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Dendrimer-based magnetic resonance imaging agents for brain cancer

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ABSTRACT Brain cancer is one of the most lethal and difficult-to-treat cancers because of its physical location and biological barriers. The mainstay of brain cancer treatment is surgical resection, which demands precise imaging for tumor localization and delineation. Thanks to advances in bioimaging, brain cancer can be detected earlier and resected more reliably. Magnetic resonance imaging (MRI) is the most common and preferred method to delineate brain cancer, and a contrast agent is often required to enhance imaging contrast. Dendrimers, a special family of synthetic macromolecules, constitute a particularly appealing platform for constructing MRI contrast agents by virtue of their well-defined three-dimensional structure, tunable nanosize and abundant surface terminals, which allow the accommodation of high payloads and numerous functionalities. Tuning the dendrimer size, branching and surface composition in conjunction with conjugation of MRI functionalities and targeting moieties can alter the relaxivity for MRI, overcome the blood-brain barrier and enhance tumor-specific targeting, hence improving the imaging quality and safety profile for precise and accurate imaging of brain tumors. This short review highlights the recent progress, opportunities and challenges in developing dendrimer-based MRI contrast agents for brain tumor imaging.

Keywords: dendrimer, contrast agent, blood-brain barrier, tumor targeting, magnetic resonance imaging

BRAIN CANCER AND THE BLOOD-BRAIN BARRIER

Brain cancer is one of the most lethal forms of cancer.

Although it represents only 2%–3% of all tumors diagnosed [1,2], the incidence of brain tumors has risen over the past decades. In general, brain tumors encompass primary brain tumors and secondary brain tumors: tumors that start in the brain are referred to as primary brain tumors, whereas tumors that stem from other part of the body and then spread to the brain are called metastatic or secondary brain tumors. Brain cancer is heterogeneous and can be classified according to the cell origin and the tumor grade [3,4]. Among the different brain tumors, glioblastoma is the most common in adults, accounting for 35%–40% of malignant brain cancer. Glioblastoma is very aggressive and has a poor prognosis. Although incremental improvements in survival have been achieved during the past decades, there is no effective cure for glioblastoma. The median length of patient survival is only 15–18 months, and just 15% of patients survive beyond five years [5].

Surgical resection is the main method of brain cancer treatment, followed by concurrent radiotherapy and chemotherapy [3]. Complete tumor resection is often difficult because of the heterogeneous and infiltrative nature of brain tumors. In addition, tumor lesions are usually hard to distinguish from the healthy brain tissue, and they are frequently located in the areas that control movement, speech, memory, cognition, etc. Therefore, extra tissue cannot be removed during brain tumor resection, unlike other solid tumors, because of the detrimental impact it would have on the neurological and cognitive functioning of the patient [6]. It is therefore of paramount importance to precisely localize and delineate

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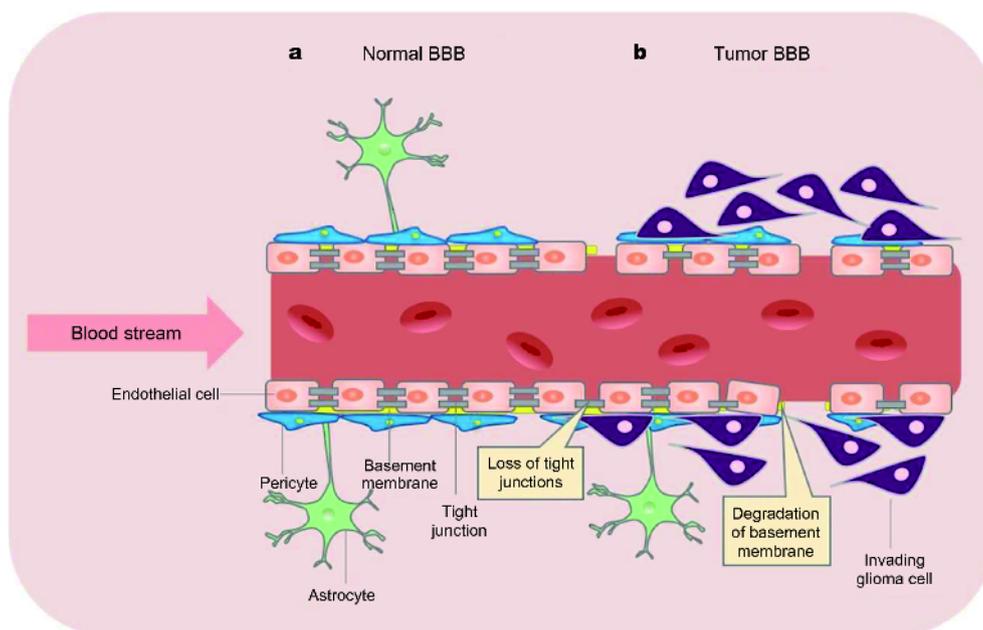* Corresponding author (email: ling.peng@univ-amu.fr)

Figure 1 Cartoon presentation of the BBB (a) in normal brain and (b) in brain cancer. In the healthy brain, vascular endothelial cells form the BBB through tight junctions, pericytes and astrocytic endfeet that surround the blood vessels. In brain tumors, glioma cells migrate along the blood vessels, thus displacing the astrocytic endfeet, disrupting the tight junctions on the endothelial cells, and degrading the basement membrane surrounding the blood vessels.

brain tumors in order to allow the maximal safe resection, in which as much tumor tissue is removed as possible without causing harm to the patient [7].

However, the delivery of imaging and therapeutic agents is the greatest challenge for the diagnosis and treatment of brain cancer because of the blood-brain barrier (BBB) (Fig. 1). The BBB is a natural defense system composed of brain capillary endothelial cells alongside astrocytes and pericytes (Fig. 1a), which together regulate the passage of nutrients, electrolyte ions, metabolites, etc., while blocking unwanted harmful and foreign substances from entering into the brain, in order to keep the brain working under normal conditions [8–10]. Although the BBB is crucial for protecting the brain and maintaining its normal function, it is a nightmare for the management of brain diseases since it greatly limits the transport of therapeutic and imaging agents—both low and high molecular weight—into the brain. This is because the healthy BBB has continuous tight junctions, a lack of fenestrations and low levels of pinocytotic uptake as well as high efflux activity, etc. (Fig. 1a). It is worth noting that the human brain has high vascular density, and hence brain cells can easily be reached by circulating drugs, as long as these drugs can effectively cross the BBB.

Fortunately for brain cancer, the BBB is disrupted as tumor growth progresses (Fig. 1b) [11,12]. This is because

growing tumors gradually invade and infiltrate the surrounding brain parenchyma, causing a progressive deterioration of the BBB, which enhances the permeability. Also, the abnormal angiogenesis and the uncontrollable cancer cell proliferation cause the tumor blood vessels to become tortuous, poorly organized and leaky [13–15], further damaging the BBB and hence enhancing the permeability. This compromised BBB can be exploited for effective delivery of therapeutic and imaging agents to the brain, which is a challenging but important task for brain cancer management [10].

It is worth mentioning that several endogenous transport mechanisms, which regulate the passage of various substances across the BBB, can be exploited to deliver therapeutic and imaging agents across the BBB. These transport mechanisms can be classified as passive diffusion, carrier-mediated transcytosis, adsorptive-mediated transcytosis and receptor-mediated transcytosis (Fig. 2). Specifically, water, oxygen and lipophilic small molecules are able to pass through the BBB *via* passive diffusion (Fig. 2a), whereas glucose, amino acids and some ions are transported across the BBB by the corresponding "carrier" proteins, a process which is referred to as carrier-mediated transcytosis (Fig. 2b). Some cationic molecules can be adsorbed onto negatively charged components at the luminal surface of endothelial cells and are then
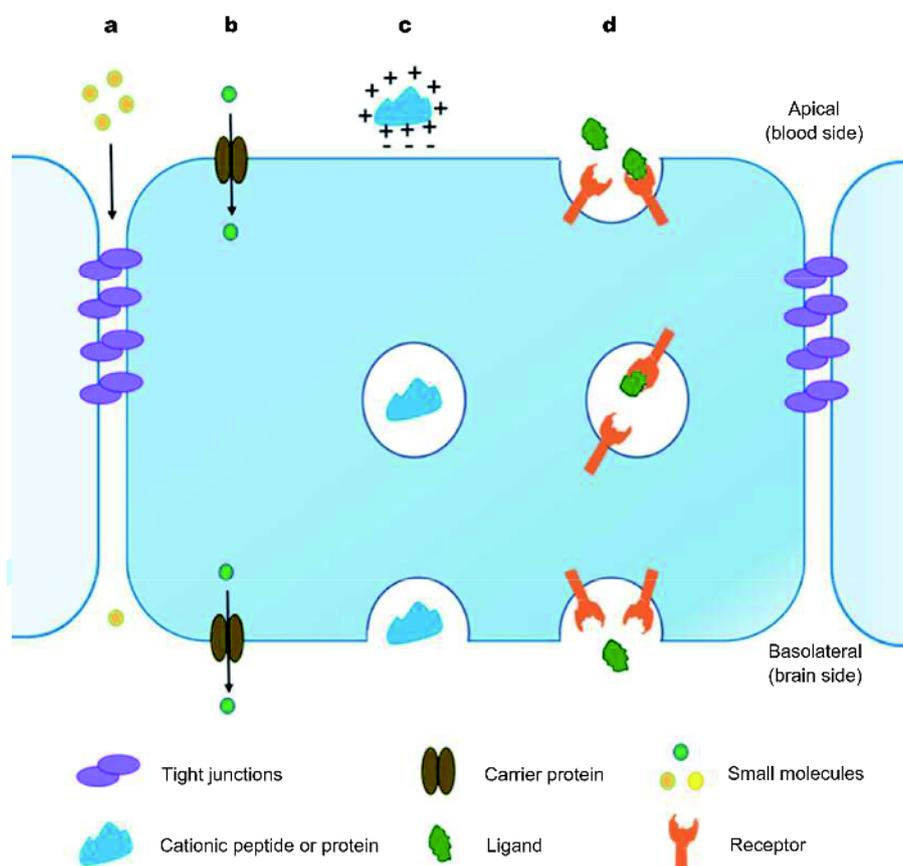
Figure 2 Endogenous transport mechanisms which regulate the passage of various substances across the BBB: (a) passive diffusion; (b) carrier-mediated transcytosis; (c) adsorptive-mediated transcytosis; and (d) receptor-mediated transcytosis.

exocytosed at the abluminal surface, a process called adsorptive-mediated transcytosis (Fig. 2c). Also, certain molecules are able to interact with receptors located on the surface of the BBB endothelium and are escorted to the brain cells *via* receptor-mediated transcytosis (Fig. 2d). Indeed, receptor-mediated transcytosis, adsorptive-mediated transcytosis and carrier-mediated transcytosis have been frequently used to transport imaging and therapeutic agents across the BBB for the treatment of brain cancer.

