

REVIEW

Progress on the diagnosis and evaluation of brain tumors

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Abstract

Brain tumors are one of the most challenging disorders encountered, and early and accurate diagnosis is essential for the management and treatment of these tumors. In this article, diagnostic modalities including single-photon emission computed tomography, positron emission tomography, magnetic resonance imaging, and optical imaging are reviewed. We mainly focus on the newly emerging, specific imaging probes, and their potential use in animal models and clinical settings.

Keywords: *Positron emission tomography; single-photon emission computed tomography; magnetic resonance imaging; optimal imaging; brain tumor; diagnosis.*

Introduction

Brain cancer, one of the top 10 causes of cancer deaths, remains a serious health threat and is largely incurable. In the United States, the annual incidence of brain cancer is approximately 15–20 cases per 100,000 people. The 5-year survival rate of patients with brain cancer has remained stable at approximately 75% over the last decade^[1]. The early and accurate diagnosis of a brain tumor is essential for disease management.

Brain tumors are typically diagnosed after symptoms including headache, nausea, personality changes, seizure, or focal neurologic impairments appear^[2]. Brain tumors include gliomas, meningiomas, and pituitary tumors, among others. Gliomas can be further divided into astrocytic tumors, oligodendroglial tumors, ependymal tumors, and mixed gliomas^[2]. Magnetic resonance imaging (MRI) and computed tomography (CT), the most common and preferred diagnostic modalities for detecting suspected primary brain tumors, can localize brain tumors and evaluate edema, hemorrhage, and hydrocephalus.

It is necessary to characterize the tumor grade to determine the optimal therapeutic approach. Histopathologic examinations remain the gold standard for establishing a glioma tumor grade; these examinations are mandatory at initial diagnosis to assess patient prognosis and to guide clinical and therapeutic management. The tumor

histological classification has been introduced by the World Health Organization and is based on the predominant cell type; the grade is based on the levels of necrosis, mitotic activity, nuclear atypia, and endothelial cell proliferation^[2–4].

Many molecular markers have been identified in brain cancers, including 1p/19q codeletion, O⁶-methylguanine methyltransferase promoter methylation, and isocitrate dehydrogenase-1 mutations^[5]. However, these markers require analysis of samples from the tumor tissues, which does not allow for a rapid diagnosis in a clinical trial. In this study, we focused on non-invasive diagnostic methods including single-photon emission computed tomography (SPECT), positron emission tomography (PET), CT, MRI, and optical imaging. The metabolic and physiologic conditions of tumors differ from those of normal tissue, including such conditions as high nutrient consumption and hypoxia, which may be exploited for tumor imaging. The specific markers and receptors that are expressed on tumor cells can be used for this aim.

Positron emission tomography

Since PET was first introduced in the 1950s, several radioisotopes and radiopharmaceuticals have been used in clinical studies including ¹⁵O-water, ¹⁵O₂,

[¹¹C]thymidine, and nucleoside analogues^[3]. However, no clear practical clinical utility has been defined for these tracers. The rapid advances in molecular biology have revealed details regarding pathogenesis and cell metabolic mechanisms. Many researchers have focused on metabolic substrates such as [¹⁸F]fluoro-2-deoxy-2-glucose (FDG), choline, and amino acids, which have been reviewed by Bénard et al.^[2].

These reagents were based on an altered tumor metabolism such as increased energy requirements and elevated protein synthesis. Such changes vary from case to case and can afford more information regarding the tumor status. However, the various tumor states also make it difficult to precisely analyze the information and determine a repeatable principle. The mechanism, application, and defects of the most commonly used PET trackers are summarized in Table 1. Although the metabolic substrates are most commonly used in clinical settings, their poor specificity and sensitivity limit their future applications. Therefore, researchers have attempted to develop more specific diagnostic probes.

Vascular proliferation plays a critical role in tumor growth and metastasis, and is an important marker in the histological grading of gliomas^[6]. Because the level of vascularization has been shown to correlate well with tumor grade and malignancy^[7], imaging angiogenesis can serve as an effective diagnostic method for cancer. The α_v -integrins ($\alpha_v\beta_3$, $\alpha_v\beta_5$) are crucial receptors for cell survival, and are highly expressed in activated endothelial cells and tumor cells but not in normal brain cells and quiescent endothelial cells. This differential expression pattern resulted in widespread studies utilizing α_v -integrins as a therapy target^[7]. RGD and the cyclic RGD peptide (c(RGDyK)) are widely used ligands for α_v -integrins for specific targeting effects.

Chen et al.^[7] labeled c(RGDyK) with ¹⁸F via *N*-succinimidyl-4-[¹⁸F]fluorobenzoate through the side-chain ϵ -amino group of the lysine residue. In a subcutaneous model and in an orthotopic glioma model, this tracer (FB-RGD) shows high tumor-muscle and T/N ratio. However, FB-RGD has rapid tumor washout and an unfavorable biliary excretion, resulting in high activity accumulation in the gallbladder and intestines^[8]. To improve the physiological behavior, Chen et al.^[8] inserted a heterobifunctional polyethylene glycol (PEG, MW = 3400) between the ¹⁸F radiolabel and the RGD moiety. The modified tracer (FB-PEG-RGD) can successfully differentiate tumor from normal brain with a high tumor/normal tissue (T/N) ratio (5.0). Other linkers, including 15-amino-4,7,10,13-tetraoxapentadecanoic acid (PEG₄) and Gly-Gly-Gly (G₃), can also be used for improving the tracer^[9]. By longitudinal microPET, FB-RGD clearly diagnosed tumors larger than 1.5 mm and provided the sensitivity and resolution to visualize and quantify anatomical variations during brain tumor growth and angiogenesis^[10].

c(RGDyK) has also been conjugated with 1,4,7,10-tetraazacyclododecane-*N,N,N',N'*-tetraacetic acid (DOTA),

and the RGD-DOTA conjugate has been labeled with ⁶⁴Cu (half-life, 12.8 h) for tumor imaging^[11]. Similarly, introducing a PEG chain between DOTA and RGD can improve the physiological behavior of the tracer^[12]. To increase the imaging effect, a tetrameric RGD peptide tracer, [⁶⁴Cu]DOTA-E[E[c(RGDfK)](2)](2) (RGD₄-DOTA) has been developed^[13]. In a U87 subcutaneous model, RGD₄-DOTA displays a significantly higher integrin-binding affinity than the corresponding monomeric and dimeric RGD analogues, and results in a high tumor/background ratio in *in vivo* PET. An RGD octamer displays an even higher integrin-binding affinity, higher tumor uptake, and longer tumor retention than the tetramer^[14]. These effects are believed to be due to the polyvalency effect^[13,14]. However, in another study, the RGD tetramer labeled with ⁶⁸Ga through 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) shows a higher binding affinity but a lower tumor uptake than the dimer^[15], indicating that there are other effects that influence the tumor uptake of the RGD multimer.

