Chapter 8
Dendrimers as Drug Carriers for Cancer Therapy

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Abstract The efficacy of anticancer agents is often limited due to treatment-related toxicity, poor pharmacokinetics, and inadequate drug accumulation in the tumor. Advances made in the field of cancer nanomedicine have made it possible to reduce the toxicity, alter the pharmacokinetics and biodistribution, increase site-specific drug delivery, and enhance the efficacy of many therapeutic agents by using nanoparticles as drug carriers. These nanocarriers can be composed of polymers, lipids, proteins, or inorganic materials. Among these delivery systems, dendrimers form a separate class of branched polymer nanoparticles that has shown great promise in cancer drug delivery. In this chapter, we describe the application of dendrimers as nanocarriers for drug and gene delivery in cancer. We discuss the structures, properties, and various synthesis methods for dendrimers suitable for anticancer drug delivery. Further, we describe various types of dendrimers with appropriate examples in different therapeutic modalities of cancer. Recent examples of drug and gene delivery using dendrimers and their advantages are also presented. The application of tumor-targeted delivery systems using dendrimers is described. The chapter concludes with a description of current challenges with dendrimer-based drug delivery and efforts made to bring these promising systems to the forefront of cancer treatment.

Keywords Polyamidoamine dendrimer · Poly(propylene imine) · Poly-L-lysine · Cancer · Drug delivery · Gene delivery · Receptor targeting

8.1 Introduction

Cancer is the leading cause of disease-related deaths worldwide, and its incidence is increasing. Chemotherapy is among the most successful therapeutic modality for treating cancers of different stages. However, chemotherapy delivery presents several challenges, such as unfavorable pharmacokinetic profiles, low aqueous solubility, narrow therapeutic index, poor membrane permeability, rapid clearance, instability in circulation, and concerns over emergence of multidrug resistance (MDR) phenotypes in cancer (Ozols 2006; Fuertes et al. 2008). Side effects caused by the toxicity of chemotherapy drugs are also an unresolved clinical issue, mainly because of the lack of good delivery agents.

Similarly, gene therapy is another therapeutic modality that is gaining much attention due to its efficiency and increased tumor specificity compared with chemotherapy. This approach, however, requires specific delivery vehicles for successful application against cancer. The major challenge is achieving therapeutic concentrations at the tumor sites, since most gene therapy agents lack sufficient stability in the circulation. This lack of stability affects tumor-specific delivery. Therefore, delivery of therapeutic molecules, i.e., chemodrugs and gene molecules, into targeted tumor tissue is an important issue in cancer therapy (Bae and Park 2011).

The global drug delivery research community is currently focused on developing safe and targeted drug delivery strategies for cancer. To improve the biodistribution of cancer drugs, nanoparticles have been designed for optimal size and surface characteristics to increase their circulation time in the bloodstream. Numerous delivery methods have been developed. Among them, nanocarrier-based delivery systems have shown promising results (Singh and Lillard 2009). Nanocarriers are able to carry their loaded drugs selectively to cancer cells using the unique pathophysiology of tumors, such as their enhanced permeability and retention (EPR) effect and the tumor microenvironment (Maeda et al. 2000). Various metallic (gold and iron oxide)-, lipid (liposomes)-, and polymer-based nanocarriers have been studied for delivery of therapeutic molecules in vitro and in vivo (Bayda et al. 2017; Hu et al. 2017; Wilczewska et al. 2012). Some lipid- and polymer-based nanocarriers have been approved by the Food and Drug Administration (FDA) for clinical trials (Bobo et al. 2016). Although many of these nanoparticles are extensively explored in biomedical applications, their stability and toxicity are major issues. Metallic nanoparticles are prone to aggregation when they interact with biological molecules leading to rapid clearance. Lipid-based nanoparticles tend to burst release the encapsulated drugs due to their dynamic architecture, causing undesired distribution of the drugs and resulting in nonspecific toxicity.

Dendrimers are alternative carriers that can overcome the abovementioned limitations. These carriers form a special class of drug delivery systems that can be constructed with a well-defined molecular structure providing special opportunities for drug and gene delivery (Abbasi et al. 2014). Dendrimers are polymer-based, three-dimensional, highly branched monodispersed molecules that can be synthesized by sequential and precise introduction of unique branching structure. Ultimately, dendrimers are highly branched and have well-defined globular structures with enormous surface functionality (Klajnert and Bryszewska 2001).

The size of dendrimers is less than 100 nm. The use of dendrimers as nanocarriers for chemotherapy drug may provide significant advantages, including high drug loading, enhancement of water solubility, and low cytotoxicity; these versatile dendrimers have the ability to encapsulate both hydrophilic and hydrophobic molecules (Madaan et al. 2014; Choudhary et al. 2017). Another important advantage is that dendrimers can mediate the delivery of single-stranded or double-stranded, natural or synthetic DNA or RNA of any kind and any size (Chaplot and Rupenthal 2014). Dendrimers have increased overall ionic interaction with DNA compared with natural polyamines, polylysine, and liposomes, and they produce very stable and highly

soluble DNA complexes (Mendes et al. 2017). Dendritic polymers also have a broader concentration range between transfection and cytotoxicity. It has been demonstrated that some of these polymers increased the efficiency of plasmid-mediated gene transfer in vivo. Dendritic polymers have been recently studied in targeted drug, gene delivery, and imaging studies (Noriega-Luna et al. 2014).

8.2 Dendrimer Synthesis Strategies and Characterization

Molecular and polymer chemistry concepts are involved in the design of dendrimers. Dendrimer construction requires a step-by-step controlled synthesis (molecular chemistry) and the creation of a repetitive structure made of monomers (polymer chemistry). The synthesis process follows either divergent or convergent methods, as represented in Fig. 8.1. Dendrimers possess more symmetric, globular, and closed packed membrane structures with higher generations, whereas lower-generation dendrimers have an asymmetric and more open structure. The number of surface functional groups and size of dendrimers vary based on generation. The active surface functional groups (e.g., -COOH, -OH, -NH2, -SH) can also be synthesized with different core molecules. The generation of surface functional groups and internal cavities plays an important role in loading and conjugation of drug, gene, and targeting ligands.

8.2.1 Divergent Method

In the divergent method, the structure initiates from a multifunctional core and builds up one monomer layer. The first-generation dendrimer is then created by reacting the core molecule with monomers that have one reactive group per monomer as shown in Fig. 8.1. The sequence is then continued by activation of the inactive groups at the periphery and conjugation of another generation of monomer molecules, resulting in the second-generation dendrimer. This process is repeated for multiple layers; each layer represents one generation. Higher-generation dendrimers with larger molecular diameters are usually synthesized using this method.