MAGNETIC RESONANCE IMAGING AND CONTRAST AGENTS

Magnetic resonance imaging (MRI) is, among various methods to image brain tumors, the most common and preferred technique to localize the tumor site and determine the tumor size [16–18]. This is because MRI provides exquisite anatomical images and functional information with high spatial resolution in a noninvasive manner yet without harmful radiation [19,20]. MRI is

therefore very important for diagnosis and preoperative imaging of brain cancer. Recently, MRI has also been applied during surgery for real-time localization of brain tumors *in situ* in order to perform the maximal safe tumor resection [7]. Consequently, precise and reliable MRI is crucial for successful diagnosis, tracking and resection of brain tumors.

Medical MRI is based on the principles of nuclear magnetic resonance (NMR) where the spatial distribution of magnetically active nuclei in the tissue is used to generate an MRI image. Similar to NMR, MRI can utilize NMR-active nuclei, including ^{1}H, ^{11}B, ^{13}C, ^{19}F, and ^{31}P, as a source of signal. Among them, water hydrogen nuclei (^{1}H) are the most commonly used in current clinical MRI. The basis of the ^{1}H-MRI signal is the precession of water hydrogen nuclei after they are excited by radiofrequency pulses in a static magnetic field with superimposed magnetic field gradients providing spatial localization. The generated signal contains information on the density, environment and spatial localization of water hydrogen
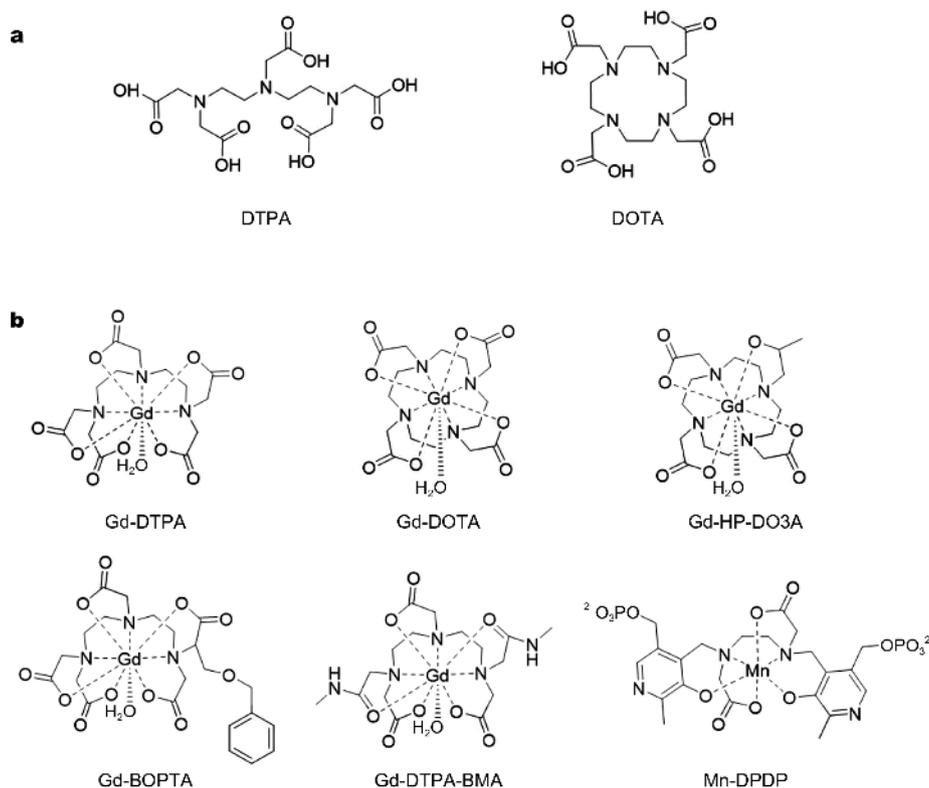
Figure 3 (a) Structures of the chelators DTPA and DOTA, which are commonly used to complex Gd^{3+} for MRI. (b) Clinically used MRI contrast agents. The Gd^{3+}-chelates include one coordinated water molecule.

nuclei, which will be reconstructed into images. Often the endogenous magnetic resonance differences between various tissues are small, and therefore a contrast agent is used in order to enhance the imaging contrast so that the tissue of interest can be distinguished from its surroundings. Such contrast agents provide more detailed and accurate morphological and functional information, which results in improved tissue contrast, precise characterization of lesions, and better evaluation of the abnormalities [21].

MRI contrast agents can be classified as paramagnetic and superparamagnetic [21,22], and the most important feature of a contrast agent is its relaxivity (r), which relates to its capacity to generate imaging signal contrast. Paramagnetic agents such as gadolinium (Gd^{3+}) and manganese (Mn^{2+}) increase both the longitudinal relaxation rate (R_1) and the transverse relaxation rate (R_2) to a similar extent, and they exhibit "positive" (bright) contrast. These agents are named as T_1 agents. Superparamagnetic agents such as iron oxide (SPIO) and ultrasmall iron oxide (USPIO) nanoparticles increase the transverse relaxation rate (R_2) much more than the longitudinal relaxation rate (R_1), and they display "ne-

gative" (dark) contrast. These agents are referred to as T_2 agents. "Positive" contrast agents are preferable to "negative" contrast agents in the clinic because signal enhancement generated by "positive" contrast agents can be more reliably assessed than signal loss generated by "negative" contrast agents.

The majority of contrast agents currently used in the clinic are based on Gd^{3+} ions. This is because Gd^{3+} is the most paramagnetically stable metal ion, and has seven unpaired electrons with a large and symmetric magnetic moment to generate high imaging contrast. However, free Gd^{3+} is highly toxic, because Gd^{3+} is similar to Ca^{2+} in size, bonding and coordination chemistry [23]. By replacing or interfering with Ca^{2+} or other metal ions associated with biological events, Gd^{3+} ions can cause neurological problems and neurotoxicity, and can also accumulate in bone and liver [24,25].

In order to sequester Gd^{3+} and neutralize its toxicity, a variety of chelating agents have been developed such as diethyltriaminepentaacetic acid (DTPA), 1,4,7,10-tetra-azacyclododecane-1,4,7,10-tetraacetic acid (DOTA) (Fig. 3a) and related derivatives. These chelators have high denticity and form complexes with Gd^{3+} via at least

hepta- or octadentate coordination (Fig. 3b), hence generating thermodynamically stable and kinetically inert complexes. Also, these Gd^{3+}-chelates will have at least one coordination site for a water molecule which is crucial for the contrast enhancement. In addition, they have a higher affinity for Gd^{3+} than other metal ions commonly present in the human body such as Ca^{2+}, Zn^{2+}, or Cu^{2+}, thereby considerably reducing the toxicity of Gd^{3+} [26]. It is worth noting that the formation of Gd^{3+} complexes with the macrocyclic ligand DOTA is more thermodynamically favorable than with the linear chelator DTPA. The corresponding Gd-DOTA complex thereby has superior thermodynamic stability and kinetic inertness compared to Gd-DTPA. Nevertheless, the Gd-DOTA complex has two diastereoisomeric forms that differ by the arrangement of the acetate arms and may exist in equilibrium in solution [27].

Examples of clinically used MRI contrast agents are shown in Fig. 3b [28–30]. In addition to Gd^{3+} agents, the Mn^{2+}-chelate Mn-DPDP is the only other metal complex approved as an MRI contrast agent. Mn-DPDP is a weak chelate that undergoes transmetallation with endogenous metal ions (Cu^{2+}, Zn^{2+}, Ca^{2+} and Mg^{2+}) and releases free Mn^{2+} which is then taken up by hepatocytes, resulting in a significant contrast enhancement. Mn^{2+} is therefore considered a more "natural" biological cation than Gd^{3+}. The main disadvantage of Mn^{2+} complexes is that they are less thermodynamically stable and kinetically inert than the Gd^{3+} complexes, as Mn^{2+} has only 6–7 coordination bonds, whereas Gd^{3+} often has 7–9 coordination bonds. It is also worth mentioning that the first MRI was obtained using an Mn^{2+} agent 30 years ago [31]. Recent interest in Mn^{2+} for cell tracking and as an MRI marker of ion channels has stimulated renewed efforts to develop Mn^{2+}-chelates as molecular imaging agents.

Standard MRI is based on the excitation of hydrogen nuclei in water molecules, which vary in abundance in the different organs and tissues of the human body, thereby achieving imaging contrast. Recently, chemical exchange saturation transfer (CEST) has been proposed as a novel technique for MRI [32]. To perform CEST, contrast agents bearing a functional moiety with a labile proton (such as a hydroxyl or amide group) can be used. A saturation pulse is then applied at its resonant frequency in order to attain a saturation state. The exchange of the proton on the CEST agent with a proton on a water molecule effectively transmits the saturation to water, thus decreasing the magnetic resonance signal from water and providing an indirect assessment of the concentration of the compound of interest. CEST has the ad-

vantages of enabling the imaging of certain compounds at concentrations that are too low to affect the contrast of standard MRI or too low to be directly discerned at typical water imaging resolution [33]. In general, CEST agents can be classified as diamagnetic and paramagnetic. The chemical shift of labile protons in diamagnetic CEST agents is very close to that of bulk water, whereas the offset values attainable for paramagnetic (PARACEST) agents can be up to two orders of magnitude larger [32]. Consequently, PARACEST agents hold great promise for improving the specificity of MRI diagnoses.

In addition to ^{1}H-MRI, ^{19}F-MRI offers the unique advantage of highly sensitive imaging because ^{19}F has a nuclear spin of 1/2 and a high gyromagnetic ratio with 100% natural abundance, Moreover, ^{19}F-NMR chemical shifts, which span over 300 ppm, depend significantly on changes in the chemical environment. This permits specific identification of ^{19}F agents by ^{19}F-MRI [34,35]. Therefore, fluorinated contrast agents will be valuable for biomedical applications of ^{19}F-MRI. It is of note that ^{19}F is almost completely absent from biological systems. The signals from exogenous fluorinated agents can therefore be easily detected and recorded without background interference. The low abundance or absence of ^{19}F in living systems means that ^{19}F-MRI requires the use of exogenous fluorinated contrast agents at concentrations higher than common for ^{1}H-MRI probes.

MRI CONTRAST AGENTS BASED ON DENDRIMER NANOTECHNOLOGY

The contrast agents that are currently in common use, such as Gd-DTPA and Gd-DOTA, are small molecular agents which have limitations of rapid renal clearance, low contrast efficiency and non-specificity, etc. Nanotechnology-based macromolecular contrast agents with nanometric size have the advantage of avoiding rapid renal clearance, thus prolonging the circulation half-life. Also, macromolecular contrast agents have greater relaxivity than low molecular weight agents because of their increased molecular weight and steric hindrance. In addition, they can also be conjugated with a targeting moeity for specific imaging. Consequently, a plethora of various natural and synthetic organic and inorganic nanomaterials differing in size, shape, structure, chemistry and architecture have been explored for use as MRI contrast agents [36–39].