Although the restricted integrin expression makes RGD attractive for integrin-positive tumor imaging, there are only a few RGD tracers under clinical evaluation. ¹⁸F-labeled galacto-RGD is the first tracer investigated in patients with squamous cell carcinoma of the head and neck and metastases of malignant melanoma, sarcomas, or osseous metastases, but not in patients with gliomas^[16,17].

One of the major obstacles of the clinical translation of ¹⁸F-labeled RGD is the laborious multistep radiosynthesis. Liu et al.^[18] provide a 1-step radiosynthesis protocol using Al¹⁸F as an intermediate, which may accelerate the synthesis of ¹⁸F-labeled RGD.

In addition, the source of positive electron nuclides is a problem for the widespread application of SPECT. ¹¹C, the most commonly used nuclide, is produced by a cyclotron and has a short half-life of 20.5 min, which restricts the use to PET centers with an in-house cyclotron facility^[19]. Other amino acids such as tyrosine can be labeled by ¹⁸F, which makes it more attractive for clinical application. However, the half-life of ¹⁸F is 110 min; therefore, the prepared dose of a radiopharmaceutical bearing this radionuclide will undergo multiple half-lives of decay during the work day. This necessitates a frequent recalibration of the remaining dose (determination of activity per unit volume) and careful planning with respect to patient scheduling. The complex radiosynthesis and short half-lives of the positive electron nuclides remain 2 hurdles that block the clinical use for most hospitals.

Single-photon emission computed tomography

Similar to a gamma camera, SPECT is a nuclear medicine tomographic imaging technique utilizing gamma rays that provide three-dimensional (3D) information. SPECT has a rather poor spatial resolution of

Table 1 The probes used in PET and their mechanisms, applications, and defects

Agent	Mechanism	Application	Defect
[¹⁸ F]Fluoro-2-deoxy-2-glucose (FDG)	GLUT-1 and GLUT-3 are overexpressed in tumor, and the expression relates to malignant change	Diagnosis, evaluation of malignancy, differentiation of recurrent brain tumors from necrosis, indication of prognosis ^[2,110] , differentiation of metastatic brain tumor from primary brain tumor ^[111]	Low sensitivity ^[2] , cannot differentiate inflammation from cancer ^[1,2] , cannot precisely determine the tumor boundaries ^[1,9]
L-[methyl- ¹¹ C]Methionine (MET)	Increased protein synthesis, activated carrier-mediated transport systems ^[113]	Diagnosis ^[114] , differentiation of gliomas from other non-tumoral brain lesions ^[115] , delineation of the tumor extent ^[19,116,117] , differentiation of tumor recurrence from radionecrosis	Overlap of several tumor grades makes the grading less useful
S-[¹¹ C]Methyl-L-cysteine (MCYS)	L-transport system	Diagnosis of solid tumors ^[118]	Not available
O-(2-[¹⁸ F]fluoroethyl)-L-tyrosine (FET)	Mediated by LAT2 (a subtype of the L-transport system) that is expressed only in tumor cells and not in inflammatory cells ^[119,120]	Diagnosis with high accuracy and sensitivity ^[121] , grading gliomas ^[122] , differentiation of gliomas from inflammation ^[123] , biopsy guidance and treatment planning of cerebral gliomas ^[124]	Not available
α-[¹¹ C]Methyl-L-tryptophan (AMT)	Metabolized by the kynurenine pathway. The initial and rate-limiting enzyme of the kynurenine pathway, indoleamine 2,3-dioxygenase (IDO), widely expressed in low-grade gliomas and glioma-neuronal tumors but restricted in high-grade gliomas ^[125,126]	Diagnosis of low-grade gliomas ^[126]	Not available
L-5-(2-[¹⁸ F]fluoroethoxy)tryptophan (FEHTP)	Carrier-mediated transport systems	Diagnosis of tumor xenografts ^[127]	Limited number of clinical trials
3,4-Dihydroxy-6-[¹⁸ F]fluoro-L-phenylalanine (¹⁸ F-FDOPA)	FDOPA can arrest in the basal ganglia but little in normal cerebral cortex and white matter ^[128]	Precise anatomic localization of brain gliomas ^[128] , imaging of low-grade tumors and evaluation of recurrent tumors, distinguish tumor recurrence from radiation necrosis ^[125,130]	Cannot differentiate low-grade from high-grade gliomas ^[29]
¹²⁴ I-labeled L-phenylalanine	Carrier-mediated transport systems	Diagnosis of glioma ^[131]	Not available
[¹¹ C]Choline	Increased lipogenesis in brain tumor requires more phospholipids, which results in the accumulation of the contents of choline-containing compounds ^[132,133]	Diagnosis, differentiation of low-grade from high-grade gliomas ^[134]	High uptake of [¹¹ C]choline in choroid plexus, venous sinuses, and the pituitary gland make it difficult to recognize the existence and/or the border of brain tumor around these structures ^[114]
3-Deoxy-3-[¹⁸ F]fluorothymidine (FLT)	Accumulates in proliferating tissues through thymidine kinase ^[135,136]	Diagnosis, differentiation of high-grade from low-grade gliomas ^[135,137] , prediction of the time to tumor progression and survival ^[136] , monitor therapy response ^[138]	Fail in the evaluation of recurrent low-grade gliomas ^[139] , increased accumulation in the normal brain with high-grade gliomas ^[137]
[¹¹ C]Acetate	Preferentially incorporated into the lipids of tumor cells and serves as an obvious carbon source ^[139]	Diagnosis of astrocytomas ^[140] , differentiation of high-grade from low-grade gliomas ^[141]	Poor grading value [#]
¹⁸ F-labeled RGD	The receptors, α _v β ₃ , α _v β ₅ , are highly expressed in activated endothelial cells and tumor cells but not in normal brain cells and quiescent endothelial cells ^[7]	Diagnosis of gliomas larger than 1.5 mm ^[10]	Limited number of clinical trials

8–10 mm, but is more sensitive and somewhat cheaper, and SPECT is available at every nuclear medicine department^[20]. At present the quantization of tissue function with SPECT is not a routine procedure. Four nuclides are used currently in SPECT imaging: thallium 201, technetium 99m, iodine 123, and indium 111^[2,21]. Several radioisotope-labeled compounds including ²⁰¹Tl, technetium-99m-hexakis-2-methoxyisobutyl isonitrile (^{99m}Tc-MIBI), and 3-[¹²³I]iodo- α -methyl-L-tyrosine (IMT) have been developed and utilized for glioma imaging, and several reviews have discussed these tracers^[2,22]. The mechanisms of action, applications, and defects of current SPECT tracers are summarized in Table 2.