PAMAM dendrimers that are widely used as nanocarriers are synthesized by the divergent method. Generally, these dendrimers are composed of an alkyl-diamine core and tertiary amine branches. Their terminal groups often end with different surface-active groups, such as -OH, -COOH, and -NH_2. The divergent method is also used to synthesize poly(propylene imine) (PPI) dendrimers with EDA and DAB as core groups, which are highly studied in biomedical applications. In this method, each step of product purification and degree of purity has limitation due to smaller molecular weight and size difference between desired dendrimer and imperfect dendrimers.

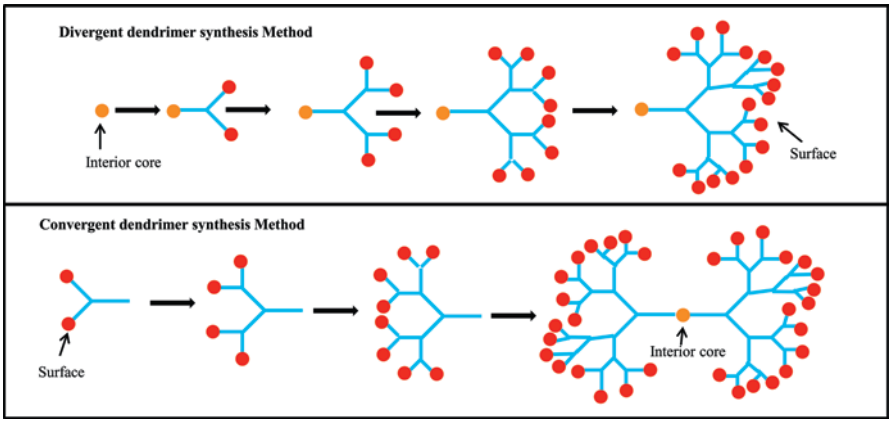

Fig. 8.1 Schematic representation of divergent and convergent dendrimer synthesis methods

8.2.2 Convergent Method

In the convergent method, dendrimer is synthesized layer by layer, but the core group terminates and the end groups react. These two (or more) peripheral branches react with a single joining unit that contains two (or more) active sites and one inactive site, as represented in Fig. 8.1. This reaction of two active sites repeats and joins with the peripheral branches. When the dendrons reach the target generation, they are then attached to a core molecule to yield the dendrimer (Xu et al. 1994; Grayson and Fréchet 2001). The molecular weight difference between desired dendrimer and its by-products is high, so it is easy to purify the required dendrimer from its by-products. Convergent method produces high-purity and homogeneity dendrimers because of lower generation and fewer reactive functional groups available. In a typical example, 5-aminolevulinic acid (ALA)-based dendrimers are synthesized by the convergent method by conjugating ALA residues to the periphery through ester linkages. Steric hindrance is generated when conjugating large dendron molecules with a small core molecule. Therefore, convergent method is useful for synthesizing lower-generation dendrimers and avoids steric hindrance.

The synthesized dendrimer structures can be characterized using different methods. The molecular weight and generation of dendrimer molecules can be identified using MALDI-TOF and ESI-MS. The chemical structure and functional groups can be identified by NMR, UV-visible, and FT-IR spectroscopy. The size and morphological properties of dendrimers can be measured by DLS analysis and TEM imaging, respectively. The internal structure of dendrimers can be confirmed with small-angle X-ray and neutron scattering and laser light scattering.

8.3 Chemistry and Structure of Dendrimers

Dendrimers are constructed with different generations; each generation increases the peripheral functional groups and cavity inside the dendrimers, as represented in Fig. 8.2. For biological applications, it is important to use biocompatible dendrimers. The most commonly used biocompatible dendrimer is polyamidoamine (PAMAM) dendrimer, which is available commercially. These PAMAM dendrimers are usually synthesized with an ammonia and ethylenediamine core. Ammonia has three branching units, while ethylenediamine has four branching units. These branching units are used to build up the different generations of dendrimers with the divergent approach, adding methyl acrylate to form amide bonds resulting from amine and ester reactions (Esfand and Tomalia 2001). The complete generations will result in amine surface functionality, whereas intermediate generations give carboxylate terminal groups to the dendrimers. The PAMAM dendrimers exist in 1–10 generations and with different surface functional groups (e.g., carboxylate, amine, alcoholic, sulfhydryl).

Another commercially available biocompatible dendrimer is poly(propylenimine) (PPI), synthesized from the butylenediamine (DAB) core molecule. The repetitive reaction is based on Michael addition of acrylonitrile to a primary amino group, followed by chemical reduction of nitrile groups into primary amino groups (Kaur et al. 2016). Another class of dendrimers based on poly-L-lysine (PLL) units, which have surface amine groups, has been explored as an antiangiogenic agent (Al-Jamal et al. 2010). PLL-based dendrimers are cationic dendrimers with amine surface groups with different generations. A PLL-based dendrimer-enhanced version of docetaxel (DTX; Taxotere®) called DEP™ docetaxel has entered Phase I clinical trials (Starpharma Holdings, Melbourne) in Australia. Starphama reported that in preclinical trials, DEP™ docetaxel showed significant tumor targeting and superior anticancer effects across a range of cancer types when compared with the clinically

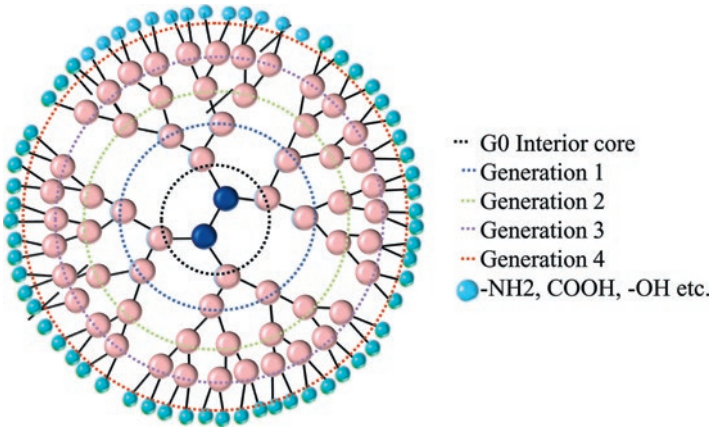

Fig. 8.2 General schematic representation of dendrimer structure. Each dotted line illustrates different generations. The chemical structures represent the surface groups of respective dendrimers