Among the different nanosystems established for MRI, dendrimers are of particular interest because of their well-defined molecular structure and multivalent cooperativity confined within a nanosize volume [36,37,40–42]. Den-
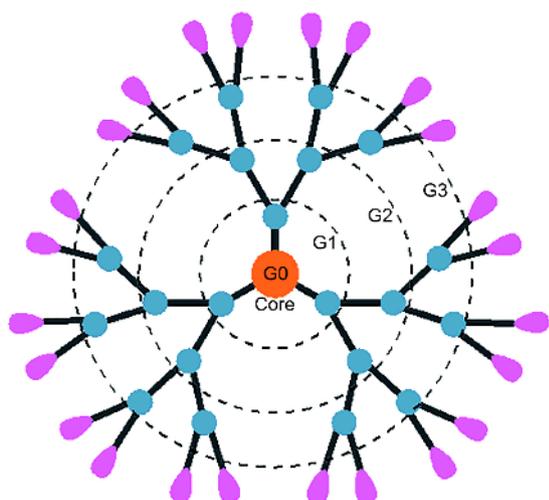
Figure 4 Cartoon illustration of a dendrimer composed of a central core, the repetitive branching units which form the generations and the terminal groups on the dendrimer surface. The central core itself is generation 0 (G_0); G_1, G_2 and G_3 refer to dendrimers of generation 1, 2 and 3, respectively.

drimers have a unique dendritic architecture with regular cascade-branched repeating units emanating from a focal point and abundant terminal groups on the surface (Fig. 4) [43–46]. A large amount of contrast agent entities can be loaded either at the dendrimer terminals or in the interior, hence increasing the imaging enhancement. Also, dendrimer contrast agents have greater relaxivity than small molecular agents, and their relaxivity can be tuned and modulated by changing the dendrimer generation. Modifications on the dendrimer surface can

further affect the relaxation of water protons, by varying parameters such as hydration, hydrogen binding, and diffusion. It is also possible to adjust the proximity of water protons to the central paramagnetic ions by tuning the distance of water molecules to the dendrimer surface. All these modifications help to generate greater contrast than small-molecule contrast agents. In addition, dendrimers have sizes in the range of 3–10 nm and are nano-object *per se*. Therefore, they can avoid rapid renal clearance and have a prolonged circulation half-life. It is noteworthy that dendrimers can be conveniently conjugated with a targeting moeity for specific imaging. Last but not least, different contrast agents and therapeutics can be easily integrated and accommodated within the same dendrimer to offer multimodal imaging and theranostics. We will give a short description of dendrimer contrast agents below.

Dendrimers are a special family of synthetic polymers and consist of three distinct structural components: 1) a central core, where dendrimer growth begins; 2) repetitive branching units, which allow for dendrimer growth in geometrically organized radial layers (named as generations); and 3) a large number of terminal functionalities exposed at the surface (Fig. 4). These structural features make dendrimers significantly different from traditional linear and hyper-branched polymers. Unlike conventional polymers which are often prepared *via* "one-pot" synthesis, dendrimers are constructed *via* stepwise synthesis, which results in precisely controlled structures. Dendrimer synthesis can be achieved using either divergent or convergent approaches (Fig. 5)
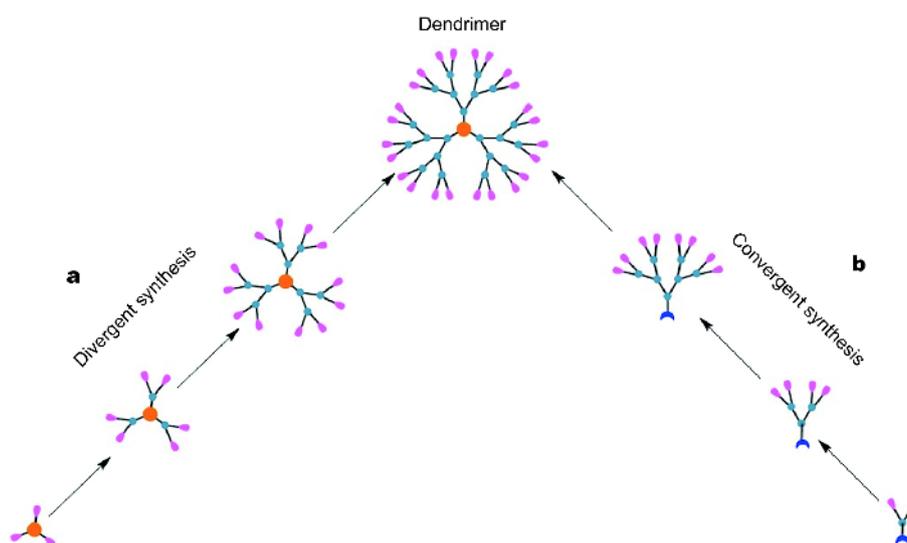

Figure 5 Cartoon presentation of dendrimer synthesis using (a) the divergent approach and (b) the convergent approaches.

[46,47]. In the divergent strategy, the dendrimer is built from a multifunctional core and then extended outward by a series of repetitive reactions (Fig. 5a). In the convergent strategy, the dendrimer is constructed from small molecules that start at the dendrimer surface and eventually end up attached to a central core through a series of inward oriented reactions (Fig. 5b).

Most dendrimers, including the most commonly studied poly(amidoamine) (PAMAM) and poly(propylamine) (PPI) dendrimers, are prepared *via* divergent synthesis. Using the divergent approach, dendrimers can be synthesized to high generations of 10 or even more. Nevertheless, during the synthesis of high-generation dendrimers, structural defects are a critical concern because they are inevitably generated from incomplete reactions or various side reactions that occur due to the growing number of reactions and steric hindrance. By using the convergent approach, we can have much better control over dendrimer synthesis, and structural defects can often be avoided. However, the reactivity to reach the interior core is often considerably reduced due to the increasing steric congestion as the dendrimer generation increases. Therefore, only low-generation dendrimers can be successfully prepared using the convergent method.

A third method, called the double-stage convergent approach, has also been developed and applied for dendrimer synthesis [47,48]. This approach involves the synthesis of building blocks using a divergent approach followed by convergent dendrimer assembly, and hence incorporates the advantages of both divergent and convergent synthesis. In this approach, the number of reaction steps required for synthesis and purification of dendrimers can be reduced, making the preparation of higher generation dendrimers more efficient compared to traditional divergent and convergent routes.

It is worth noting that "click" chemistry has been successfully implemented in dendrimer synthesis, offering simplified and accelerated synthesis, and at the same time, diverse structural complexity [49,50]. "Click" reactions give a high yield within a short reaction time, yet can be performed under mild conditions and with high atom economy. High yield and completion of each synthetic step is crucial to maintain the purity and consistency of a dendrimer for applications ranging from molecular devices, sensors and catalysts to drugs, imaging agents and drug-delivery systems [51,52].

In 1994, Lauterbur and co-workers [53] pioneered the first study on dendrimer-based MRI agents. In that study, the DTPA chelating agent was conjugated to the terminal amine of PAMAM dendrimers of generations 2 and 6,

followed by complexing with $GdCl_3$ to deliver the dendrimer contrast agent **1** (Fig. 6). By virtue of their large molecular weight, these dendrimer contrast agents exhibited higher longitudinal relaxivities compared to the small molecular agent Gd-DTPA. Also, their blood circulation half-lives increased considerably. Importantly, these dendrimer agents showed superior *in vivo* imaging compared to the small molecular agent Gd-DTPA. This seminal work of Lauterbur was an important starting point for developing dendrimer-based MRI contrast agents.

Further studies using PAMAM [54,55] and PPI dendrimers (Fig. 7) [56] confirmed that the molecular and ionic relaxivities increase according to the dendrimer molecular weight and generation number, while the molar relaxivities achieve a saturation limit beyond generation 7 [55]. Nevertheless, conjugation of the Gd^{3+}-chelate to a large dendrimer does not significantly affect the water exchange rate at the metal center, and the constants for the water exchange rate remain almost similar for all the dendrimer agents studied [57]. Also, protonated dendrimers often generate a more rigid and open structure, and hence a further increase in relaxivity [58,59]. Moreover, Ali and colleagues [60] reported a pH-sensitive agent based on a PAMAM dendrimer of generation 5 which bears a DOTA-derived chelating ligand, DOTA-4AmP, at the dendrimer surface. The relaxivity of this agent was more than doubled when the pH was changed from 9 to 6.

Studies of the biodistribution of PAMAM dendrimer contrast agents [61–63] demonstrate that the glomerular filtration rate of smaller generation dendrimers (G_3 to G_5) is high, and these smaller dendrimers are rapidly excreted, while G_5 and G_6 are retained long enough to visualize normal fine vasculature. Higher-generation dendrimers G_7 to G_9 show lower levels of renal uptake than G_6, and G_8 and G_9 exhibit much higher levels of accumulation in the liver. Consequently, G_7 stands out as the most suitable agent for imaging the tumor vasculature because it is retained the longest in the circulation, allowing longer image acquisition times. Similar trends can be also found with PPI dendrimer agents [64].

The biodistribution of dendrimer agents is determined by their size, but their surface composition and modification also play a critical role in affecting the circulation half-life, pharmacokinetic and toxic profile, and targeting specificity [36]. For example, the terminal groups have a strong effect on dendrimer toxicity. Dendrimers with terminal amines are more toxic than those with terminal carboxylic acid groups [65]. Higher generations and in-
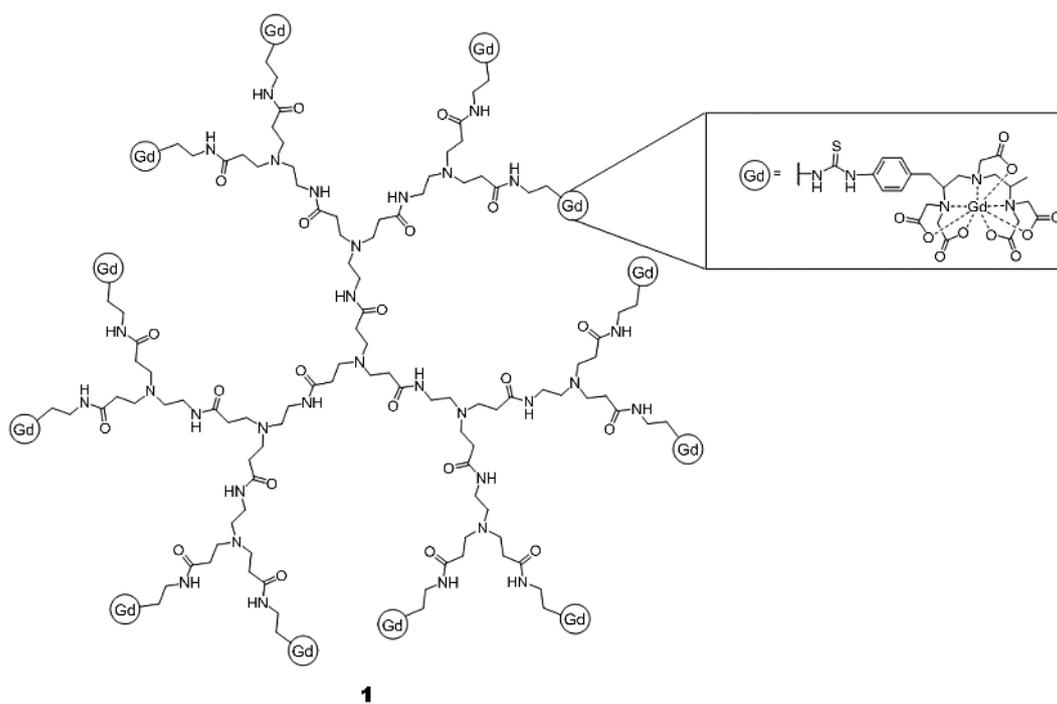
Figure 6 The first dendrimer-based contrast agent for MRI developed by Lauterbur and co-workers: Gd-DTPA functional moieties were appended to the termini of PAMAM dendrimers [53].
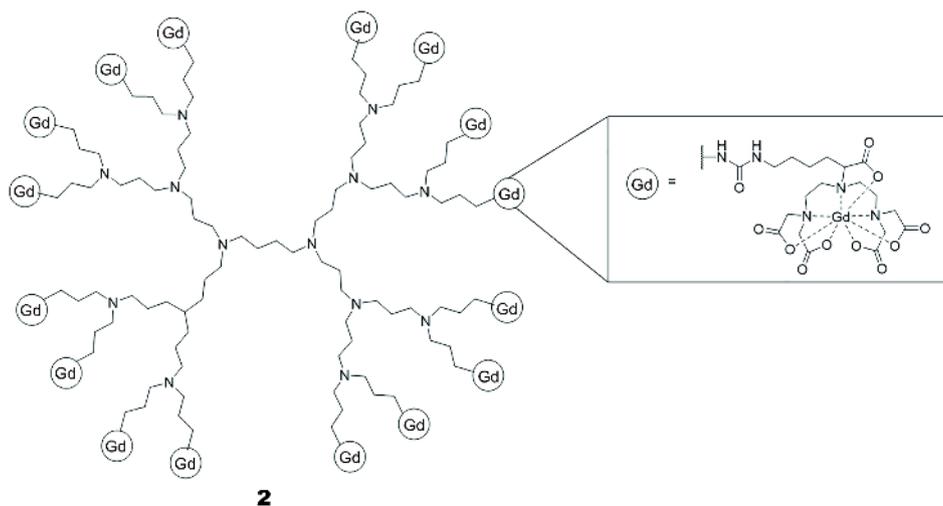