Similar to PET tracers, most SPECT tracers are either radionuclides alone or metabolism substrates that are labeled with radionuclides. The changing tumor metabolic status leads to differential uptake of the SPECT tracers. Through the calculation of T/N ratio or other index, the tracers can be used for the diagnosis and grading of gliomas and for predicting patient prognosis. For example, the significant correlation between the T/N ratio of ^{99m}Tc-MIBI and patient survival makes MIBI useful for predicting patient prognosis^[23]. It has been shown that a higher T/N ratio correlates with a shorter patient survival time. In 4 groups with different T/N ratios (1.0–2.0, 2.1–3.0, 3.1–4.0, and >4.0), the survival times differ significantly from each other (except for the 2 lower groups)^[24].

Although the longer half-lives and simple preparation make SPECT more attractive than PET, the nuclides used in SPECT mostly involve those with a high number in the periodic table of elements, which restricts the compounds that can label these nuclides. At present, most SPECT trackers are chelates with radionuclides. The poor targeting and specificity of these chelates prohibit widespread use.

Magnetic resonance imaging

First introduced in 1973, MRI is an essential method in clinical and research settings. This technique is based on the basic principles of nuclear magnetic resonance (NMR), and takes advantage of the tissue contrast generated from the NMR signals received from hydrogen nuclei located in different physiological environments throughout an organism^[25]. MRI has excellent soft-tissue specificity, and can be used to identify many types of lesions in the brain and spinal cord. The predominant factors that influence the amount of signal and the extent of contrast received from a sample include spin-lattice/longitudinal relaxation time (T_1), transverse relaxation time (T_2) and spin density (ρ)^[25], which differ from one tissue to another and are responsible for the contrast between different tissues. To better distinguish tumor from normal tissue, the patients are often injected with a contrast agent such as gadolinium chelates and iron particles, which alter the T_1 and/or T_2 of

the protons in the vicinity of the agent and, thus, help generate image contrast (bright/dark) for diagnosis^[25].

Gadolinium

Gd chelate is the most widely used contrast agent for MRI. In clinical settings, Gd is administrated mostly as Gd diethylenetriaminepentaacetic acid (DTPA). The enhanced effect in Gd-DTPA MRI is dependent on tumor cell density, vascularization, necrosis, and the degree of blood-brain barrier (BBB) alteration^[26]. In addition to the Gd-DTPA accumulation, the high extraction fraction of Gd-DTPA correlates with a high capillary permeability as determined from Evans Blue staining. Therefore, Gd-DTPA can be used for evaluating tumor vasculature permeability^[27]. The size, shape, and location of experimental brain tumors can be evaluated by Gd-DTPA-enhanced MRI^[26,28]. In a rat glioma model, Gd-DTPA shows improved glioma enhancement over Gd-DOTA^[29]. The tumor can be distinguished from normal tissue, and tumor growth can be monitored^[30]. Short scan times and macroscopic tumor/edema separation make Gd-DTPA an attractive contrast agent for clinical use^[31].

Because Gd-DTPA does not penetrate the BBB, Gd-DTPA MRI can be utilized to evaluate BBB defects. A strong correlation between the concentration of Gd-DTPA in the brain tissue (measured by longitudinal relaxation, R_1) and the tumor model has been established, and use of Gd-DTPA provides new information regarding pathogenesis or etiology, and leads to improved methods in monitoring the efficacy of treatments in intracranial diseases^[32]. In clinical use, a strong correlation between the degree of enhancement by Gd-DTPA with the degree of BBB breakdown and the development of peritumoral edema has been demonstrated^[33]. These results imply that the edema originates in the area of BBB breakdown.

In a clinical study, tumor cells were observed in 87 of 89 specimens taken from areas with Gd-DTPA-enhancement on MRI. Seventy-four percent of these specimens (64 of 87) showed a malignancy with a growth fraction of more than 5%. However, tumor cells were observed in 27 of 56 specimens taken from areas without Gd-DTPA enhancement. Eighty-five percent of these specimens (23 of 27) showed a growth fraction of less than 5%. Five patients who were in a clinically stable condition or had radionecrosis showed a constant pattern of enhancement or slightly increased enhancement. Therefore, Gd-DTPA MRI can be effectively used for the diagnosis of tumor viability and malignancy after treatment^[34].

Differentiating tumor from areas of radiation necrosis is often difficult. A fast spoiled gradient refocusing technique in the open-configuration intraoperative MR system can be used to assess the rate of regional enhancement of the treated tumor bed and to localize specific sites for pathologic sampling^[35]. It determines that the

