreased; relatively increased in spinal localization	NA
5. Meningeal tumors		
Meningiomas (25–30 %)	Variable (0.2- to 1.8-fold)	Increased (1.3- to 3.6 fold)
	(^{68}GA)DOTATOC-PET detects somatostatin receptor expression in meningiomas	
Hemangiopericytoma (<0.5 %)	Decreased	Increased
6. Tumors of the region of the sella		
Craniopharyngioma (<2 %)	Variable depending on histological type	NA
Adenomas of the hypophysis (5–8 %)	Specific binding to D2-receptors on adenomas of hypophysis from perisellar meningiomas and craniopharyngiomas specific increases in monoaminooxidase activity on ^{11}C-Deprenyl-PET differentiates adenomas of hypophysis from perisellar meningiomas by specific increased monoaminooxidase activity	^{18}F-FESP-PET differentiates
7. Tumors of cranial nerves		
Neurinoma (6–8 %)	Iso–or hypometabolic	Only slight increase
8. Lymphomas		
Primary CNS lymphoma (2–5 %)	Increased; allows differential diagnosis from toxoplasmosis	Increased
9. Metastatic tumors (20 % of all brain tumors)		
Lung, breast melanoma, gastrointestinal, hypernephroma	Variable, screening for metastasis with ^{18}F-FDG is not recommended	NA
	(^{66}GA)-DOTATOC-PET details somatostatin receptor positive metastasis of carcinoid tumors	

Adapted with Permission from Jacobs et al. (2003a, b)

Legend: *NA* not available, *18F-FESP* (18F) fluoro-ethyl-spiperone, *68GA-DOTATOC* 68-GA-1,4,7,10-tetraazacyclododecan-N,N′,N″, N‴-tetraaceticacid-D-Phe-Try-octreoid, *PET* positron emission tomography

[a]18F-FDG in comparison to cortical cerebral metabolic rate of glucose (CMRGlc)

[b]11C-methionine in comparison to contralateral control region

(3) the design of labeled peptides binding specifically to the cell adhesion receptor integrin α(v)β (Weissleder and Mahmood, 2001) or other tumor-specific antigens and of labeled bone marrow-derived endothelial precursor cells to allow highly specific tumor visualization and the study of glioma angiogenesis and neovascularization (Haubner et al., 2001; Sundaresan et al., 2003; Schaller et al., 2008), (4) the generation and in vivo characterization of transgenic mice with gliomas induced by signaling through Ras and Akt pathways (Schaller et al., 2008), and (5) the construction of bifunctional imaging marker and therapeutic genes to allow direct assessment of therapeutic gene expression in culture and in vivo models by directly corresponding assays (Jacobs et al., 2003a, b; Schaller et al., 2008). Especially, the design of small tumor-specific antibody fragments is an attractive way for specific detection of tumor cells by imaging in vivo as well as for targeted therapy by radioimmunotherapy.

Many of the current experimental protocols investigating new drug and treatment strategies for experimental gliomas included MRI, optical or PET imaging of either the distribution of therapeutic agents (Jacobs et al., 2003a, b), or therapy-induced tumor-changes (Jacobs et al., 2003a, b; Voges et al., 2003; Schmidt et al., 2004), with the overall attempt of designing image-guided treatments (Voges et al., 2003; Schaller et al., 2008). Most intriguing for clinical application is the design of multifunctional nanoparticles that can be detected both by MRI and fluorescence imaging, allowing for noninvasive preoperative assessment of the tumor and for intraoperative visualization of tumor margins by optical imaging (Schaller et al., 2008).

PET receptor ligand studies have generated a wealth of knowledge regarding disease pathogenesis and potential therapeutic targets for novel pharmaceutical agents. PET offers the opportunity to use an in vivo technique to study the pharmacodynamics and biodistribution of new agents and to ensure they target the organs or compartments of interest, for example, in case of neuropharmacology, the ability of drug to cross the BBB and bind to specific receptors in the brain (Schaller, 2004; Schaller et al., 2008). The study of drug occupancy can provide information regarding the occupancy of the binding sites for a particular dose of the drug and its pharmacokinetics (Schaller et al., 2008). This will help determine optima drug dosing regimens.