Figure 7 PPI dendrimer-based MRI contrast agent bearing Gd-DTPA terminal functionalities.

creased doses of cationic dendrimers are usually associated with more toxicity [66]. PEG is frequently appended as a surface modification to mask the surface charge of dendrimers in order to reduce toxicity, while at the same time increasing the circulation half-life, hence improving the biocompatibility. Conjugating dendrimer contrast agents with targeting moieties will modify the

pharmacokinetics and biodistribution of the agents, thereby enhancing both the imaging efficiency and specificity. The development of stimulus-responsive MRI contrast agents that are sensitive to variables in the physiological and pathological microenvironment will also facilitate specific molecular imaging.

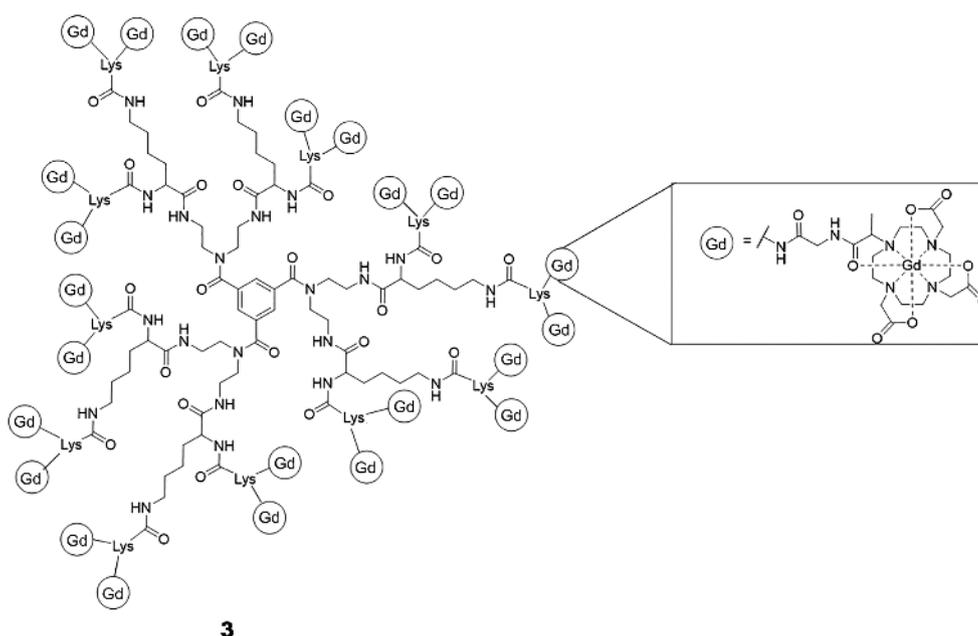Overall, tuning the dendrimer chemistry, core,

Figure 8 Gadomer®-17, developed by Schering AG, has entered into clinical trials for MRI.

branching, generation and surface coating in conjunction with conjugation of contrast agents has led to different dendrimer-based MRI agents with altered relaxivities [36,37,40,41,67]. Gadomer®-17 (Fig. 8), a dendrimer comprising a trimesoyl triamide core and polylysine dendritic branching with terminal payloads of Gd-DTPA [68], is the first dendrimer-based product to be clinically evaluated as a contrast agent for magnetic resonance angiography [69,70]. It has high vascular permeability, and is able to visualize intratumoral vasculature [71] and acute myocardial ischemia [72]. Gadomer®-17 showed promising results in clinical studies, but further clinical evaluation was discontinued. This contrast agent is currently commercially available for preclinical research. In this short review, we will mainly focus on dendrimer-based MRI constrast agents related to investigating and imaging brain cancer.

DENDRIMER-BASED CONTRAST AGENTS FOR MRI OF BRAIN TUMORS

An ideal dendrimer contrast agent for brain cancer imaging should possess a number of key features, namely biocompatibility, the ability to transport and protect the imaging functionality from degradation and/or rapid clearance, the potential to cross the BBB and reach the brain, and specific targeting and delivery of the agent to the desired cell population of interest. Also, the agent should be excreted or degraded after imaging.

As described in the introduction, brain tumors often have leaky vasculature and a disrupted BBB. This means that nanosized dendrimer agents are able to cross the BBB and reach tumor lesions *via* the leaky vasculature (Fig. 9a). Moreover, lymphatic drainage is often defective in tumor tissues. Therefore, nanosized agents which enter into the tumor tissues cannot be filtered out and will stay and accumulate there, leading to enhanced permeability and retention (EPR) in the tumor (Fig. 9a). This is also called passive tumor targeting [14,73]. EPR-mediated passive targeting results in high local concentrations of nanosized agents within tumor lesions, which in turn enhances the imaging quality, sensitivity and resolution.

Active targeting approaches that circumvent the compromised or intact BBB are often required for better and more efficient delivery to the brain. A promising strategy is to explore the endogenous transport mechanisms for delivery across the BBB. Due to the tight junctions, paracellular diffusion is almost impossible for macromolecular dendrimer agents. Carrier-mediated transport is also only applicable for shuttling specific hydrophilic small-molecule nutrients like amino acids and glucose. Adsorptive-mediated transport is inherently nonspecific, although it has been used to transport dendrimer agents across the BBB. This is because adsorptive-mediated transcytosis is mainly mediated by the interaction of positively charged peptides (e.g., cell-penetrating peptides) or proteins (e.g., albumin) with negatively charged
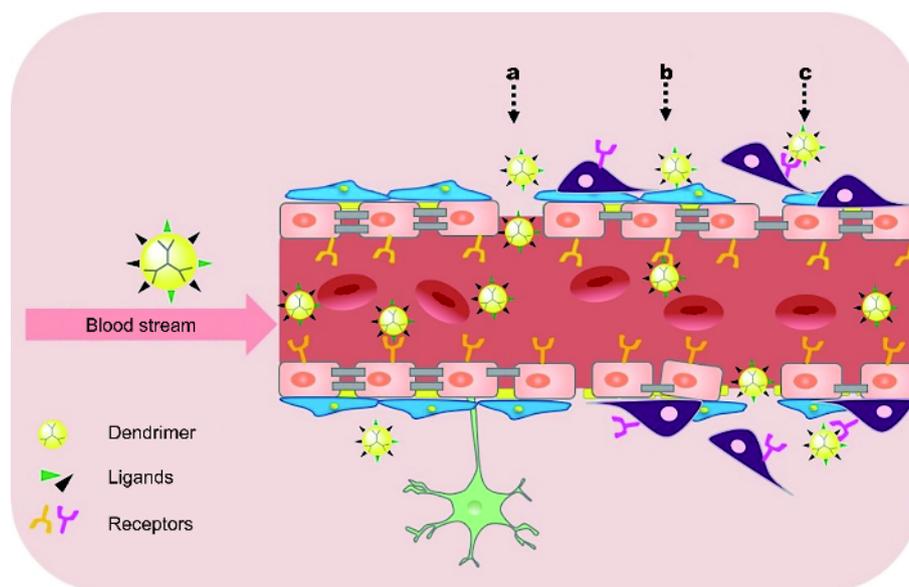
Figure 9 A dendrimer-based contrast agent can cross the BBB and reach the brain tumor *via* (a) the EPR effect, (b) receptor-mediated transcytosis and (c) receptor-mediated cellular uptake, triggered by ligands on the nanoparticle surface.

components on the cell membrane, triggering endocytosis and subsequent intracellular transport before eventual release into the brain in a nonspecific way [74–76]. Receptor-mediated transcytosis is the method of choice for crossing the BBB since it depends on the interaction of ligands with their specific receptors on the surface of the BBB endothelium (Fig. 9b). The discovery of several receptors that are uniquely expressed on brain capillary endothelial cells (BCECs) has made receptor-mediated transcytosis a highly promising delivery route. For example, receptor-mediated transcytosis has been demonstrated for transferrin, low-density lipoprotein receptor-related protein (LRP), insulin, insulin-like growth factors and leptin. Taking advantage of these properties, scientists have functionalized nanoparticles with receptor targeting ligands such as transferrin [77], LRP, rabies virus glycoprotein peptide [78], and snake neurotoxin peptide [79] to up-regulate their BBB permeability.

Finally, active tumor targeting can be further realized by introducing a targeting moiety onto the delivery system, which can interact with and bind to ligands or receptors present on the cancer cell surface [80–83]. In this way, the imaging agent can be delivered specifically to the target cancer cells after crossing the BBB (Fig. 9c), thereby achieving higher delivery efficacy while sparing other cells to reduce toxicity. Different targeting agents such as antibodies, peptides and small molecular ligands have been applied to construct active targeting systems for tumor homing [84–86]. These systems can be further developed

to create nanoplatforms for brain tumor targeting.

In this section, we will present examples of dendrimer-based MRI contrast agents related to brain tumor imaging. We will also report combinations of different imaging techniques for multimodal imaging and integration of therapy and imaging modalities to develop theranostics for treating brain tumors.