Table 8.1 Commercially available dendrimers and their surface functional groups

Dendrimer name	Surface functional group
Polyamidoamine	-OH, -COOH, -NH_2 C12 hydrophobe
Poly(propylen imine) tetramine dendrimer	-NH_2
Poly(ethylene glycol) linear dendrimer	Boc-protected amine
bis-MPA dendrimers	Acetylene, azide, Boc-protected amine, hydroxyl, carboxylic
Hyperbranched PEG dendrimers	Hydroxyl
PEG-core dendrimers	Hydroxyl, acetylene, ester
Phosphorous dendrimers	Dichlorophosphinothioyl
Poly-L-lysine	Amines
Poly(etherhydroxylamine)/poly(ester amine)	Amines, hydroxyl

approved drug, Taxotere®, a commercial Taxol formulation (Fox et al. 2009). Polyester dendrimers are another class of neutral surface charge biocompatible dendrimers that is mostly useful in drug delivery applications. It has low toxicity to normal tissues. Other chemical structures of peptide dendrimers, carbohydrate dendrimers, melamine dendrimers, and phosphorus dendrimers are also biocompatible and are being tested for various biomedical applications. Table 8.1 shows the commercially available dendrimers with various surface functional groups.

The structure and functional groups of dendrimers play a crucial role in drug and gene delivery applications. Water-soluble and biodegradable dendrimers are more biocompatible. Chemotherapeutic drugs can be functionalized with dendrimers through different methods, such as covalent conjugation, electrostatic interaction, and hydrogen bonding (Madaan et al. 2014). The surface functional groups of dendrimers (e.g., -COOH, -SH, -NH_2) can be used for direct conjugation with drugs through different chemical reactions (EDC/NHS or disulfide linkage) (Badalkhani-Khamseh et al. 2018). The drug molecules can also be conjugated with dendrimers using tumor microenvironment-sensitive linkages (hydrazone, disulfide) (Wang et al. 2016a, b). The carboxylate and amine groups possess negative and positive surface charges, respectively; those charges can be utilized to load drug molecules through electrostatic interaction.

Higher-generation dendrimers contain a cavity in their internal structure. These cavities can be utilized to load small molecules via coordinate bond or hydrogen bond formation (Choudhary et al. 2017). Nucleic acid molecules, such as plasmid DNA, siRNA, and shRNA, exhibit a negative surface charge due to their phosphate backbones. Thus, amine-functionalized dendrimers exhibiting positive surface charge can easily condense with negatively surface charged gene molecules, forming a compact complex that results in increased transfection efficiency (Palmerston et al. 2017). To increase the targeting efficiency, the dendrimers surface can be functionalized with proteins, aptamers, peptides, affibodies, and antibodies through chemical or physical interactions (Saad et al. 2008). To enhance the accumulation of the dendrimer nanoparticles in the tumor tissues, the dendrimers are usually modified with PEG molecules of different molecular weights. PEGylation prolongs the blood circulation, resulting in improved pharmacokinetics and biodistribution.

8.4 Biocompatible Dendrimers

The structural properties of size, charge, hydrophobicity, and functional groups are important parameters for in vitro and in vivo membrane and tissue interactions and biocompatibility. In vitro cationic dendrimers interact with anionic cell membranes and tissues, which causes toxicity by disturbing the cell membrane. The surface charge of dendrimers increases with increasing generations; lower-generation dendrimers are less toxic than are higher-generation dendrimers. With increasing concentration of dendrimers in the treatment, the toxicity increases (Duncan and Izzo 2005). Therefore, it is important to find the optimal tolerable concentrations of dendrimers when used in vivo.

Dendrimer toxicity is usually evaluated by hemolytic properties, membrane fluidics, in vitro cytotoxicity, and antibacterial activity. The surface modification of dendrimer with lipid molecules also results in toxicity, even at lower concentration (Albertazzi et al. 2013). Dendrimer toxicity can be reduced by choosing a biocompatible and biodegradable core and branched monomers and functionalizing active surface groups with biocompatible molecules such as PEG, amino acids, and carbohydrates.

Neutral and anionic dendrimers electrostatically cannot interact with biological tissues. Hence these dendrimers are optimal for clinical applications. An interesting study demonstrated that G4 PAMAM dendrimers show less toxicity and poor immune response and increase deeper tissue diffusion activity in the central nervous system (CNS) (Albertazzi et al. 2013). Another article reported that generation 4 and generation 8 viologen-phosphorus dendrimers are not toxic to B14 cells, but show cytotoxicity towards N2a cancer cells (Ciepluch et al. 2012). Polyester-based dendrimers degrade inside the body, which reduces the toxicity (Feliu et al. 2012). Disulfide linkage dendrimers also degrade in the intracellular environment due to the reducing nature of cells, resulting in negligible toxicity.

The *in vivo* toxicity of dendrimers resembles the *in vitro* toxicity. At lower concentrations, cationic PAMAM dendrimers shows minimal toxicity, while at higher concentrations, they cause liver toxicity. When the cationic groups are replaced with neutral polyethylene oxide, polyester dendrimers exhibit reduced *in vivo* toxicity (Jain et al. 2010).

8.5 Dendrimers for Drug Delivery

Dendrimers offer unique cavity-like structures inside their branches, enabling them to carry drugs by encapsulation. In addition, the presence of numerous peripheral functional groups in dendrimers can be utilized for conjugation of drugs. Dendrimers can carry hydrophilic drugs, hydrophobic drugs, or both (Nanjwade et al. 2009). The small size and globular architecture of drug-loaded dendrimers favor their easy permeability through vasculature pores and successful entry into the tumor milieu

for delivery of the drug payload. Thus, passively targeted conventional dendrimers use enhanced permeability and retention (EPR) effects for tumor-directed delivery of cancer drugs, which in turn reduces the exposure of normal tissues to drugs.

Paclitaxel is a hydrophobic anticancer drug. Delivery of this drug into the human body is a challenge, due to solubility and dispersibility. Dendrimers can be used as alternative carriers to deliver paclitaxel. Yang et al. utilized polyamidoamine-alkali blue (PTX-P-AB) dendrimer loaded with paclitaxel. Using pharmacokinetics, they found that PTX-P-AB dendrimer delivery increased lymphatic absorption and AUC values in lymph nodes compared with Taxol®. They concluded that PTX-P-AB was able to function as both lymphatic tracer and lymphatic targeting vector (Yang et al. 2016). Recently, another group reported that PTX conjugation was performed through enzyme-sensitive linker glycylphenylalanylleucylglycine tetra-peptide by an efficient click reaction to PEGylated peptide. This enzyme-sensitive PTX delivery increases cytotoxicity against 4 T1 cancer cells while reducing dendrimer toxicity to normal cells when compared with free PTX. In vivo studies also supported extended circulation time in tumors, showing therapeutic effects in the 4 T1 breast cancer model (Li et al. 2017).