Table 2 The probes used in SPECT and their mechanisms, applications, and defects

Tracers	Mechanism	Application	Defects
Thallium 201	²⁰¹ Tl accumulation in viable tumor cells relates to a combination of factors including blood flow, variability in the expression of the Na/K-ATPase pump, and disruption in the blood-brain barrier ^[2,142] . ²⁰¹ Tl uptake increases minimally in normal brain tissue or other non-tumor tissues such as radionecrosis ^[143]	Identify tumor recurrence and sensitively differentiate tumor recurrence from radionecrosis ^[144-146] , prediction of early progression of gliomas ^[143]	Fails to predict glioma grade ^[18]
[^{99m} Tc]Hexakis-2-methoxyisobutyl isonitrile (^{99m} Tc-MIBI)	MIBI is a lipophilic cationic compound that accumulates in the cytoplasm and mitochondria, and the increased mitochondrial density in tumor cells is the foundation of tumor imaging with ^{99m} Tc-MIBI ^[149,150]	Diagnose gliomas, differentiation of gliomas from benign lesions ^[151,152] , determination of recurrent gliomas ^[153] , determination of brain tumor grade and distinguish tumor recurrence from radiation necrosis ^[154] , determination of tumor proliferative activity ^[155] , predict prognosis ^[2,2]	
[^{99m} Tc]-1,2-bis(bis(2-Ethoxyethyl)phosphino)ethane (^{99m} Tc-tetrofosmin)	^{99m} Tc-tetrofosmin is rapidly cleared from the blood and accumulates in the myocardium and brain tumors but rarely in brain ^[145]	Distinguish tumor recurrence from radionecrosis ^[156] , differentiate glioma grades ^[157] , evaluate proliferative activity and predict recurrence potential ^[157,158]	
[^{99m} Tc]Glucoheptomate (^{99m} Tc-GHA)	Although the uptake mechanism is not clear, disruption of the blood brain barrier and resultant enhanced permeability is predominant in the brain tumor accumulation of ^{99m} Tc-GHA ^[159]	Good visualization of tumor margins ^[159] , differentiation of glioma recurrence from post-radiation gliosis and high-grade from low-grade gliomas ^[159] , compared with ²⁰¹ Tl, ^{99m} Tc-GHA correlates more closely with surgical findings with regard to the location of the tumor margin, the extent of tumor invasion, and intratumoral necrosis ^[160]	Not suitable for the evaluation of tumors close to the nasal mucosa, the uptake of ^{99m} Tc-GHA depends on blood-brain barrier disruption ⁶
^{99m} Tc-RGD	The receptors, $\alpha_v\beta_3$, $\alpha_v\beta_5$, are highly expressed in activated endothelial cells and tumor cells but not in normal brain cells and quiescent endothelial cells ^[7]	Diagnosis of tumor xenografts ^[161]	Limited number of clinical studies
³ [¹²³ I]- α -Methyl-L-tyrosine (¹²³ I-MT)	Mediated by LAT2 (a subtype of the L-transport system) that is expressed only in tumor cells and not in inflammatory cells ^[119,120]	Differentiation of recurrence from benign lesions ^[162] , monitor the radiotherapy and gene therapy effect and detect and delineate recurrent gliomas ^[163-167]	Early washout from the brain limits the diagnosis to the first 45 min after injection. In combination with the changing blood pool activity during that period, this leads to an activity distribution that changes during SPECT acquisition, which can cause imaging artifacts ^[168] . Fails to establish a relationship with either histological grade or survival time ^[164,169,170]
¹²³ I-HPA	Not available	High sensitivity and specificity	Low T/N ratio in low-grade gliomas ^[171] , no difference between low-grade and high-grade gliomas ^[168]
[¹¹¹ In]Pentetreotide	Not available	Increased tumor uptake is observed in patients with glioblastomas, lymphomas, medulloblastomas, neurocytomas, or metastatic tumors ^[2,21]	No increased uptake in low-grade astrocytomas, no correlation between [¹¹¹ In]Pentetreotide uptake and tumor grade

Gd uptake correlates with histologic data, which indicates that dynamic MRI in an open-bore magnet is a promising method for localizing potential sites of active tumor growth in patients treated for malignant astrocytomas and metastatic brain lesions. A 3D approach using co-occurrence matrix analysis is proposed to increase the sensitivity and specificity of brain tumor characterization. When 3D images are compared with images obtained with the conventional 2D method, a better discrimination is obtained between necrosis and solid tumor and between edema and solid tumor^[36].

Cerebral blood volume (CBV) maps can be calculated from MRI signals. The maximum CBV ratios of low-grade (I, II) gliomas are significantly lower than the maximum CBV ratios of high-grade (III, IV) gliomas, indicating that the MRI-derived CBV values are effective in determining glioma grade^[37,38]. However, the differentiation is not precise because there are no significant differences between grade I and II, grade II and III, and grade III and IV tumors. In addition to CBV mapping, dynamic susceptibility contrast imaging sets also contain information regarding the flow and permeability properties of the tumor microvascular system. When combined with conventional MRI, dynamic susceptibility contrast techniques can offer important functional information regarding glioma biology^[39].

Other Gd chelates have been synthesized for improved imaging effects, for example, Ga tetraphenylporphine sulfonate (TPPS)^[40]. In contrast to Gd-DTPA, Gd-TPPS increases the signal intensity on both T₁- and T₂-weighted MR images. The accumulation of Ga-TPPS in glioma shorts T₁ by 53% and T₂ by 34% compared with Gd-DTPA. Consequently, gliomas appear hyperintense on T₁-weighted images and hypointense on T₂-weighted images, which can more accurately distinguish gliomas from normal brain tissue and peritumorous edema. Another Gd chelate, gadobenate dimeglumine (Gd-BOPTA), can clearly visualize gliomas with a higher contrast to normal brain and a higher signal-to-noise ratio, and retain longer than Gd-DTPA^[41,42]. Macromolecular contrast medium such as albumin-Gd-DTPA is useful for the grading of tumor angiogenesis and the monitoring of anti-angiogenesis interventions^[43]. However, no agent is superior to Gd-DTPA.

In addition, a diagnosis by Gd chelates is based on BBB disruption and enhanced tumor penetration, which is suitable for high-grade gliomas. For low-grade gliomas and metastatic tumors, the visualization is far from satisfactory. To solve this problem and enhance the selectivity of Gd chelates, specific ligands can be conjugated. A peptide that specifically recognizes tenascin-C (which is highly upregulated in gliomas) can be conjugated to Gd-DOTA^[44]. The peptide-modified Gd-DOTA shows a higher uptake by glioma cells. Another strategy may also be useful for target imaging; a monoclonal antibody against 9L glioma cells has been conjugated to Gd-DTPA, which can then differentiate among

tumor, perifocal edema, and radiation injury^[45]. Ye et al.^[46] administered the biotinylated monoclonal antibody CL3 to visualize accumulation in the tumor. Avidin is administered as the chaser after 24 h, then streptavidin-modified Gd-DTPA is dosed after another 30 min. The blood level of streptavidin-modified Gd-DTPA is increased and the tumor signal is enhanced. However, this strategy is complex and may not be suitable for clinical use.

Nanoparticles can be loaded onto Gd chelates, and provide relatively high concentrations of contrast agents that are more suitable for non-invasive imaging. For tumors, nanoparticles can passively target and accumulate by the enhanced permeability and retention (EPR) effect, leading to an improved contrast of tumor to normal tissues. Conjugation with specific ligands could further improve the targeting effect and increase contrast. Many nanoparticles have been evaluated for Gd chelate loading and tumor imaging, such as liposomes, nanotubes, fullerenes, dendrimers, and polymers.

Liposomes

Liposomes are the first nanoparticles that have been evaluated for Gd loading. The accumulation of Gd-loaded liposomes in the tumor is significantly higher than free Gd-DTPA and corresponds more closely to the histologically proven vascularized portions^[47]. However, normal liposomes tend to be taken up by the liver and spleen^[48]. PEGylation can effectively enhance the blood circulation time and increase accumulation in the tumor^[49]. Furthermore, modification with tumor-targeted ligands can increase the tumor imaging effect and decrease the required dose.