Passive *versus* active targeting
As mentioned above, nanoparticles can accumulate within tumor lesions by EPR-mediated passive targeting. In order to achieve brain tumor targeting, researchers have used specific functional entities to overcome the BBB and actively target cancer cells [84,85,87]. It is expected that dendrimer-based imaging agents would accumulate and be enriched at the tumor lesions *via* the EPR effect and/or receptor-mediated transcytosis, then bind to ligands or receptors present on the brain cancer cell surface (Fig. 9). In this way, the imaging agent can be delivered specifically to the cancer cells after crossing the BBB, thereby achieving higher delivery efficacy while sparing healthy cells to avoid adverse effects. Below, we will highlight both passive and active targeting approaches for delivering dendrimer-based contrast agents to brain tumors with recent examples.

Passive targeting
Following the seminal work of Lauterbur on PAMAM dendrimer-based contrast reagents [53], improved con-
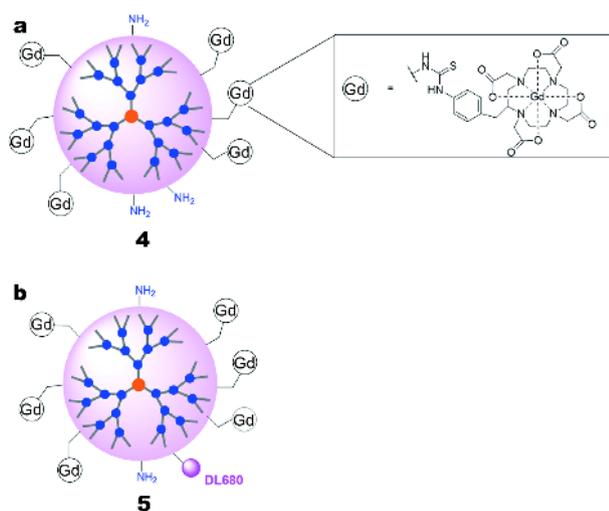
Figure 10 PAMAM dendrimers bearing (a) the contrast function Gd-DOTA and (b) both Gd-DOTA and the fluorescent dye DL680 at the terminals [89,90].

jugation of Gd-DTPA to dendrimers was achieved using non-aqueous chemistry [88]. The *p*-NCS-benzyl functional group was used as a rigid linker to join Gd-DTPA or Gd-DOTA to the dendrimer in order to ensure that the slow rotation of the rigid dendrimer molecule can be transmitted to the surface MRI functions. Recently, a PAMAM dendrimer of generation 5 bearing the contrast function Gd-DOTA (**4**) at the terminals (Fig. 10a) showed glioma-specific accumulation in animal models [89,90]. This can mainly be ascribed to the defective BBB of the brain tumor and the EPR effect of the nanoparticulate agents. Furthermore, the effectiveness of MRI imaging was confirmed using *in vivo* and *ex vivo* optical imaging with the dendrimer agent (**5**), which carries both the Gd-DOTA payload and the fluorescent dye DL680 (Fig. 10b).

As we know, brain cancer disrupts the BBB as it progresses [91], and the blood-brain-tumor barrier (BBTB) is established once the BBB becomes damaged. The BBTB often contains large pores, which make the BBB more permeable to nanosized macromolecules than the endothelial barrier in the microvasculature of most normal tissues. In order to probe the pore size in the BBTB of brain tumors, Sarin *et al.* [92,93] labelled PAMAM dendrimers of generation 5 to 8 with Gd-DTPA at the terminals, and investigated their transport in brain tumors in mouse models using dynamic contrast-enhanced magnetic resonance imaging. The size of the G_7 dendrimers bearing Gd-DTPA functions was 11 ± 1 nm, and that of the G_8 dendrimers was 13 ± 1 nm. The functionalized G_5–G_7 dendrimers with sizes less than 12 nm were able to pass through the pores of the BBTB in RG-2 glioma xenograft mice, whereas larger G_8 dendrimers could not. In addition, dendrimers with long blood half-lives were able to accumulate more effectively within glioma cells. This finding suggests that the physiological upper limit of the pore size in the BBTB of the microvasculature in malignant solid tumors is approximately 12 nm. This is in line with the results obtained from a later study on the permeability of solid tumors using nanoparticles of different sizes [94], where the optimal size of nanoparticles for tumor penetration was found to be around 12 nm [95]. Therefore, effective delivery into malignant brain cancer cells can be achieved with nanoparticles less than 12 nm in diameter and with a long blood circulation time.

Among the various dendrimers explored, PAMAM, PPI and poly(lysine) dendrimers are most frequently studied for establishing MRI agents because of their good biocompatibility and easy chemical conjugation as well as their commercial availability [36]. Nevertheless, polyester and polyether dendrimers have also been explored for constructing MRI agents [96–98]. In addition, placing the Gd^{3+} ion at the dendrimer core was further proposed to increase the relaxivity (Fig. 11) [97,98]. However, the
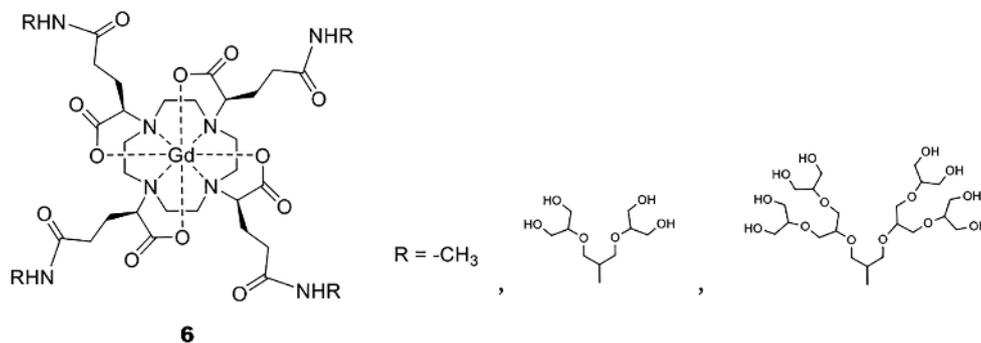

Figure 11 Gd^{3+} incorporated at the dendrimer core to increase the relaxivity for MRI [97].
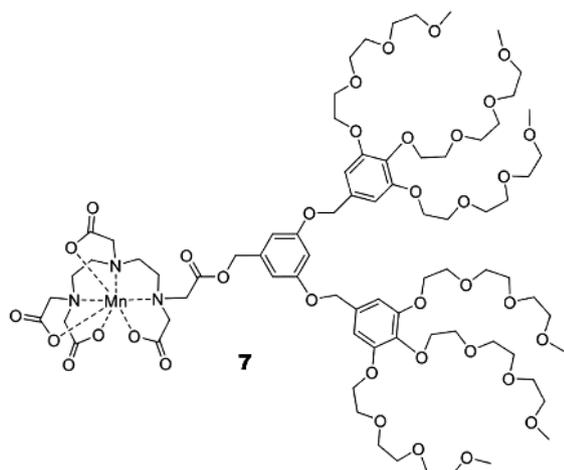
Figure 12 The dendritic manganese contrast agent 7 [99].

water exchange rate will also be slowed down, and this will tend to negate any theoretical increase in relaxivity.

Besides Gd^{3+}, a dendritic Mn^{2+}-enhanced contrast agent (Fig. 12) was also developed for brain targeting [99]. This is because Mn^{2+} has natural human biochemistry: the uptake of extracellular Mn^{2+} by neurons is often through NMDA receptors for glutamate and/or voltage-gated calcium channels, and the uptake of Mn^{2+} is directly coupled to the physiological activity and functions of the cells. Mn^{2+} can therefore generate contrast in a manner that depends on cellular activity, which means that Mn^{2+}-based contrast agents have potential for investigating neuronal activation and architecture. The dendritic Mn^{2+}-conjugate 7 displayed high water solubility and revealed neither *in vitro* neuronal toxicity at concentrations up to 1 mmol L^{-1} nor *in vivo* toxicity at 20 mmol L^{-1} in a rat model. The excellent safety profile of this dendritic manganese contrast agent is likely due to the slow release of the paramagnetic ions. Also, its relaxivity is higher than that of the commercial agent Mn-DPDP, which suggests that it may have potential applications in studying and treating brain and neurodegenerative diseases.

It is worth noting that passive targeting *via* the EPR effect is unlikely to be an effective and viable approach for imaging and treating brain cancer [100], because the BBB is a formidable obstacle even if it is disrupted by a developing tumor. The extravasation of nanoparticulate agents and their accumulation in the tumor *via* the EPR effect depends entirely on nanoparticle diffusion, which is difficult to control. Also, some agents display extremely low rates of diffusion. In addition, tumor blood vessel permeability is not homogeneous even within the same

tumor. Last but not least, the elevated interstitial fluid pressure can block passive diffusion of nanoparticles into the brain parenchyma. Consequently, there is great interest in active targeting strategies for delivery of nanoparticles to brain tumors.

Active targeting
Considerable efforts have been devoted to developing active targeting approaches to overcome the BBB and specifically target brain cancer cells. As described above, receptor-mediated transcytosis is a naturally-occurring pathway through which endogenous and exogenous molecules can cross the BBB. Ligands that have been explored for targeting brain tumors *via* receptor-mediated transcytosis include transferrin, LRP, neurotoxins, peptides, nucleic acids and antibodies.

Chlorotoxin (CTX) is a 36-amino acid peptide that specifically binds to brain glioma cells [101], thus allowing the penetration of gliomas through tissue barriers. Its targeting specificity stems from its selective binding to membrane-bound matrix metalloproteinase-2 isoforms (MMP-2) [102], which are expressed at high levels in gliomas and related cancers, but not in healthy brain cells. An ^{131}I-labelled CTX is currently undergoing clinical trials for treatment of brain cancer [103]. Huang et al. [104] reported a dendrigraft poly-L-lysine dendrimer (DGLs) equipped with CTX as the glioma tumor targeting ligand and Gd-DTPA as the imaging payload for MRI (Fig. 13). This agent markedly enhanced the cellular uptake in C6 glioma, but not in normal cells. In addition, when mice were treated with this agent, the imaging contrast was significantly improved compared to the unmodified counterpart and the commercial control. Remarkably, the enhanced signal from this agent was maintained much longer than the controls, illustrating its potential for improving imaging and diagnosis. This discovery is particularly relevant to brain glioma, which is difficult to image and diagnose because of the BBB.