Targeted dendrimer nanocarriers have been developed for tumor-specific delivery of drugs by modifying the dendrimers with ligands that have specific affinity toward certain cancer cell surface receptors. Anchoring poly(propylene imine) dendrimers with folate, dextran, or galactose resulted in targeted delivery of anticancer drug paclitaxel (PTX) in HeLa and SiHa cells (Kesharwani et al. 2011). However, the therapeutic efficacy of PTX was different for folate-, dextran-, and galactose-modified dendrimer formulations (IC50 values of 0.05, 0.2, and 0.8 μM, respectively). They showed that the folate-anchored dendrimer-PTX formulations had the highest targeting potential. All of these formulations showed higher cell-killing efficiency than did free PTX.

Another study demonstrated that LFC131 peptide functionalized PAMAM dendrimers encapsulated with common anticancer drug doxorubicin (Dox). Researchers studied CXCR4 receptor targeting in breast cancer cells and observed significantly increased therapeutic efficiency with targeted dendrimers over nontargeted dendrimers. Further, the targeted LFC131-PAMAM reduced migration of BT-549-Luc breast cancer cells toward chemoattractant (Chittasupho et al. 2017). In another report, peptide-based capsid-like mimic dendrimers increased tumor penetration and drug accumulation in solid tumor tissue. These Dox-loaded delivery systems facilitate capsid-like components, nanostructures, and pH-responsive controlled drug release. Capsid-like structures increased the accumulation of Dox and in vitro and in vivo therapeutic effects in 4 T1 tumor-bearing BALB/c mice while reducing toxicity (Li et al. 2016).

Dendrimer nanoparticle delivery of other classes of anticancer drugs, such as cisplatin and 5-fluorouracil (5-FU), has also been studied (Tran et al. 2013). Cisplatin and 5-FU were loaded onto neutral surface PEGylated polyamidoamine (PAMAM) dendrimer (G 3.0) and negatively charged carboxylated PAMAM dendrimer (G 2.5). The formulations of cisplatin and 5-FU showed therapeutic activity

against NCI-H460 lung cancer and MCF-7 breast cancer cell lines, respectively (Tran et al. 2013).

In a recent report, ursolic acid (UA), a natural triterpene acid, was used for suppression of tumor growth and metastasis. However, bioavailability was low due to hydrophobicity. To increase UA dispersibility, a low-polyamidoamine (low-PAMAM) dendrimer-based formulation was developed through self-assembly. This dendrimer-UA complex enhanced cytotoxicity, attenuated the migration and adhesion of SMMC7721 liver cancer cells, and suppressed metastasis. Moreover, in vivo study revealed an improvement in blood circulation time for the dendrimer-UA complex that ultimately resulted in tumor growth inhibition in a mouse model (Shen et al. 2018).

Camptothecin (CPT), a poorly water-soluble plant alkaloid isolated from *Camptotheca acuminata*, is widely used as a cancer treatment. Cheng et al. reported that PAMAM dendrimer-CPT complexes could enhance the aqueous solubility, a major issue during drug formulation, of CPT in clinical trials (Cheng et al. 2008). In another ester-linked glycine and β-alanine spacers conjugated G3.5 PAMAM dendrimer-SN38 (7-ethyl-10-hydroxycamptothecin) was used against colorectal cancer metastases. This G3.5-SN38 conjugate has advantages in that the drug is covalently conjugated to the dendrimer rather than complexed, which increases stability in gastric and intestinal environments and reduces uncontrolled release. Further, CPT delivery in HT-29 cells using G3.5-glycine-SN38 and G3.5-βAlanine-SN38 formulations showed IC50 concentrations of 0.60 and 3.59 μM, respectively (Goldberg et al. 2011).

8.6 Dendrimers for Gene Delivery

RNA interference (RNAi) is a process in which a desired gene is silenced or part of its expression is knocked down through a complementary RNA introduced into the cell. RNAi is a conserved biological process among multicellular organisms, in which double-stranded RNA is processed by the enzyme dicer into ≈21–23 bp double-stranded fragments known as small interfering RNAs, or siRNAs (Carthew and Sontheimer 2009). This process forms an "RNA-induced silencing complex" (RISC), which scans mRNAs for homology and, upon sequence-specific binding, promotes the destruction of target mRNAs through enzymatic activity integrated in the complex (Tijsterman and Plasterk 2004). In cancer, therapeutic RNAi is moderated by introducing small interfering RNA (siRNA), short hairpin RNA (shRNA), or microRNA (miRNA) complementary to the target mRNA in cancer cells. The siRNA are known to be highly specific in inactivating the targeted gene. Thus, they can act as therapeutics or can inactivate genes that can enhance the activity of a co-anticancer agent, such as a small molecule inhibitor or a chemotherapeutic. RNAi agents are vulnerable to enzymatic digestion and rapid removal from the circulation upon systemic administration.

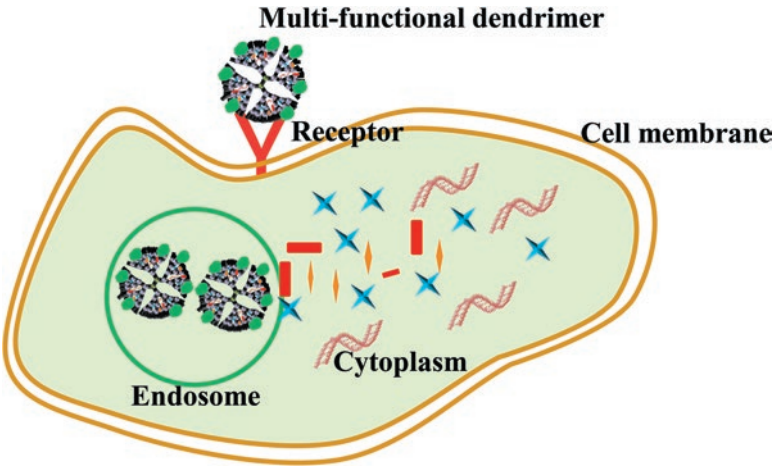

Fig. 8.3 Schematic illustration of multifunctional dendrimer uptake by cells through receptor-mediated endocytosis and entry into endosomes. After endosomal disruption, therapeutic and diagnostic molecules enter the cytoplasm