CD105 (endoglin) is a co-receptor of the transforming growth factor β superfamily, and is present on the endothelial cells of both peri- and intratumoral blood vessels. In particular, CD105 is largely expressed in small and likely immature tumor vessels as demonstrated in breast, prostate, and gastric cancers and in gliomas^[50,51]. Zhang et al.^[51] modified Gd-DTPA-loaded liposomes with CD105-targeted immunoglobulins (CD105-Gd-SLs) to evaluate angiogenesis in glioblastomas. In a preliminary study, the CD105-Gd-SLs signal was significantly higher than that of unmodified liposomes, which primarily resulted from the new microvessels as demonstrated by immunohistochemistry and immunofluorescence. The circulation time also expanded because the CD105-Gd-SLs signal continuously increased over 120 min, whereas unmodified liposomes decreased to baseline at 60 min. The expression of a specific angiogenesis marker, the endothelial integrin $\alpha_v\beta_3$, has been shown to correlate with tumor grade. The LM609 monoclonal antibody, a specific ligand of $\alpha_v\beta_3$, can be conjugated with liposomes for detection of tumor grade^[49].

Dendrimers

The polyamidoamine (PAMAM) dendrimer is the most widely used dendrimer for Gd loading. PAMAM covalently conjugates with DTPA and loads onto Gd. The dendrimer can also be modified with target ligands such as lactoferrin, chlorotoxin, angiopep-2, RGD, and the T7 peptide^[52–55]. These modified PAMAMs show improved diagnostic value over unmodified PAMAMs; the gliomas were clearly visualized. As mentioned earlier, the expression of many targets is related to tumor grade and malignancy, which can be utilized for tumor evaluation. However, there are no reports concerning this application.

Other nanocarriers

Fullerenes, nanotubes, and polymers can load onto Gd and be used as MRI contrast agents^[56–58]. There are preliminary studies showing the potential application for Gd loading and tumor detection; however, there has been no evaluation of the correlation of signal enhancement with the histological examination of gliomas.

Superparamagnetic iron nanoparticles

The particle sizes of superparamagnetic iron nanoparticles (SPIOs) range from 50 to 500 nm, while the particle sizes of ultra-small SPIOs (USPIOs) are less than 50 nm. SPIOs are composed of iron oxides, magnetite (Fe_3O_4), maghemite ($\gamma\text{Fe}_2\text{O}_3$), or other ferrites, and have no magnetic properties outside of an external magnetic field because of their size^[59,60]. SPIOs are often coated with dextran, phospholipid, PEG, poly amino acid, or other materials to prevent destabilization and agglomeration of the colloidal suspension and to make the nanoparticles soluble in aqueous or biological media^[59]. For example, ferumoxtran, with a mean hydrodynamic diameter of 31 nm, is composed of a monocrySTALLINE magnetite and a maghemite core of 4.3–6.0 nm coated with the low molecular weight dextran. Ferumoxides are larger than ferumoxtran and are also coated with dextran. Because of their negative enhancement effects on T_2 - and T_2^* -weighted sequences, SPIOs are widely used as contrast agents for MRI.

Iron oxide is specifically taken up by macrophages, which may involve scavenger receptor SR-A-mediated endocytosis^[61]. Dextran-coated SPIOs, the most widely used SPIO, may be phagocytosed by Kupffer cells and portal macrophages, which is useful for liver imaging^[62].

The differences in particle size and surface characteristics significantly affect physicochemical and pharmacokinetic behavior. On humanized monocyte THP-1 cells, ferumoxide is shown to undergo a more intense macrophage uptake than ferumoxtran-10, most likely because of its larger size^[59]. Anionic SPIO is composed of a maghemite monocrySTALLINE core of 8 nm covered with meso-2,3-dimercaptosuccinic acid. The mean hydrodynamic diameter is 24 nm^[63]. Compared with

ferumoxtran, a higher cellular uptake of anionic SPIO is observed in many cell lines including tumor cells, dendritic cells, lymphocytes, and epithelial cells, which indicates that anionic SPIOs are promising for cellular tracking^[63]. Modification with a specific ligand could also significantly enhance cellular uptake. The Tat peptide-conjugated SPIO has been successfully used to label hematopoietic progenitors and oligodendrocyte progenitors^[64,65]. Transfection agents can also improve the uploading of ferumoxides in various cells^[66].

However, in vivo nanoparticles are usually taken up by macrophages in the liver (by Kupffer cells), spleen, and bone marrow, resulting in the imaging of these tissues. To target other tissues, the particles need to have a decrease in the uptake by the liver and spleen and display a long blood half-life. As referenced earlier, ferumoxtran has a more reduced uptake by macrophages than ferumoxides. This leads to a long ferumoxtran half-life that can be used for brain tumor imaging. The short plasma half-life of ferumoxides results in the lack of or only minimal tumor signal changes, and ferumoxides therefore fail in brain tumor imaging^[67]. Anionic SPIOs also display a short half-life; therefore, there is no obviously enhanced uptake of anionic SPIOs in vivo^[63]. Conversely, ferumoxtran exhibits a long half-life and high tumoral accumulation in vivo (0.11% of the injected dose per gram of tissue in brain tumors), which is sufficient for detection by MRI^[68].

The mechanism of SPIO transport into the tissue by macrophages may include endocytosis by activated blood monocytes that migrate into pathologic tissues, transcytosis of USPIOs across the endothelium and migration of USPIOs into the tissues followed by progressive endocytosis of these USPIOs by in situ macrophages, or transport of USPIOs into the pathologic tissue, in some cases via the inflammatory neovasculature irrigating the tissues^[59,69,70]. Tumor characterization with USPIOs is thought to be based on extravasation through leaky capillaries and interstitial accumulation. In PC3 xenograft-bearing mice, there is an early peak at 1 h on T_2 imaging and a second peak at 12 h on T_1 imaging after injected ferumoxtran, which suggests an initial intravascular distribution of particles and a secondary extravasation through the interstitium at 12 h^[63].

Depending on the EPR effect, USPIOs can distribute in vascular compartments. However, the USPIOs cannot cross the disrupted BBB. Therefore, USPIOs can be used for the imaging of tumor-associated microvessels and the tumor-associated inflammatory lesion, and for assessment of tumor microvascular heterogeneity^[59]. A clinical study has demonstrated that ferumoxtran-10 can be used to image intracranial tumors^[67]. The antiangiogenic therapy can alter the BBB in tumors and result in a change in tumor enhancement; thus, USPIO also provides an important complementary diagnostic tool for the evaluation of tumor response to antiangiogenic therapy^[71].