LRP mediates the transport of a variety of ligands across the BBB, such as lipoproteins, extracellular matrix proteins, proteases and protease inhibitor complexes [105]. LRP receptors are highly expressed on brain capillary endothelial cells (BCECs) and on many types of glioblastoma [106]. Angiopep-2, a 19-amino acid peptide, is a ligand of the LRP receptor and demonstrates a high BBB transcytosis efficacy [107]. For example, an angiopep-2-paclitaxel conjugate, ANG1005, has been developed to deliver the anticancer drug paclitaxel across the BBB to treat glioma. ANG1005 has been subjected to several Phase I clinical trials [108], and Phase II trials are
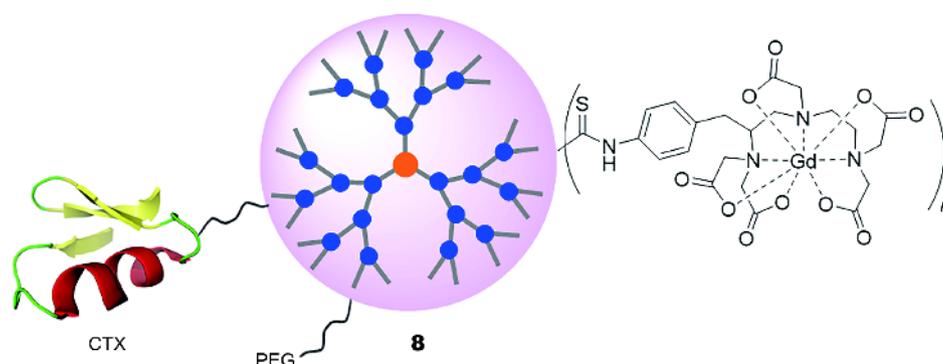
Figure 13 Dendrigraft poly-L-lysines (DGLs) conjugated with Gd-DTPA and the CTX peptide for targeted MRI of brain tumors [104].

underway (http://angiochem.com). Angiopep-2 is therefore frequently chosen as the BBB-permeable ligand for nanoparticle delivery to the brain. Li and co-workers [109] conjugated angiopep-2 to a dendrimer of generation 5, along with the MRI reporter Gd-DTPA and the fluorescent dyes Cy5.5 and rhodamine, to create a dual MRI/fluorescent imaging agent (Fig. 14a). This agent combines BBB permeability, passive and active targeting functions to visualize glioblastomas, and high sensitivity. It therefore holds promise for pre-operative localization of brain tumors and for making image-guided tumor resection possible during surgery.

Additionally, Li and co-workers [110] conjugated cyclic RGD peptide to the above dendrimer agents to further improve the tumor targeting and imaging quality (Fig. 14b). The RGD tripeptide is the ligand of $\alpha_V\beta_3$ integrin, which is often present on tumor cells, and is also highly expressed on activated endothelial cells of the tumor neovasculature, but not the normal vasculature [111]. Therefore, integrin is a molecular target for tumor diagnosis and therapy [112]. Dendrimer nanosystems decorated with the RGD targeting peptide showed effective delivery in glioblastoma cancer models [113,114]. The dual-targeting imaging agent **10** developed by Li and co-workers is expected to first target $\alpha_V\beta_3$ integrin on the tumor vasculature *via* binding of the cyclic RGD peptide [115]. Increased local concentration of **10** within the tumor vasculature then facilitates interaction of the BBB-permeable angiopep-2 peptide with the LRP receptors on the vascular endothelial cells, which promotes transport across the BBB *via* LRP receptor-mediated endocytosis. After the agent **10** penetrates the BBB, it then targets the brain tumor because the tumor cells express high levels of both $\alpha_V\beta_3$ integrin and LRP receptor. *In vivo* imaging studies showed that **10** efficiently crossed the intact BBB in normal mice, and also facilitated precise detection of
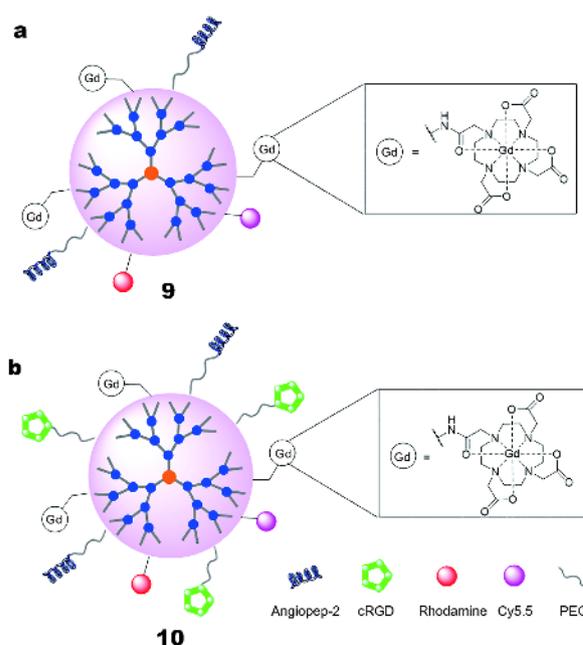

Figure 14 PAMAM dendrimers decorated with the BBB-permeable angiopep-2 peptide, the MRI reporter Gd-DTPA, and the fluorescent dyes Cy5.5 and Rhodamine, without (a) and with (b) the cyclic RGD peptide as active targeting agents to visualize brain tumors [109,110].

the boundary of the orthotopic U87MG human glioblastoma xenografts with high contrast and sensitivity. Overall, this dual and sequential targeting contrast agent **10** holds great promise for the noninvasive visualization of brain tumors even when the BBB is intact, which may facilitate real-time optical image-guided brain tumor resection during surgery.

Although active targeting is valuable for delivering nanosized agents to brain tumors, it cannot guarantee 100% success in all cases. An example is the comparative study performed on liver cancer and brain cancer models
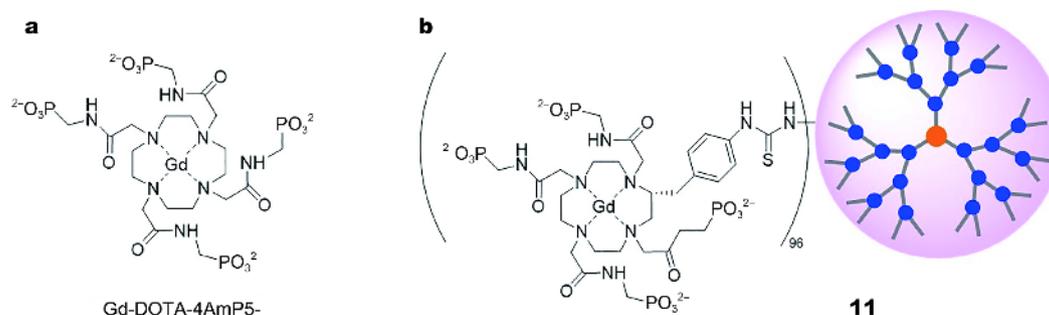
Figure 15 pH-sensitive MRI agents: (a) Gd-DOTA-4AmP^{5-} [126] and (b) a dendrimer conjugated with Gd-DOTA-4AmP^{5-} at the termini [60].

using a dendrimer nanosystem to target the transferrin (Tf) receptor (TfR), which is overexpressed on cancer cells and brain cells [116]. TfR is important for the transcytosis of Tf-bound iron across brain capillary endothelial cells in humans [117], and it is highly expressed in the BBB. However, it is also expressed on hepatocytes, etc. In that comparative study, the PAMAM dendrimer-based MRI contrast agent carrying a Gd-DTPA payload was decorated with the peptide T7, a high-affinity ligand of TfR [118]. This T7-conjugated dendrimer contrast agent targeted the liver cancer more effectively than the non-targeting system [116]. However, it did not show any specificity for brain cancer. These results highlight that circumventing the BBB is a significant challenge to effective brain delivery.

Stimulus-responsive targeting
Contrast agents which are responsive to physiological conditions can expand the range of MRI beyond anatomical and functional imaging by further conveying information at the cellular or molecular level. These agents, which are referred to stimulus-responsive or smart contrast agents, are sensitive to specific fluctuations in the local environment, such as pH, biological metal ion concentration or enzymatic activity, and are capable of reporting on such changes by inducing alterations in the MRI signal [119–124]. The change in relaxivity of these agents before and after stimulation is the most important factor in evaluating the efficiency of a stimulus-responsive contrast agent [125]. We present here stimulus-responsive dendrimer agents that are sensitive to common microenvironmental variables *in vivo* such as pH, metal ions, redox potential and enzymes. These stimulus-responsive agents have the potential to offer more specific and accurate functional MRI.

pH-responsive
Cancer, inflammation and various ischemic diseases are

often associated with a lower extracellular pH, and this can be used for early detection of disease or to monitor treatment efficacy. It is therefore of great importance to non-invasively and reliably assess tissue pH. One of the earliest and most extensively studied pH-sensitive MRI agents is Gd-DOTA-4AmP^{5-} (Fig. 15a), which was developed by Sherry and coworkers [126]. The pH-sensing mechanism of this agent is based on protonation of the four phosphonate groups, which results in a 1.5-fold increase in relaxivity over the pH range 9.5–6.0. The phosphonate groups have pK_a values in the range 6.5–8.0 and facilitate catalytic exchange of protons from the Gd^{3+}-bound water molecule when protonated.

Sherry and colleagues [60] further reported that the relaxivity and responsiveness of Gd-DOTA-4AmP^{5-} was improved by conjugation to a PAMAM dendrimer (Fig. 15b), which generated nanoparticles with ~96 pH-responsive Gd^{3+}-chelates. This pH-responsive dendrimer-based contrast agent produced a 2.2-fold increase in relaxivity over the pH range 9.5–6.0. Also, Aime and coworkers [127] investigated Gd-DOTA-functionalized poly-L-ornithine, which forms random assemblies at low pH, but generates ordered helical structures at higher pH. Collectively, these results demonstrate the potential for the development of effective, pH-responsive MRI agents. It should be mentioned that the pH variation in most physiological or pathophysiological processes is relatively small, and may not create a large enough change in relaxivity. This may be a limitation for pH-responsive contrast agents.

Metal ion-responsive
Metal ions are critical for many biological signaling events, redox homeostasis, and metabolism. Much effort has been dedicated to synthesizing MRI agents that respond to biologically important metal ions like Ca^{2+}, Zn^{2+}, Fe^{2+}, and Cu$^{+/2+}$ [121]. However, only ions with concentrations above the micromolar range can be detected
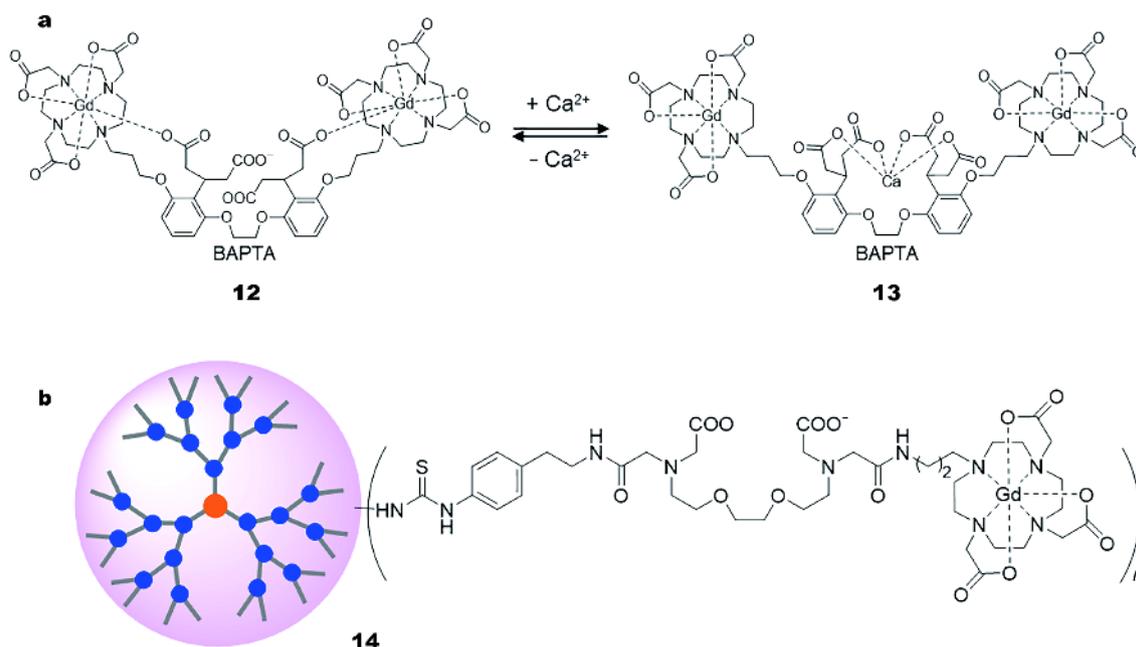
Figure 16 (a) Ca^{2+}-sensitive MRI contrast agent developed by M eade *et al.* [128]; (b) dendrimer-based Ca^{2+}-sensitive MRI contrast agent [129].