Dendrimers, due to their unique structure and cationic functionality, can readily complex with and condense nucleic acid therapeutics. They can carry RNAi agents, protect them from enzymatic digestion, and prolong the circulation time. In addition, dendrimers allow various surface modifications for targeted delivery of RNAi agents (Tambe et al. 2017). Different dendritic structures, such as polyamidoamine (PAMAM), poly(propylene imine) (PPI), poly-L-lysine, poly(etherhydroxylamine) (PEHAM), poly(ester amine) (PEA), and polyglycerol, have been used as gene delivery systems (Table 8.2). Among various dendrimers, PAMAM has been widely considered an efficient carrier for gene transfer and, recently, for RNAi delivery (Yang et al. 2015a, b). Figure 8.3 illustrates the uptake of multifunctional dendrimers by cells and the entrance of the therapeutics and image agents into the cytoplasm via endosomal escape.

The nucleic acid to dendrimer charge ratio, nitrogen to phosphate ratio (N:P ratio), and heat activation of the dendrimer are important parameters that determine dendrimer-nucleic acid complexation and efficient transfection. In a typical example, PAMAM (G4, G5) dendrimers were complexed with plasmid DNA, and their ability to transfect cells in vitro and in vivo was evaluated (Navarro and Tros de Ilarduya 2009). Compared with naked DNA, these PAMAM dendrimers were more effective in protecting DNA from DNase and transfection efficiency. The N:P ratio of 10:1 between dendrimer and DNA was optimal in inducing efficient transfection. Moreover, heat-activated dendrimer showed enhanced efficiency in transfection compared with nonactivated or intact dendrimer-DNA complexes. In vivo studies demonstrated that intravenously administered G4 and G5 heat-activated dendrimer-DNA complexes showed increased gene transfection efficiency compared with nonactivated complexes (Navarro and Tros de Ilarduya 2009).

Table 8.2 Example of dendrimers utilized for drug and gene delivery

Dendrimers	Therapeutic agent	References
Polyamidoamine	Paclitaxel	Yang et al. (2016) and Li et al. (2017)
Poly(propylene imine)	Paclitaxel	Kesharwani et al. (2011)
Polyamidoamine	Doxorubicin	Chittasupho et al. (2017)
Peptide-based capsid-like mimic dendrimers	Doxorubicin	Li et al. (2016)
PEGylated and carboxylate polyamidoamine (PAMAM)	Cisplatin and 5-fluorouracil (5-FU)	Tran et al. (2013)
Polyamidoamine	Ursolic acid (UA)	Shen et al. (2018)
Polyamidoamine	Camptothecin (CPT)	Cheng et al. (2008) and Goldberg et al. (2011)
Polyamidoamine	Plasmid DNA	Navarro and Tros de Ilarduya (2009) and Huang et al. (2007)
Amine-terminated PAMAM	siRNA	Jensen et al. (2011) and Shen et al. (2014)
PAMAM G4.0-PEG-1,2-dioleoyl-sn-glycero-3-phosphoethanolamine	siRNA	Biswas et al. (2013)
Poly(propylene imine)	siRNA	Tietze et al. (2017)

Similarly, siRNA delivery using dendrimers requires optimization of key parameters, such as the N:P ratio between dendrimer and siRNA and the generation of dendrimer used for complexation with siRNA. Jensen et al. studied various dendrimer generations to obtain optimal complexation efficiency with siRNA. They observed that dendrimer generations with low charge density (e.g., G1) lacked siRNA condensation ability. Higher efficiency in dendriplex (dendrimer-siRNA complex) formation was observed with G4 and G7 dendrimers with high charge densities. Among the dendrimer generations studied, flexible G1 and rigid G7 dendrimers displayed unfavorable thermodynamic properties. The researchers concluded that G4 dendrimer showed better dendriplex formation ability than did other dendrimers used in siRNA encapsulation (Jensen et al. 2011).

The N:P ratio plays a crucial role in forming complex and release kinetics. Although a dendrimer-siRNA complex with an appropriate N:P ratio exhibits therapeutic efficacy, it may cause toxicity. To improve safety, dendrimer-siRNA complexes can be coated with liposomes. These liposome-coated complexes are called dendrosomes. Dutta et al. reported successful delivery of siRNA targeted to E6 and E7 oncogenes in cervical cancer cells using a novel dendrosome nanocarrier DF3. In the first step, a dendrimer-siRNA complex (viz., 4D100) was optimized for transfection efficiency in cells. 4D100 was toxic to cells, but when it was encapsulated in liposomes to form dendrosomes (DF3), the toxicity was negligible. Compared with other formulations tested in their study, DF3-containing siRNAs showed considerable knockdown of the target genes (E6 and E7) in cervical cancer cells (Dutta et al. 2010).

PEGylation into dendrimers increased the transfection efficiency. Shen et al. studied G5.0 and G7.0 PAMAM dendrimers conjugated with PEG 5000 Da for the

delivery of syndecan-4-specific siRNA and caveolin-1 protein in C2C12 mouse myoblasts and the HepG2 human hepatocellular carcinoma cell line. PEGylation was increased to give the polyplexes higher syndecan-4 siRNA transfection efficiency with low immune-recognition response and cytotoxicity. The results showed a significant improvement in the cellular uptake of PEG-PAMAM dendrimer polyplexes in HepG2 with the downregulation of syndecan-4 and upregulation of caveolin-1 (Shen et al. 2014).

In another study, DOPE lipid was utilized for conjugation along with the PEG molecule. A triblock copolymeric system composed of PAMAM G4.0-PEG-1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (PAMAM-D-PEG-2K-DOPE) was synthesized and condensed with siRNA. The hydrophobicity of DOPE allows cellular interaction for enhanced cell penetration and to achieve increased siRNA condensation. The PAMAM-D-PEG-2K-DOPE micellar nanocarrier formed stable complexes with siRNA with serum stability and increased cellular uptake of siRNA, leading to better target gene knock-down when compared with the PAMAM G4.0 dendrimer. Further, PAMAM G4.0-D-PEG-2K-DOPE/PEG-5K-PE micelles showed potential for drug/siRNA codelivery (Biswas et al. 2013).