Evaluation of Therapy by Positron Emission Tomography

The development and clinical testing of targeted biological therapies for brain tumors present new opportunities and new challenges. The efficacy of traditional cytotoxic agents, which may produce detectable tumor regression, is typically measured by response rate or survival (Schaller et al., 2008). However, new biologic therapies have led to targeted molecular therapies that may permit improvement in therapeutic efficacy and reduced toxicity; thus, requiring new measures of activity (Schaller et al., 2008): For example, signal transduction pathways that are inappropriately regulated in brain tumors include growth factors and their receptors (e.g. EGFR, VEGFR or PEDGR), which regulate cellular interactions with the microenvironment and intracellular oncogenic pathways. Improved functional neuropathology and molecular imaging may, therefore, permit identification of patient subgroups for which clinical responses may be enriched (Table 2.4).

In the early postoperative period, (^{18}F)-FDG-PET can be used to differentiate residual tumor tissue from postoperative surgical effects (Schaller et al., 2008). It seems clear that a decline in tumor tissue uptake of (^{18}F)-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 (Schaller et al., 2008).

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. (^{18}F)-FDG uptake suggests the presence of viable brain tumor tissue (at least when high tumor uptake of (^{18}F)-FDG was

Table 2.4 New findings in brain tumors with possible relation to molecular imaging in human primary brain tumors

1. Experimental
Signal transduction pathways
Growth factors and their receptors: e.g. epidermal growth factor receptor, vascular endothelial growth factor receptor and platelet-derived growth factor), which regulate cellular interactions with the microenvironment and intracellular oncogenic pathways
Low-molecular-weight inhibitors
To target many kinases and may have advantages in terms of delivery. Monoclonal antibodies may have greater specificity, but face delivery restrictions
2. Clinical
18F-FLT PET
Potential to monitor treatment response and to serve as a prognostic marker?
18F-Fluoromisonidazole PET
A role in directing and monitoring targeted hypoxic therapy?
68Ga-DOTA-TOC PET
Potential?

Adapted with Permission from Schaller et al. (2008)
Legend: *DOTATOC* 68-GA-1,4,7,10-tetraazacyclododecan-N,N′,N″, N‴-tetraaceticacid-D-Phe-Try-octreoid, *FLT* 3′-deoxy-3′-[^{18}F] fluorothymidine, *PET* positron emission tomography

noted before therapy), while absence of (^{18}F)-FDG uptake suggests that necrosis may be present (Schaller et al., 2008). An increase in brain tumor metabolism compared to studies before therapy predicts longer survival (Schaller et al., 2008). This is explained by predominant killing of low energy-consuming cells or stimulation of quiescent cells, either tumor or normal, to become metabolically more active. In other terms, the increased regional metabolism means that within a certain volume of a specific tissue, the ratio and density of normal cells to tumor cells improved.