due to the low sensitivity of MRI. As Ca^{2+} is in high concentration and involved in neuronal signaling, several groups have developed Ca^{2+}-responsive MRI contrast agents. Meade and colleagues [128] reported the first Ca^{2+}-responsive agent, which consisted of two Gd-DO3A chelates joined by a modified BAPTA (1,2-bis(*o*-amino-phenoxyl)-ethane-*N,N,N',N'*-tetraacetic acid) linker (Fig. 16a). When no Ca^{2+} was present, the BAPTA carboxylate arms bound the chelated Gd^{3+}, and the relaxivity was 3.26 (mmol L^{-1})$^{-1}$ s^{-1}. When Ca^{2+} was added, the carboxylate arms selectively bound the Ca^{2+}, allowing the Gd^{3+} to bind bulk water, and increasing the relaxivity to 5.76 (mmol L^{-1})$^{-1}$ s^{-1}. Recently, Gündüz *et al.* [129] reported a dendrimer contrast agent that is sensitive to Ca^{2+} (Fig. 16b). Ca^{2+} was able to trigger the longitudinal relaxivity response of this agent. MRI studies in the rat cerebral cortex indicated that the diffusion properties of this agent are advantageous as compared to the monomeric equivalents.

Enzyme-responsive
Enzymatically modulated contrast agents have the potential to assess enzyme activity as well as detecting the location of enzymes and their related physiological properties. Meade *et al.* [130,131] developed the first examples of enzyme-sensitive contrast agents to illustrate the principle of enzymatic activation of MRI contrast

agents by β-galactosidase. In addition, Chen *et al.* [132] developed an MRI agent based on a PAMAM dendrimer of generation 5, which carried multiple copies of the Gd-DOTA payload, the fluorescent dye Cy5, and the peptide ACPP, which is composed of the cell-penetrating peptide Arg$_9$ and a gelatinase-cleavable peptide segment (Fig. 17). Gelatinases (MMP2/MMP9) belong to the matrix metalloproteinases (MMPs) and are involved in neurovascular impairment after stroke. *In vivo* detection of MMP2/MMP9 activity may deliver information about disruption of the BBB, hemorrhage, and injury or death of neurons. The dendrimer agent **15** was used to follow the time-course of MMP2/MMP9 activity in the brain at the cellular level *via* a combination of fluorescent imaging and MRI. This gelatinase-specific, non-invasive agent will therefore be useful for detecting pathological processes *in vivo* and for validating potential treatments.

In this section, we presented dendrimer-based contrast agents which undergo changes in relaxivity according to specific fluctuations of pH, metal ion concentration or enzymatic activity in the local environment. Together, these agents can provide valuable information on the existence and execution of numerous essential physiological and pathological processes at the cellular and molecular levels. In general, each agent is responsive and specific to a relevant physiological or pathophysiological process, and hence provides specific and more accurate
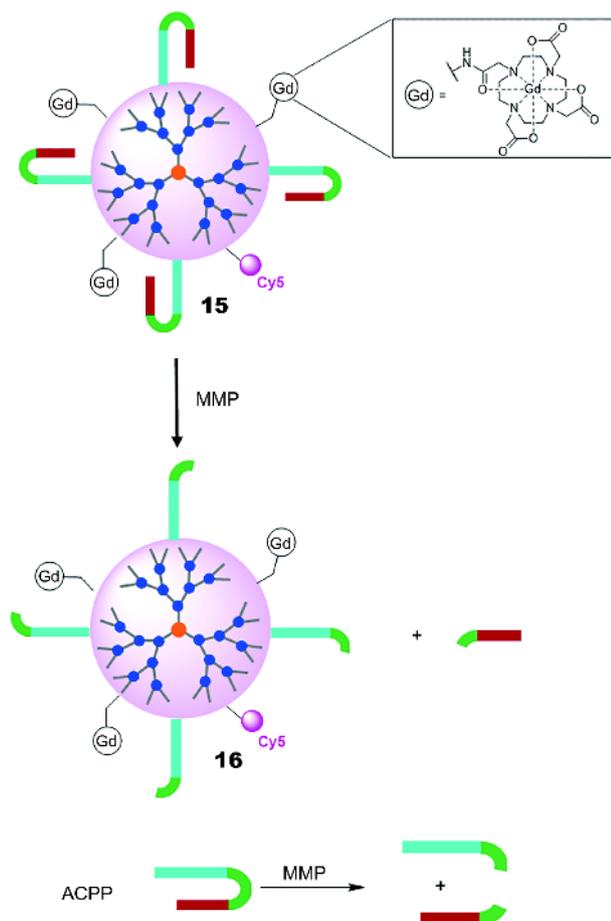
Figure 17 A PAMAM dendrimer of generation 5 was decorated with Gd-DOTA as an MRI reporter, Cy5 as a fluorescent dye, and the cell-penetrating peptide ACPP, which has a gelatinase-cleavable peptide segment [132].

imaging information. Nevertheless, these stimulus-responsive contrast agents have their own advantages and disadvantages. The pH-responsive agents are less specific, as many biological events or disease lesions are associated with low pH, such as cancer, inflammation, and ischemia. Also, most biological and pathophysiological processes result in relatively small pH changes, which may not create significant changes in the relaxivity of the contrast agent. The ion-responsive agents are more specific because ion-related biological processes require distinct ions, thus permitting the identification of pathophysiological events with more precision. Because of the low sensitivity of MRI, only ions with high concentrations can be detected. The most specific agents are the enzyme-responsive agents, as most enzymes have specific biological and physiological functions and activities. Nevertheless, the effectiveness of these agents may also be limited if the substrate or enzyme concentration is too low to be detected by MRI. All MRI contrast agents suffer from common limitations and drawbacks related to the low sensitivity and detection threshold of MRI. Using dendrimer platforms to load multiple copies of the responsive agent is expected to circumvent the sensitivity issue, thus enhancing the MRI signal. Moreover, different imaging modalities can be accommodated within the same dendrimer, such as **15** in Fig. 17, providing multimodal imaging for better and more accurate imaging.

Numerous studies have demonstrated the potential of responsive contrast agents to detect changes in diagnostically relevant variables *in vitro*. However, very few of these agents have been followed up *in vivo*. Feedback from *in vivo* MRI studies will be crucial for designing new and more versatile agents with structures that can be easily modified to deliver greater changes in relaxivity in response to physiological variables.

Chemical exchange saturation transfer (CEST) for MRI

As described above in the section on MRI, CEST is emerging as a novel technique [32], which enables the imaging of certain compounds at low concentrations [33]. The incorporation of metal ions results in paramagnetic CEST (PARACEST) agents, which have chemical shifts that are further away from water [32]. PARACEST agents have also been covalently linked to nanocarriers, including linear polymers, dendrimers and other macromolecules [133–136], to further improve the sensitivity of detection *in vivo*. For example, Ali *et al.* [137] conjugated the PARACEST agent Eu-DOTA-Gly$_4$ to a PAMAM dendrimer of generation 5 (Fig. 18a), and demonstrated the CEST effect in the tumor tissue of a mouse mammary carcinoma model. Later, Ali *et al.* [138] created another dendrimer-based PARACEST agent by appending both Eu-DOTA-Gly$_4$ and the fluorescent dye DL680 to the PAMAM dendrimer (Fig. 18b). This dendrimer agent exhibited effective *in vivo* CEST MRI in a glioblastoma mouse model, which was further validated using *in vivo* and *ex vivo* fluorescent imaging.

Recently, McMahon and co-workers [139] created dendrimers conjugated with salicylic acid as platforms for CEST. 5-Aminomethyl salicylic acid was selected as a CEST contrast agent because it has a near optimal exchange rate for detection. PAMAM dendrimers were surface-conjugated with salicylic acid, and subsequently with DOTA functions for future radiolabeling or positron emission tomography (PET)/MRI, and then the remaining amine terminals were capped with acetyl groups
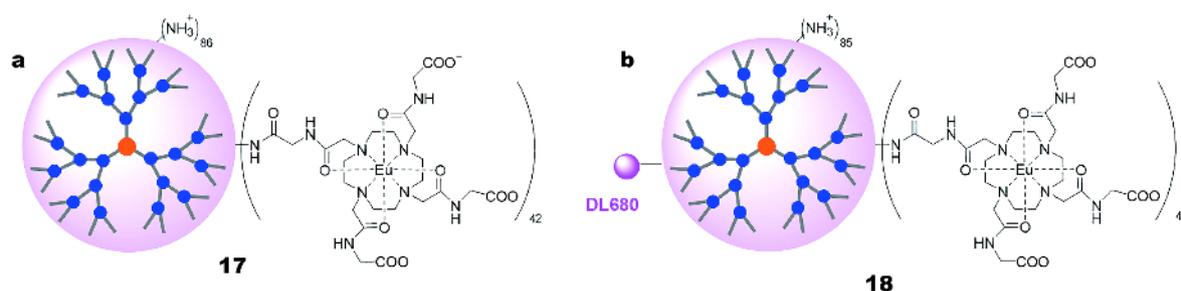
Figure 18 PAMAM dendrimers bearing (a) Eu-DOTA-Gly$_4$ [137] and (b) both Eu-DOTA-Gly$_4$ and the fluorescent dye DL680 [138] as PARACEST agents.
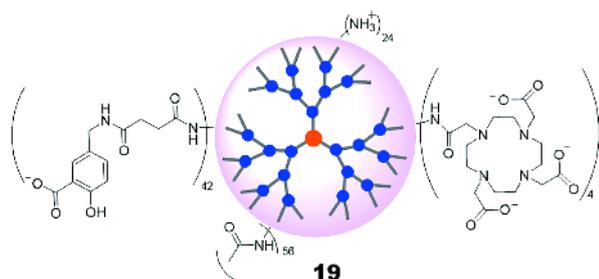

Figure 19 Salicylic acid was covalently attached to the surface of PAMAM dendrimers to generate the CEST effect in MRI [139].
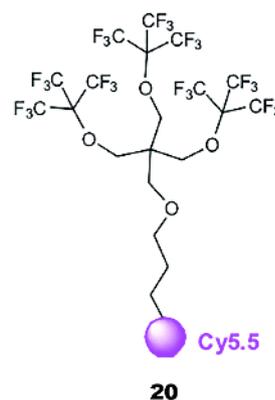

Figure 20 Bimodal dendrimer contrast agents bearing numerous equivalent fluorine nuclei at the dendrimer termini for ^{19}F-MRI and the near-infrared cyanine dye Cy5.5 at the dendrimer core for optical fluorescent imaging [140].