Although both Gd chelate and USPIOs can enhance contrast, because of the low diffusivity and endocytosis

by metabolically active cells, USPIOs are superior for the delineation of tumor margins^[72]. In a clinical trial, Enochs et al.^[72] compared the Gd chelate and USPIOs in the diagnosis of 4 patients with brain tumors (3 patients had high-grade primary gliomas and 1 had metastatic melanoma). The results show that whereas the Gd chelate diffuses into the normal surrounding brain tissue with a half-life of hours, causing a progressive blurring of the tumor margins, the USPIOs remain localized to the tumor over a period of several days. However, research has demonstrated that ferumoxtran-10 is mainly distributed in reactive cells such as astrocytes and macrophages rather than in tumor cells^[73]. In a rodent model, Moore et al.^[68] demonstrated that dextran-coated SPIOs accumulated predominantly in tumor cells and tumor-associated macrophages. This finding mainly contributes to a promising application for tumor delineation. Several studies with SPIO nanoparticles in patients with brain tumor show a sharply marginated enhancing rim at the periphery of brain tumor that persisted for several days^[74].

The plasma half-life of ferumoxtran is approximately 24–30 h in clinical studies. Therefore, the lesions can be visualized on the postoperative MRI at 48 h or more after ferumoxtran infusion. These data suggest that ferumoxtran can be used for assessing residual tumors after surgical elimination without the need to re-administrate contrast materials^[73]. Comparing postoperative imaging with preoperative imaging can effectively determine the residual tumor. However, postoperative Gd contrast enhancement differs significantly in individuals because the enhancement depends on the imaging modality, timing, dose of the contrast medium, type of surgery, degree of injury in surrounding brain, dose of steroid, and whether intraoperative parenchymal hemorrhage has occurred^[73]. These multiple factors make it difficult to assess residual tumor. However, ferumoxtran might lessen this problem with a long half-life and no re-administration requirement.

In addition, the half-life can be further extended by PEG modification. Cole et al.^[75,76] modified cross-linked starch-coated SPIOs with PEG. The liver and spleen concentrations of PEG-modified SPIOs are reduced approximately 12-fold and 2.5-fold, respectively, at 1 h compared with unmodified SPIOs. Enhanced, selective magnetic brain tumor targeting ($t = 1$ h) of PEG-modified SPIOs has been confirmed in 9L-glioma tumors with an injected dose/g tissue up to 1.0%, which achieves a 15-fold improvement over unmodified SPIOs (0.07% injected dose/g tissue). MRI and histological analyses visually confirm enhanced targeting and also suggest a limited contribution of passive mechanisms to tissue retention of nanoparticles.

SPIOs can only passively distribute in the tumor and cannot transport through the BBB, making it difficult to visualize the infiltrative portion of brain tumors. Modification with specific ligands may solve this

problem, and provide more specific imaging. Many ligands such as transferrin, lactoferrin, RGD, and folic acid have been evaluated for enhanced tumor imaging^[77]. Xie et al.^[78] conjugated lactoferrin with SPIOs for glioma MRI detection. Lactoferrin is considered to be a specific ligand for a low-density lipoprotein receptor that is highly expressed both on the BBB and tumor cells^[79]. Lactoferrin-conjugated SPIOs have a higher uptake than SPIOs by C6 glioma cells. In vivo studies further demonstrate that lactoferrin-conjugated SPIOs can more clearly visualize gliomas, and lactoferrin-conjugated SPIOs are clearly observed around the vascular region of the tumor slices after 48 h.

The magnetic properties of SPIOs have also been evaluated for drug delivery to specific sites^[80]. The high surface area-to-volume ratio enables the loading of large amounts of drugs. Thus, SPIOs can be used for not only diagnosis but also, simultaneously, therapy (also referred to as theragnosis). It has been demonstrated that loading with chemotherapeutic agents has marginal effects on the physical (size and zeta potential) and magnetization properties of the SPIO. The drug encapsulation efficiency for doxorubicin and paclitaxel is approximately 74%–95%, which is suitable for drug delivery^[81]. Like most nanoparticles in drug delivery, drug-loaded SPIOs can sustain the release of the cargo and passively accumulate in tumors by the EPR effect^[82]. However, this is not specific enough for tumor theragnosis. Thus, dual-modified SPIOs have been developed for drug loading and active targeting. Methotrexate is a widely used chemotherapeutic agent, and chlorotoxin is a peptide that exhibits a high affinity for tumors of neuroectodermal origin^[83]. Sun et al.^[84] conjugated SPIOs with both methotrexate and chlorotoxin. The dual modification enhanced tumor cell uptake and apoptosis. More importantly, the modifications prolonged the retention of the SPIOs in the tumor, indicating its promising application in theragnosis and even in therapeutic effect monitoring. Another study has modified SPIOs with RGD, and has shown enhanced glioma uptake and apoptosis^[85]. To better monitor the distribution of SPIOs and the therapeutic effect, a near-infrared dye can be simultaneously loaded into the SPIO^[86]. However, the monitoring accuracy should be evaluated.

The toxicity and biocompatibility is crucial for nanoparticles that cannot be avoided. In general, USPIOs have a good biocompatibility, and the uptake by macrophages does not associate with the activation^[59]. The Kupffer cells incorporate the Fe of USPIO into the body's iron store such as ferritin and hemoglobin. Thus, the USPIOs are biodegradable and do not have long-term toxicity. However, the uptake of citrate-coated SPIOs by macrophages results in a significant increase in oxidative stress. The stress is transient because it decreases after 1 day of incubation; however, the proliferation rate is not apparently affected. The systemic safety of several iron oxide nanoparticles has been

evaluated after injection in humans, indicating that these products have a satisfactory safety profile according to standard toxicological and pharmacological tests. Although there has been some research involving the complement system, there are few convincing data to support the hypothesis^[59,62].

There are many other alloy particles or metallic particles that show potential biomedical applications. However, these particles, such as FePt, FePd, and FeCo, can be coated with many materials similarly to the SPIOs and show no essential differences from the SPIOs^[87–89]. Therefore, these particles are not discussed here.

Optical imaging

Recent advances in medicine and biology have identified the mechanisms of many diseases and physiological processes. A large number of molecules and pathways involved in disease processes have been discovered. However, the particular roles of these molecules and pathways need to be further explored, which results in the need for effective tracking methods. Optical *in vivo* imaging, a sensitive, non-invasive and non-ionized technique, is emerging as a key technology to satisfy the challenges of advanced molecular imaging in preclinical research and patient care^[90,91]. Optical imaging is a promising method for cancer diagnostics.