In a different study, to improve siRNA therapeutic efficiency, single-chain fragment variables (scFvs) were conjugated to poly(propylene imine) dendrimers and functionalized with maltose (mal-PPI) for siRNA delivery. Using biotin–neutravidin bridging, researchers conjugated mal-PPI with epidermal growth factor receptor variant III (EGFRvIII), monobiotinylated anti-EGFRvIII scFv fused to a *Propionibacterium shermanii* transcarboxylase-derived biotinylation acceptor (P-BAP). Compared with the control polyplex with nonspecific scFv-P-BAP, the polyplex with EGFRvIII scFv delivered siRNA exclusively toward tumor cells by receptor-mediated endocytosis. The authors concluded that the use of EGFRvIII scFv-modified mal-PPI-based polyplexes is an effective strategy for tumor targeted delivery of siRNAs (Tietze et al. 2017) (Table 8.2).

8.7 Dendrimers for Receptor-Targeted Delivery

Cancer cells often overexpress specific receptors. By exploiting the receptor–ligand affinity, drug delivery systems can be modified using specific ligands for those receptors to achieve cancer cell-targeted drug delivery. Receptor-targeted drug delivery has been extensively explored for active targeting. However, active targeting is achieved with a carrier surface functionalized with active targeting ligands that have high binding affinity towards a specific cell type, tissue, or organ. Some targeted delivery systems are currently in clinical trials (Vhora et al. 2014). To improve the specificity of dendrimer nanocarrier systems, ligands specific to cancer cell receptors can be conjugated. Commonly used ligands include transferrin, folic acid, peptides (e.g., Arg-Gly-ASP), and aptamers.

Transferrin (Tf) is an iron-chelator protein and has affinity toward transferrin receptors (Tf-R), which are overexpressed by many cancers. This ability of cancer cells allows them to internalize Tf in high levels compared with levels in normal

cells. Tf-conjugated targeted dendrimer drug delivery systems exploit these high Tf levels. A typical example of Tf-ligand-based targeted dendrimer drug delivery was reported by Huang et al. (2007). They constructed a high-branching nanoscopic PAMAM dendrimer conjugated with Tf using a bifunctional polyethylene glycol linker and tested the uptake in brain capillary endothelial cells (BCECs) and in Balb-C mouse brains. The transfection efficiency of the PAMAM-PEG-Tf/DNA complex was much higher than that of PAMAM/DNA and PAMAM-PEG/DNA complexes in BCECs. The luciferase activity obtained from DNA complexed with PAMAM/Tf complex was 2.25-fold higher than that from DNA complexed with PAMAM in mouse brains after IV administration. At the 10:1 weight ratio of PAMAM/DNA, Tf gene expression for the PAMAM-PEG-Tf/DNA complex was approximately twofold higher than that of the PAMAM/DNA and PAMAM-PEG/DNA complexes in the brain (Huang et al. 2007).

Integrins (e.g., integrin alpha-v beta-3) are overexpressed in activated endothelial cells, newborn vessels, and some tumor cells, but are not present in resting endothelial cells and most normal organ systems. Arg-Gly-Asp (RGD) peptide specific to integrins has also been explored for targeted dendrimer-based drug delivery toward tumor neovasculature and tumor cells. Kong et al. studied the use of cyclic-RGD peptide c(RGDfK) ligand for targeted delivery of PAMAM by modifying PAMAM G4.0–25% C12 with fluorescein isothiocyanate. RGD-modified PAMAM showed significantly higher cellular uptake than did non-RGD-modified PAMAM, as confirmed by fluorescence microscopy assay with 22RV1 cells. PEGylation successfully reduced the toxicity of PAMAM in 22RV1 cells with high expression of integrin alpha-v beta-3. No apparent toxicity was observed with the modification with c(RGDfK). Drug release was observed in targeted tumor sites, and the therapeutic efficiency of 10-hydroxycamptothecin was enhanced with RGD-modified dendrimer delivery in 22RV1 cells and MCF-7 cells compared with their non-RGD counterparts (Kong et al. 2014).

Folic acid is a well-known ligand for selective targeting of drugs into folate receptor-positive tumor cells. Conjugating folic acid molecules to dendrimers allows them to target tumor cells with folate receptor expression to enhance the therapeutic efficiency of the drug. Jain et al. (2014) reported the use of FA-conjugated poly-L-lysine (PLL) dendrimers (FPLL) to which the water-soluble drug doxorubicin (Dox) was conjugated through a pH-sensitive linker. FPLL showed significant antiangiogenic activity in human umbilical vein endothelial cells (HUVEC), compared with nontargeted dendrimer. When compared with free Dox, the FPLL formulation showed higher levels of accumulation in MCF7 xenografts in a mouse model and enhanced therapeutic activity, leading to significantly prolonged survival (Jain et al. 2014).

FA-conjugated dendrimers are also under study for the construction of multifunctional architectures for targeted cancer drug delivery and as diagnostic tools. Two or more functional end groups can be introduced to dendrimers to allow conjugation with multiple agents, such as drugs, genes, fluorescent dyes, MRI agents, and targeting ligands (Fig. 8.4). A recent study demonstrated the use of folic acid (FA)-conjugated PAMAM G5.0 dendrimers as a platform for constructing a

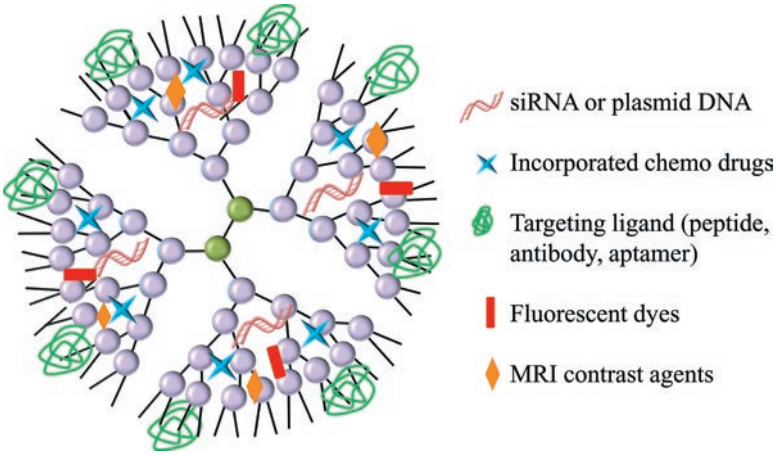

Fig. 8.4 Schematic representation of multifunctional dendrimer nanoparticles functionalized with therapeutic molecules (drugs and genes), diagnostic agents (fluorescent dyes and MRI agents), and targeting ligands