(Fig. 19). The resulting agents produced strong CEST contrast at 9.4 ppm from water with the proton exchange tunable from 1000 to 4,500 s^{-1}, which should facilitate sensitive detection. When these agents were used to monitor convection-enhanced delivery into U87 glioblastomas in xenograft mice, the contrast persisted for more than 1.5 h and was distributed over ~50% of the tumors [139]. This shows that salicylic acid-modified dendrimers are promising new contrast agents for medical applications.

CEST has the potential to deliver novel contrasts by using different agents based on endogenous, natural, or synthesized compounds. Importantly, for clinical applications, these agents include drugs at pharmacological concentrations. Compared to conventional MRI, CEST achieves unprecedented sensitivity at the molecular level and can potentially increase the specificity of MRI diagnoses. In addition, several PARACEST agents can be detected during a single MRI scan, which may facilitate the evaluation of multiple biomarkers. This could further increase the specificity and accuracy of MRI-based tumor detection [32].

^{19}F-MRI
Unlike ^{1}H-MRI, ^{19}F-MRI offers the unique advantage of highly sensitive imaging because of the absence of en-

dogenous ^{19}F-NMR signals, which means that there is no background signal interference during MRI recording [34,35]. Also of importance is that ^{19}F has the potential to offer quantitative MRI. Recently, Wang *et al.* [140] developed a bimodal dendritic imaging agent bearing numerous equivalent fluorine nuclei for ^{19}F-MRI and a near-infrared cyanine dye at the dendrimer core for optical fluorescent imaging (Fig. 20). There was a high correlation between the ^{19}F-MRI signal and the NIR fluorescence intensity *in vitro* and *in vivo*. This agent also enables quantitative ^{19}F-MRI, confirmed by complementary fluorescence analysis. Consequently, dendrimer-based ^{19}F-MRI agents hold great promise for quantitative and sensitive multi-modal molecular imaging.

MRI-based multimodal imaging
Dendrimer nanoplatforms are capable of accommodating large amounts of imaging agents to enhance imaging sensitivity, and, at the same time, combining several different imaging techniques for multimodal imaging to increase the precision and reliability of diagnosis and

treatment. Multimodal imaging agents have been created to combine MRI with other imaging techniques such as fluorescent optical imaging, computer tomography (CT), PET and single photon emission computed tomography (SPECT). Some of the dendrimer-based agents mentioned above such as **5**, **9**, **10**, **15**, **16**, **18** and **20** have been used for MRI and optical fluorescent bimodal imaging, because fluorescent probes enable accurate and sensitive high-contrast cellular/tissue imaging. Bimodal imaging probes integrating the high-resolution *in vivo* MRI and optical fluorescence imaging into a single dendrimer platform can overcome the limitations of conventional contrast agents and facilitate quantitative MRI and fluorescence imaging. Many dendrimer-based MRI agents have therefore been developed which carry both MRI functional entities and near-infrared fluorescent dyes such as cyanine [140], rhodamine [92,93], quantum dots [141], etc, for more accurate and sensitive multi-modal imaging [41]. Often, fluorescent imaging enables the precise delineation of tumor margins and hence more complete resection of the tumor during surgery.

CT facilitates direct quantification of the contrast agent because X-ray attenuation (i.e., signal intensity) is linearly related to the concentration of the contrast agent. Regino *et al.* [142] reported an imaging agent based on a dendrimer of generation 8 bearing Gd-DTPA function as a dual CT and MRI contrast agent for monitoring convection-enhanced delivery to the brain. Visible enhancement of both CT and MRI was observed using this agent, and MRI offered greater sensitivity than CT. Although CT provides less anatomical contrast and lower sensitivity than MRI, the dual CT/MRI agent can act as a surrogate marker for convection-enhanced delivery of macromolecular therapeutic agents to the brain.

PET and SPECT can deliver functional information with high sensitivity. However, both of them are limited by low anatomical resolution. MRI offers high-resolution anatomical images, particularly when a specific contrast agent is used. Therefore, PET and SPECT in combination with MRI have been integrated within the dendrimer platform and applied to improve the precision and accuracy of imaging and diagnosis [41]. For example, Luo *et al.* [143] developed a dendrimer platform carrying DOTA functionalities to chelate Mn^{2+} for MRI and ^{99m}Tc for SPECT imaging along with folic acid as the ligand for targeted tumor imaging. The success of the multimodal imaging approach may permit earlier and more accurate detection of tumors, as well as providing further insights into the molecular mechanisms underlying cancer development.

MRI-based theranostics

Besides carrying several probes for multimodal imaging, dendrimers can also combine imaging and therapeutic agents within the same nanoparticle to create theranostic platforms. Yang *et al.* [144] reported a PAMAM dendrimer covalently linked to gadolinium-functionalized nanographene oxide (Gd-NGO). The aim of this nanosystem was to deliver chemotherapeutic drugs and gene-targeting microRNAs (miRNAs) to cancer cells, while also carrying an MRI contrast agent to identify the location and extent of BBB openings and quantitate drug delivery to tumor tissues. The positively charged surface of the dendrimer-Gd-NGO conjugate simultaneously adsorbed the anticancer drug epirubicin (EPI) and bound to the miRNA Let-7g (Fig. 21). The so-constructed theranostic agent **21** had considerably higher transfection efficiency than nanoparticles loaded with only EPI or miRNA, and also more strongly inhibited the growth of cancer cells in human glioblastoma (U87) models. These findings indicate that **21** may be a promising non-viral vector for combining chemotherapy, miRNA delivery and molecular imaging diagnosis in clinical applications.

CONCLUSION AND PERSPECTIVES

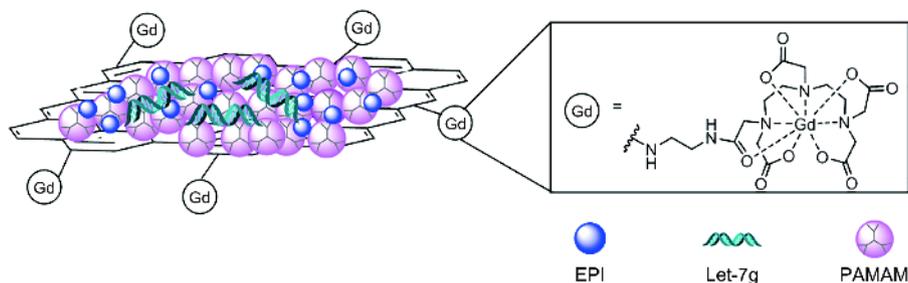Since the first clinical contrast agent Gd-DPTA was ap-

Figure 21 PAMAM dendrimer conjugated with gadolinium-functionalized nanographene oxide (Gd-NGO) entraps the anticancer drug epirubicin (EPI) and interacts with the negatively charged miRNA Let-7g as a theranostic nanosystem [144].

proved in 1988, low molecular weight agents have been crucial for improving MRI contrast. Nevertheless, macromolecular agents have been added to the arsenal in order to address the limitations of these low molecular weight agents, i.e., fast renal clearance, non-specificity and low imaging efficiency. Dendrimers, a special family of synthetic macromolecules, have generated tremendous interest as core platforms for constructing MRI agents. Dendrimers have tunable chemistry, which permits a precise "chemical makeup", and can carry a high payload within a nanosized three-dimensional space. The size and shape of the dendrimer, as well as the surface distribution of the desired functionality, can be adjusted to enhance the imaging sensitivity and impart targeted imaging. By virtue of their large molecular weight and steric hindrance, dendrimer agents have greater relaxivity than low molecular weight agents such as Gd-DPTA and Gd-DOTA. In addition, the large number of modifiable terminals and the possibility of precise chemical decoration with multiple modifications and/or target molecules, permits dendrimers to act as effective and specific agents. Surface modification includes targeting moieties for precise and sensitive molecular imaging and PEGylation to improve circulation half-lives and biodistribution. Also, combinations of different imaging techniques for multimodal imaging or integration of therapeutic and imaging modalities as theranostics within a single dendrimer can be easily realized and have been applied to brain cancer.

As previously discussed, brain cancer is particularly challenging to manage because the BBB impedes the delivery of therapeutic and imaging agents for treatment and diagnosis. Luckily, the BBB is often disrupted by the uncontrolled cancer cell proliferation and the abnormal angiogenesis in the tumor, resulting in leaky tumor blood vessels, and hence the EPR effect. These features have been exploited to develop nanosized agents for therapeutic and imaging purposes. In this vein, dendrimers with precise nanostructure and cooperative multivalence yet differing in core, branching, generation and surface composition have been investigated as MRI agents for brain cancer, and some of them show promise. To further improve the imaging quality, active targeting has been integrated into dendrimer platforms to circumvent the BBB (both compromised and intact) as well as specifically targeting brain cancer. Although targeted agents have proved to be superior to non-targeted systems in general, there is no guaranteed success, highlighting the challenge of overcoming the BBB for effective brain delivery. Dendrimer contrast agents with specific functionalities to respond to pH change, metal ions and enzyme activity

have also been explored for molecular imaging. In addition, CEST and PARACEST agents have been further developed to improve imaging specificity and sensitivity.

It is worth noting that although most dendrimer contrast agents have been constructed using commercially available dendrimers such as PAMAM, PPI and polylysine dendrimers, the potential diversity of the dendrimer core, the branching units, and the internal and surface functionalities is immense for creating dendrimer-based MRI contrast agents, multimodal imaging agents or theranostics. In addition, most investigations into dendrimer contrast agents have been performed in the context of preclinical imaging studies in animal models. Translation of the results and knowledge obtained from these model systems to the clinic lies a long way ahead. Regulatory agencies also face a number of challenging issues, such as consistent characterization and reproducible production, which are required for the real-world implementation of contrast agents with outstanding properties. Feedback from biologists and clinicians to chemists will be essential for establishing new and more effective agents which can deliver the required imaging precision and quality.

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树型分子的磁共振造影剂在脑肿瘤成像中的应用

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摘要 脑癌由于其特殊的生理位置和血脑屏障, 是一种难以治疗的致命肿瘤. 手术切除是目前脑癌的主要治疗手段, 然而成功的手术需要精确的肿瘤定位和肿瘤成像以确保完全切除癌细胞并避免伤害正常脑组织. 磁共振成像是诊断脑癌最常见的成像方法, 但通常需要造影剂来增强成像对比度和精准度. 树型分子因其独特的精准三维结构, 可调控的纳米尺寸和众多的表面官能团, 可作为构建磁共振成像造影剂的优选材料. 藉由调整树型分子的尺寸、使用不同的分支结构和表面官能团修饰 (包括磁共振成像功能团和脑肿瘤靶向基团), 可以改善造影剂的弛豫性, 克服血脑屏障并增强肿瘤靶向能力, 从而改善成像质量以获得精准的脑瘤成像, 并提高造影剂的安全性. 本文简要总结了基于树型分子的磁共振成像造影剂在脑癌成像应用中的挑战、最新进展、机遇和未来发展趋势.