Optical *in vivo* imaging involves bioluminescence and fluorescence. However, because bioluminescence does not require fluorescent probes, we focus here on fluorescence. The very low autofluorescence of tissues in most of the body means that optical imaging can achieve a high signal-to-noise ratio. The lower limits of detection for optical imaging may reach picomolar or even femtomolar concentrations for an optical reporter or contrast agent. The reporters and contrast agents are often molecules and particles modified with fluorescent groups such as green fluorescent protein and Cyt. For ultraviolet and visible light, absorption by hemoglobin and other molecules may reduce optical signals by approximately 10-fold per centimeter of tissue, leading to poor tissue penetration (1–2 cm)^[90,92]. The penetration of near-infrared fluorescence (NIRF), with emission wavelengths between 650 and 900 nm, is substantially better because of the low absorption of light and reduced tissue autofluorescence^[91]. In addition, NIRF has good tissue penetration, low toxicity, and a quick clearance, and is not radioactive. As a result, the sensitivity for NIRF imaging agents is greatly enhanced, potentially allowing for tomographic optical imaging signals to be detected at depths of 7–14 cm^[90,93]. These qualities make the NIRF a promising contrast agent for cancer imaging.

The differential absorption of light by tissues also produces images that are weighted toward optical reporters and probes that are located closer to the surface of a subject. While this limitation is being overcome with

3D imaging and analysis techniques such as fluorescence molecular tomography (FMT), optical techniques typically allow for the relative quantification of imaging signals rather than the absolute quantification possible with PET. Despite these challenges, optical techniques have growing roles in molecular imaging research and clinical translation.

Optical technologies

Optical coherence tomography

Optical coherence tomography (OCT) captures 3D images from optical scattering media (e.g., biological tissue), and OCT can sometimes provide submicrometer resolution. However, the imaging depth in OCT is limited by optical scattering rather than absorption, because scattering tends to attenuate and randomize the light. Depending on the wavelength of light, this technique can achieve imaging depths of up to 2 mm in most tissues. This technique has been used clinically for some applications such as eye examinations, and has been tested *in vivo* and *ex vivo* for cancer diagnostics. To generate sufficient contrast, the imaging agents for this modality require large scattering cross sections^[94].

Photoacoustic tomography

Photoacoustic tomography (PAT) is a hybrid imaging modality that provides strong optical absorption contrast and high ultrasonic resolution. Because the spatial resolution beyond one optical transport mean free path (~1 mm) is determined by ultrasonic parameters, the maximum imaging depth and resolution of PAT are scalable when diffusive photons are available. One can greatly increase the penetration depth of PAT with near-infrared light because the optical absorption of hemoglobin and scattering of tissues are weak in this regimen. Therefore, a proper combination of PAT with the appropriate contrast agent can accurately detect and diagnose tumors. As in the case of OCT, the imaging depth depends on the wavelength of light, but the imaging depth (~30 mm) is higher than that of OCT. This modality is currently being evaluated *in vivo*. In addition, contrast agents for PAT require large absorption cross sections^[94].

Two-photon microscopy

Two-photon microscopy is a fluorescence-based technique that obtains images of living tissue up to about 1 mm in depth; it typically uses red-shifted light to minimize scattering in the tissue, and the background signal is strongly suppressed owing to multi-photon absorption. This technique is being tested *in vivo*. Cho et al.^[94] reported that surgery with molecular fluorescence imaging can be efficient for the complete removal of tumors. These results suggest a possible breakthrough in the application of two-photon microscopy for

molecular imaging to overcome the disadvantage of imaging depth.

Surface-enhanced Raman spectroscopy (SERS) imaging

SERS utilizes the enhancement of Raman scattering by molecules adsorbed on surfaces of metal nanoparticles. The increment can be as much as 10¹⁴–10¹⁵; hence, Raman-active dyes placed on the surface of gold and silver colloids will exhibit greatly amplified Raman signals. The ability of gold colloids to easily conjugate with targeting ligands enables the detection of tumors *in vivo* using this technique. Contrast agents for this modality also require Raman active molecules (i.e., SERS probes) on the surface of gold or silver colloids^[94].

Optical probes

Many NIRF dyes are synthesized for optical imaging. To achieve the target diagnosis and enhance tumor accumulation, dyes are often conjugated with specific molecular biomarkers or encapsulated into target delivery systems. However, there are several limitations. Conventional organic dyes cannot be easily synthesized to emit different colors because the excitation and emission wavelengths depend on their chemical structure, and tuning to a precise wavelength requires sophisticated chemistry. The narrow excitation spectra and broad emission spectra mostly cross into the red wavelengths. This makes it difficult to use these dyes for multiplex imaging. Organic dyes exhibit photobleaching, which makes them unsuitable for imaging purposes. In an aqueous environment, the quantum yield of organic fluorophores is typically less than 15%^[95].

Because of these limitations, although organic dyes have been widely used as contrast agents in both laboratory and clinical research, inorganic nanoparticles are receiving increasing attention as future contrast agents because of their superb properties^[94] such as tunable emission wavelengths, the ability to be designed with controlled properties, easy surface modification, and suitability for multiple modalities. The high sensitivity and light stability are also attractive properties of inorganic nanoparticles. In addition, inorganic nanoparticles can load other molecules to serve as probes for multiple modalities, which could more accurately diagnose potential tumors.

Dyes

Compared with inorganic probes, organic near-infrared (NIR) fluorescent dyes are very attractive candidates for imaging, because of the emergence of newly developed NIR dyes with improved photophysical properties and their availability for large-scale chemical synthesis. These organic dyes can conjugate with various types of specific molecules such as chemical small molecules, amino acids, proteins, nucleotides, DNA primers,

double-stranded DNA, and antibodies, which have been applied in molecular imaging. Owing to the availability of covalent or noncovalent conjugation with cancer-targeted organic and biological molecules, NIR dyes are being actively investigated as a promising approach to image cancer specifically and sensitively. In addition, 2 classes of native dyes that can preferentially accumulate in the tumor cells without the need for chemical conjugation to tumor-targeting ligands have been recently identified and have shown unique optical and pharmaceutical properties for cancer imaging. These advances have expanded the current concept of tumor-targeted imaging, and hold significant promise to contribute to cancer diagnosis and treatment^[96].

Initially, to enhance the tumor-targeting effect NIR dyes are directly conjugated with ligands. Chen et al.^[97] conjugated Cy5.5 with RGD to image the U87 glioma model. The conjugate leads to elevated cell-associated fluorescence on integrin-expressing tumor cells and endothelial cells. *In vivo* studies have shown that the tumor uptake reached a maximum at 2 h post-injection with a high signal-to-noise ratio, and slowly washed out over time. This indicates that the strategy is promising for non-invasive imaging of brain tumor.