multifunctional theranostic system for targeted cancer imaging and therapy (Zhu et al. 2014). PAMAM-entrapped gold nanoparticles were covalently conjugated with fluorescein isothiocyanate, PEG-modified-a tocopheryl succinate, and PEGylated folic acid (Au-TOS-FA-DENPs). In vitro cellular uptake assay and flow cytometric study with U87MG and L929 cells showed that the conjugate could be specifically delivered to cancer cells that overexpressed folic acid receptors via receptor-mediated binding and endocytosis. In CT imaging, the CT value of U87MG with high expression of folic acid was significantly enhanced with Au-TOS-FA-DENPs. Further, a significant decrease in U87MG-HFAR cell viability was observed when treated with Au-TOS-FA-DENPs compared with the nontargeted counterpart. An in vivo study reported no toxic side effects and obvious tumor inhibition in BALB/c nude mice (Zhu et al. 2014).

Aptamers are an attractive class of ligands that exhibit many desirable properties for constructing a targeted drug delivery system. These ligands are short, single-stranded oligonucleotides obtained through the process of systematic evolution of ligands by exponential enrichment (SELEX). These oligonucleotides are known for their high binding affinity and target specificity, low immunogenicity, and versatile synthetic accessibility. Aptamer–dendrimer bioconjugates have been created for targeted delivery of chemotherapeutic drugs and gene silencing agents for cancer therapy. Recently, one report demonstrated that AS1411 aptamer functionalized on PAMAM dendrimer, which conjugated with 10-bromodecanoic acid (10C) and 10C-PEG. These aptamer-functionalized dendrimers targeted nucleolin ligand and specifically knocked down Bcl-xL protein with shRNA plasmid delivery. The aptamer-modified dendrimer significantly improved the transfection efficiency when compared with the nontargeted dendrimer in A549 cells. This improved transfection efficiency led to increase gene silencing and apoptosis (Ayatollahi et al. 2017).

MicroRNA delivery has been explored using aptamer conjugated-dendrimer systems. In a recent study, micro-RNA-34a (miR-34a), a potent endogenous tumor suppressor in NSCLC, was encapsulated into S6 aptamer-conjugated dendrimer to form a lung cancer-targeted gene delivery system (PAM-Ap/pMiR-34a NPs). The aptamer conjugation to PAM significantly improved the pMiR-34a cellular uptake and transfection efficiency in NSCLC cells. They showed that PAM-Ap/pMiR-34a NPs enhanced the regulation of targeted genes BCL-2 and p53 in vitro. PAM-Ap/pMiR-34a NPs significantly inhibited the cell growth, migration, and invasion and induced significant apoptosis of lung cancer cells compared with nontargeted NPs (Wang et al. 2015).

Recently, we demonstrated that FA-conjugated PAMAM dendrimer can be successfully used for folate receptor alpha (FRA)-targeted combinatorial delivery of CDDP and HuR siRNA for lung cancer therapy. Here, CDDP and HuR siRNA were encapsulated through a hydrolysis method and electrostatic interactions, respectively. Further, folic acid was conjugated to the surface of dendrimer functional groups for FRA-targeted delivery. The combinatorial delivery showed significant enhancement in therapeutic activity of CDDP and HuR siRNA in nonsmall cell lung cancer cell lines (H1299 and A549). Normal lung fibroblast cells (MRC9) that had low FRA expression levels did not show significant toxicity with our formulation. The FRA-targeted combined delivery also showed significantly higher therapeutic efficiency of CDDP and HuR siRNA than did nontargeted delivery (Amreddy et al. 2017).

Despite these promising outcomes, there are some challenges in using targeted dendrimers for drug delivery. First, a clear understanding of the differential expression and accessibility of cell surface receptors in the target cancer is required. Second, the ligand density in the dendrimer should be optimal for efficient interaction with the cell surface receptors. The proper choice of ligands and ideal conjugation chemistry will increase the target specificity. For example, aptamers should be carefully designed to avoid any multimerization interactions with drugs, as maintaining proper confirmation of aptamers after conjugation with dendrimers is crucial in determining the receptor specificity. Another important step is the choice of conjugation chemistry between ligands and the dendrimer host that should control the stability and release of ligand appropriately in the in vivo environment.

8.8 Dendrimer–Light Interaction Therapies

8.8.1 Photodynamic Therapy

Photodynamic therapy (PDT) is a type of phototherapy that kills bacteria, fungi, and viruses. It is used in treatment of skin, head, and neck, lung, and bladder cancers. PDT involves three components: photosensitizer (PS, a chemical substance), light, and reactive oxygen species (ROS). The PS is administered into the desired tissue and followed by irradiation with NIR light with the specific wavelength of excitation of the PDT agent (PS). Irradiation will cause generation of ROS in the presence

of molecular oxygen. This kills cancer cells, as ROS damages the cell membrane and organelles, resulting in cell death. Most potential PS molecules are not solubilized in aqueous media; when administered into the body, PS molecules interact with normal tissues resulting in nonspecific toxicity. The targeted delivery of PS molecules which can be achieved through nanocarrier-based delivery is an alternative. Dendrimers are one of the best carriers of PS molecules.

PS molecules can be encapsulated into dendrimers by methods such as electrostatic, physical interaction, and covalent conjugation. In a typical example, the anionic and cationic phosphorus dendrimer were used to encapsulate methylene blue and rose bengal (RB) PSs through electrostatic and π–π interactions, respectively. These polyanionic and polycationic combinations resulted in better stability and therapeutic effect, and identified to be better PDT carriers (Dabrzalska et al. 2015). Encapsulation of PS by physical interaction can occur in organic and aqueous solvent mixtures at different ratios. The RB PS physically interacts with PAMAM dendrimer when incubated in a mixture of methanol and water. The resulting PAMAM–RB dendrimer showed improved phototoxicity in mouse lymphoma cell lines (Karthikeyan et al. 2011). The photosensitizer chlorin e6 (Ce6) covalently conjugated to G4.5 PAMAM dendrimers showed enhanced photodynamic therapeutic effect than did free Ce6 (Bastien et al. 2015). Another approach implemented a PAMAM dendrimer-PS complex in image-guided PDT (Yang et al. 2015a, b). In this approach, the Ce6 PS-conjugated polyethyleneimine-PEGylated ceria nanoparticles increased solubility and stability of Ce6 PS in an aqueous environment. Furthermore, the enhanced cellular uptake and therapeutic effect of Ce6 was observed by image-guided therapy approach.