Changes in proliferation pattern can be assessed by monitoring (^{18}F)-thymidine uptake (reflecting 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 that are likely to be refractory to noninvasive treatments could allow more selective targeting or 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 (^{11}C)-MET-PET is suitable for follow-up of the treatment effects (Woesler et al., 1997; Schaller et al., 2008). For example, a dose-dependent reduction in uptake of (^{11}C)-MET in low-grade astrocytomas up to 1 year after brachytherapy has been demonstrated, whereas (^{18}F)-FDG uptake is unchanged (cited in Schaller et al., 2008). Others have found no (^{11}C)-MET uptake in six of seven cases of radionecrosis that are difficult to assess using MRI or CT (cited in Schaller et al., 2008). (^{11}C)-MET-PET had a sensitivity of 77.8 % and specificity of 100 % for differentiation recurrence of metastatic brain tumors from post-radiotherapy changes (cited in Schaller et al., 2008). However, (^{11}C)-MET-uptake may also be elevated in other conditions where there is a disruption of the BBB, such as cerebral hematoma or even necrotic areas caused by radiotherapy (Voges et al., 1997; Schaller et al., 2008). Glucose metabolism may be normal or low in lower grade tumors compared with surrounding cortex. Combined use of (^{11}C)-MET- and (^{18}F)-FDG-PET enhances the discrimination between recurrent tumor and post radiotherapy changes. Remarkably, the protein synthesis rate, determined by using (^{18}F)-tyrosine-PET, remains unchanged in 80 % of patients after radiotherapy (Schaller et al., 2008). Four hours after irradiation, the increase in tumor (^{18}F)-FDG uptake compared to the preirradiation study is significantly 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 (^{11}C)-MET- and (^{18}F)-FDG-PET studies following interstitial brachytherapy: (^{11}C)-MET is superior to (^{18}F)-FDG in delineating residual of recurrent tumor tissue. This finding confirms earlier data on the comparison of (^{18}F)-FDG and amino acid in visualization of untreated low- and high-grade astrocytomas.

Several PET studies have tried to establish a relationship between metabolic response and prognosis after initiation of chemotherapy in patients with glioblastoma multiforme. The change of (^{18}F)-FDG uptake induced by chemotherapy can be correlated with survival. Both positive and inverse correlation can be found between metabolic responses and survival, making these data inconclusive. In a more recent study, methionine is found to be superior to (^{18}F)-FDG in monitoring the treatment effects in low-grade astrocytomas (Schaller et al., 2008).

Prognosis

(^{18}F)-FDG-PET is used to predict the survival of untreated patients and to confirm suspected recurrence of high-grade astrocytomas. It was shown that (^{18}F)-FDG may differentiate recurrence from other therapy-related changes. Further tumor (^{18}F)-FDG uptake lower than adjacent cortical tissue is associated with a longer survival time than observed in tumor (^{18}F)-FDG uptake higher than in the adjacent cortex (Schaller et al., 2008). A relationship between glucose metabolism as assessed by (^{18}F)-FDG uptake as risk of malignant evolution in low-grade astrocytomas. It has also been shown the presence of areas of increased (^{18}F)-FDG uptake in a histologically proven low-grade astrocytoma predicts an adverse clinical course patients with hypermetabolic tumors demonstrated a median survival of 7 months after (^{18}F)-FDG-PET compared with 33 months for those with hypometabolic tumor. It has been demonstrated PET can be used to separate high-grade astrocytomas into subgroups (hypometabolic 78 % 1 year survival) (cited in Schaller et al., 2008; Schaller, 2008). Residual or recurrent high-grade astrocytomas showed high glucose utilization present with a mean survival period of 5 months, whereas in those tumors showing lower utilization, mean survival was 19 months (Schaller, 2008).

Presently, the experience with other tracers than (^{18}F)-FDG is limited, but in a quantitative evaluation of (^{11}C)-MET-uptake with low-grade astrocytomas, the patients with a low tumor uptake in the baseline study demonstrated a significantly better prognosis than those with a high uptake (Stockhammer et al., 2007). The prognostic information by amino acid PET studies has been provided by the presence, but not by the intensity, of uptake (Schaller et al., 2008). The current data suggest caution in relating high amino acid uptake values to poor prognosis despite the capability of amino acid imaging to help determine the presence and extent of astrocytomas.

Advances in Molecular Analysis and Characterization

Innovative techniques using complementary DNA and oligonucleotide microarrays (gene chips), tissue microarrays (tissue chips), and differential immunoabsorption have provided high throughput and potentially comprehensive approaches for the molecular characterization of human gliomas. Alterations of several tumor suppressor genes and oncogenes have already been identified as being critical to glioma transformation and progression. These approaches have led, for example, to the subclassification of glioblastoma multiforme into distinct subtypes based on the molecular signatures of the tumors. Improved and efficient molecular profiling of primary brain tumors is advancing diagnosis/prognosis. Identifying targets for novel and rational therapeutic approaches opens the window for clinical routine use of molecular imaging for primary brain tumors in the near future.