As the conjugation of ligands with dyes is often restricted by the chemical structure of the dyes, encapsulation is a potentially useful technique. The dyes are protected by the carriers, and targeting can be achieved through modification of the carriers. Pang et al.^[98] encapsulated a dye-DiR into polymersomes and surface modified them with lactoferrin. The system can successfully visualize gliomas to a greater extent than free dyes. The imaging effect can be improved through better targeting strategies, such as a cascade targeting strategy^[99]. Because the carriers can load drugs and dyes simultaneously, this strategy is useful for theragnosis, especially if the carriers are environmentally responsive.

Quantum dots

Quantum dots (QDs) are nanocrystals composed of atoms from groups II–VI, III–V, or IV–VI of the periodic table, with typical sizes in the range of 1–100 nm^[100]. Compared with organic dyes and fluorescent proteins, QDs offer a broader range of emission spectra that cover both the visible and NIR wavelengths, large absorption coefficients across a wide spectral range, tunable size, and high photostability^[94,101]. Therefore, QDs have been extensively explored as contrast agents for fluorescence imaging modalities, including single- and multiphoton microscopy and photoacoustic imaging^[94].

Advances have allowed for the precise control of particle size, shape, and internal structure^[101]. These advances make QDs suitable tools for investigating the interaction of particles with cells and exploring the influencing factors such as size, shape, and surface modification, which were well reviewed by Aggarwal et al. and Mahnoudi et al.^[102,103]. QDs with suitable size, shape,

and surface characteristics may avoid opsonization and achieve a long half-life in blood. However, for targeting to and accumulating in tumors, especially brain tumors, it is essential that QDs are conjugated with ligands or are encapsulated with target delivery systems.

RGD is a ligand for integrin $\alpha_v\beta_3$ that is overexpressed in brain tumors. Cai et al.^[104] functionalized QDs with RGD to image the tumor vasculature. Each QD was modified with 30–50 RGD peptides. Conjugation with RGD facilitated the uptake of QDs by U87 cells. In a subcutaneous U87 model, the fluorescence intensity of RGD-QDs in the tumor site is almost 4-fold higher than that of unmodified QDs. In addition to higher fluorescence intensity, a higher T/N ratio was achieved by target imaging. At 6 h post-injection, the T/N ratio of RGD-QDs is 4.42 ± 1.88 , while that of QDs is only 0.84 ± 0.21 .

Gao et al.^[105] encapsulated QDs with an ABC copolymer that was surface modified with a tumor-target ligand. This system has successfully been used for prostate cancer imaging. Similarly, Papagiannaros et al.^[106] encapsulated QDs in phospholipid micelles. In vivo, the signal-to-noise ratio achieved 15, which is powerful enough to precisely detect the tumor.

Lim et al. developed a type of perfluorodecalin (PFD)/(InGaP/ZnS QDs) composite nanoemulsions using a rational materials design approach. ^{19}F molecules in the PFD/(InGaP/ZnS QDs) nanoemulsions provided an ^{19}F -based MRI capability, while fluorescent QDs dispersed in PFD nanodroplets provided an optical imaging modality^[107]. However, there is a lack of in vivo evaluation.

Gold nanoparticles

Gold nanoparticles can show strong extinction peaks in the visible and NIR regions. Such strong peaks originate from the collective oscillations of their conduction electrons in the presence of an incident light, a phenomenon commonly known as localized surface plasmon resonance (LSPR). Because of LSPR, gold nanoparticles can strongly scatter and/or absorb light, the magnitude of which is largely determined by the size and internal structure (solid vs hollow). It is relatively straightforward to control these parameters to tune the LSPR properties of gold nanoparticles for a specific imaging modality. For example, gold nanoparticles with relatively large scattering cross sections are ideal for enhancing the contrast in OCT, whereas photoacoustic imaging requires the use of gold nanoparticles with large absorption cross sections. In addition, gold nanoparticles can have large absorption coefficients against the X-ray source used for CT imaging, exhibit a photoluminescence capability suitable for multiphoton microscopy, and even enhance the intensity of Raman-active molecules for the SERS technique. To date, 4 different types of gold nanoparticles have been tested in preclinical settings as contrast agents for molecular imaging: nanospheres, nanocages, nanorods, and

nanoshells^[94]. Hollow gold nanospheres have been used for photoacoustic imaging of brain vasculature^[108]. Gold nanoparticles 11 nm in size were used for CT imaging of brain tumor^[109]. Because of the presence of BBB in normal brain, gold nanoparticles are able to distribute only into brain tumor, resulting in high resolution.

Rare-earth doped nanoparticles

Owing to their luminescence properties, rare-earth ions are often used for optoelectronic applications, with notable examples including the neodymium-doped yttrium aluminum garnet (Nd:YAG) laser and erbium-doped fiber amplifiers used in optical-fiber communication systems. Similar to QDs, rare-earth doped nanoparticles exhibit sharp emission peaks (sharper than QDs), long fluorescence lifetimes, high quantum yields, and excellent photostability. For these reasons, rare-earth ions possess great potential for the non-invasive and real-time in vivo disease diagnosis. The synthesis and optical properties of rare-earth-doped nanoparticles have been actively studied, and their application in biomedicine is being explored and expanded^[94]. However, there are few studies on their application for brain tumor imaging.

Prospects

Recent advances in molecular biology have led to an increased focus on molecular markers. These markers, modified with suitable radioactive isotopes, may serve as target PET or SPECT probes. However, the cost factor and the injection of radioactive material limit their biological application. Poor resolution also restricts their clinical use in the early diagnosis of brain tumors. More convenient and safe imaging methods with better resolutions are needed.

MRI is a widely used and promising modality. The introduction of contrast agents significantly enhances the diagnostic accuracy with clear delineation. The specific ligands or biomarkers that modify contrast agents can further elevate the T/N ratio and can be used for analysis of specific receptors or pathways, which is useful for planning the therapy design. However, compared with PET and SPECT the sensitivity of MRI is deficient^[95], although this may be improved by the development of better contrast agents.

Optical imaging is considered to have both high resolution and high sensitivity, and is the future trend for non-invasive diagnostic tools. The development of dyes and particles is rapid and is providing more suitable properties for optimal imaging. However, the safety of these compounds is still in question. The large-scale production of molecular imaging probes is also a burden on clinical imaging.

Because there are various advantages and disadvantages of these different imaging modalities, combining modalities is a promising strategy. At present, an

instrument that combines CT and optical imaging is commercially available. The CT image can provide accurate tissue information on the location of fluorescent probes. Although there have been several reports on the probes that can be used for a dual-modality diagnosis, *in vivo* studies are limited. However, dual-modality imaging holds future promise as an application for the early and accurate diagnosis of tumors.

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Conflict of interest

The authors have no conflicts of interest to declare.

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