Oligodendroglial tumors harboring combined 1p and 19q loss 81p/19q LOH are characterized by a favorable prognosis and response to chemotherapy and radiotherapy, but detection of 1p/19q LOH relies on postoperative procedures. (^{18}F)-FDG-PET has the potential to predict 1p/19q LOH in WHO grade II gliomas preoperatively in tumors whose appearance on initial magnetic resonance images is consistent with that of low-grade glioma (Schaller et al., 2003a, b, c, d, 2008).

Although cell division is the most distinguishing function of growth in primary brain tumors, probing membrane biosynthesis with PET and 1-(^{11}C)acetate or a choline tracer may yield information as helpful as protein or DNA synthesis. Because astrocytic gliomas frequently carry epidermal growth factor receptor (EGFR) mutations at a frequency that is related to grade; a PET tracer that is specific for this mutated receptor could be useful for grading and prognosis. Methods for imaging angiogenesis are being developed (Schaller et al., 2003a, b, c, d, 2008); (^{18}F)-labeling of a cyclic RGD-containing glycopeptides, cyclo(-Arg-Gly-Asp-D-Phe-Lys(sugar amino acid)-), with 4-nitro-phenyl 2-(^{18}F)fluoropropionate has been reported. (^{18}F)-labeled annexin V is being tested as a new PET agent for quantization tumor cell death and predicting response to therapy. Annexin V binds to surface membranes that have exposed phosphatidyl serine residues resulting from programmed cell destruction.

Pharmacoselective Potential of Molecular Imaging in Neurooncology Drug Development

Novel targeted drugs such as small molecular inhibitors of receptors and signaling pathways in the biology of primary brain tumors are showing some activity in initial studies. As we learn more about these drugs and how to optimize their use as single agents and in combination with radiation, chemotherapy, and other targeted molecular agents, they will likely play an increasing role in the management of this devastating disease. Such molecules can be labeled with positron emitting isotopes and the emitted radiation is detected using sensitive PET cameras.

It is now possible to measure in vivo and normal tissue pharmacokinetics of anti-cancer drugs and investigate their mechanism of action. Radiolabelling of tracers can be used to measure specific pharmacodynamic endpoints and target identification (Schaller et al., 2008; Sandu and Schaller, 2010; Sandu et al., 2011a, b). Increasing evidence shows how these technologies, when added to early drug development, can rapidly reduce the time for entry into humans and early identification of mechanisms of action. With the move towards more segmented markets and identification of specific subgroups, PET's use for noninvasive biomarkers will become increasingly important.

Conclusion

Energy metabolism and amino acid transport and incorporation are important components of the pathophysiology of gliomas. Molecular imaging is providing such regional biologic information. Imaging brain tumors is straightforward and proliferation imaging with PET is very promising. However, neither has been exploited thoroughly enough to allow judgment of their potential benefit to the practice of neurooncology. Although protein and DNA based cell division is the most distinguishing feature of tumor growth, probing membrane biosynthesis with PET may also yield helpful information. Because astrocytic gliomas frequently carry EGFR mutations at a frequency that is related to grade, a PET racer, like (^{18}F)-fluoromisonidazole, that is specific for this mutated receptor, could be useful for grading and prognosis. Methods for imaging angiogenesis are being developed. As molecular pathways leading to and sustaining neoplasia become well understood, so will our capacity to measure them in vivo and intervene to the patient's advantage. Against the background of a thorough molecular imaging assessment of brain tumors, developments of novel therapeutic approaches based on gene therapy will involve the continued monitoring of therapy.

Not every patient can be studied with molecular imaging, and it is not necessary to do so; but molecular imaging technologies should be used in selected patients to advance our understanding of the complex pathophysiology of astrocytoma formation. This will allow the development and assessment of new therapeutic modalities including molecular target and gene therapies ("imaging-guided therapies").

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