Receptor-targeted PS delivery also improves therapeutic effect in cancer treatment and reduces the toxicity to normal tissues. A study reported that HER2 peptide and 5,10,15,20-tetrakis(4-hydroxyphenyl)-21H,23H-porphine (PS) were covalently conjugated to the PAMAM dendrimer. This HER2 receptor-targeted PS delivery increases cell uptake and PDT effects in HER2 overexpressed SKOV3 ovarian cancer cells in vitro and in vivo compared with when administered in HER2-negative expressing MCF7 cell line (Narsireddy et al. 2015).

The PEGylation of dendrimers improves dispersibility and prolong circulation that improves cytotoxicity. The PEGylation of PAMAM and PPI dendrimers also increases protoporphyrin-IX stability in physiological conditions resulting in higher cytotoxicity in the desired tissue (Kojima et al. 2007). Dendrimers in other forms of complex called micelles also increase the PDT effects. The polyanion dendrimer porphyrins (DPs) complexed in other micelles composed with PEG-b-P(Asp) effectively delivers PS, leading to enhanced PDT efficacy (Stapert et al. 2000).

8.8.2 Photothermal Therapy

Photothermal therapy (PTT) involves a photo-activating agent that will generate heat upon light irradiation to kill cancer cells. Unlike PDT, PTT does not generate singlet oxygen. Normal tissues can bear up to 42 °C heat, whereas cancer tissues

cannot tolerate this temperature. Some of the nanoparticles are inherent to act as photothermal agents. Metallic-based gold nanorods, gold nanoshells, and graphene oxide materials are the typical examples for photothermal agents. For effective delivery and treatment, these nanoparticles can be combined with polymer-based nanoparticles. Gold nanorods absorb the radiation in the near-infrared region, whereas gold nanoparticles absorb at the visible region. Hence, nanorods show better therapeutic efficiency in photothermal therapy. The dendrimer-stabilized gold nanorods show better photothermal effects than do dendrimer-stabilized gold nanoparticles.

The safety of photothermal agent development is also an important parameter in terms of clearance from the body, which requires photothermal agents small in size and effectively efficient. Wang et al. developed ultrasmall-Au DSAuNRs, which showed better safety and higher photothermal affect in vivo (Wang et al. 2016a, b). Photothermal ablation can also be utilized to deliver other therapeutic molecules (genes and drugs), to further increase the controlled therapeutic effect. Zhang et al. described PAMAM dendrimers grown onto mesoporous silica-coated gold nanorods via a divergent method. The dendrimers were loaded with siRNA and Dox therapeutics. Upon NIR light irradiation, siRNA and Dox were released along with the PTT effect, resulting in multitherapeutic effects in cancer cells and reduced toxicity to normal cell lines (Zhang et al. 2017). The CTAB is known to be toxic in higher concentrations; replacing CTAB surfactant combined with dendrimer reduces the toxicity to normal tissues. Li et al. synthesized gold nanorods by CTAB and replaced the CTAB with PAMAM dendrimer, followed by conjugation with arginine-glycine-aspartic acid (RGD) peptides for targeted delivery. They reported selective photothermal effects in the $\alpha_V\beta_3$ overexpressing A375 cell line compared with MCF7 cell lines expressing less $\alpha_V\beta_3$ (Li et al. 2010).

8.8.3 Boron Neutron Capture Therapy

Neutron capture therapy is a noninvasive therapy used for localized tumor treatments. It is mainly useful for brain and head and neck tumors. The mechanism involves a two-step process. First, nontoxic neutron-capturing agents are administered into tumor. Usually, boron-10 (^{10}B) and gadolinium-157, 155 ($^{157, 155}$ Gd) metals act as neutron-capturing agents. This step is followed by irradiation with a neutron beam on targeted tissue. Then those metal substances convert into radioactive substances that decay helium and energy as by products. The energy thus generated helps to kill the cancer cells. For effective administration of ^{10}B into targeted tissue, nanocarrier systems are useful tools (Laramore et al. 1994; Aromando et al. 2009).

Polymer-based dendrimer nanoparticles are also used in boron neutron capture therapy for cancer. Different generations of PAMAM dendrimers are utilized for ^{10}B delivery. The ^{10}B molecules can be conjugated with PAMAM dendrimers via different chemical methods. Barth et al. reported that the boronated molecule isocyanato

polyhedral borane [Na(CH3)3NB10H8NCO] was conjugated to second and fourth generation of amine-terminated PAMAM dendrimers (Barth et al. 1994). In further studies, boronated dendrimers were conjugated with SPDP-derivatized MoAb to improve the tumor accumulation in the therapeutic range. Another report demonstrated that fifth-generation PAMAM dendrimer was conjugated with boronated molecules and chimeric MoAb cetuximab, which directs to EGFR- and EGFRvIII-expressed brain tumors (Wu et al. 2004).

PEG conjugations on dendrimers will effect pharmacodynamic properties. Receptor-targeted boron delivery further increases the boron accumulation and BNCT effect and reduces the uptake by reticuloendothelial system. Shukla et al. conjugated boronated poly(ethylene glycol) (PEG) with third-generation PAMAM dendrimers. The PEGylated boronated complexes showed more tumor uptake than did the corresponding non-PEGylated dendrimers (Shukla et al. 2003).

8.9 Dendrimers in Imaging and Diagnosis

Dendrimer molecules do not have inherent diagnostic and imaging properties. To create an imaging and diagnostic tool with dendrimer molecules, dendrimers are externally loaded or encapsulated with imaging and diagnostic agents. Usually, gadolinium (Gd III) and superparamagnetic iron oxide (SPIO) nanoparticles provide magnetic resonance imaging (MRI) contrast. The loading of these agents is useful for tracking dendrimers inside the body. Gadolinium-diethylene triamine pentaacetic acid (Gd-DTPA) and gadolinium-tetraxetan (Gd-DOTA) chelates conjugated with different generations of dendrimers increase MRI signal intensity (Zhu et al. 2008; Rudovský et al. 2006). Higher-generation dendrimers will give better MRI contrast than will lower-generation dendrimers, since more metal chelates can be conjugated to higher-generation dendrimers.

Another important imaging technique is X-ray computed tomography (CT). Iodinated and gold nanoparticle-based agents are being used in CT imaging (Cormode et al. 2014). In a typical example, different generations of G3, G4, and G5 dendrimers with amine surface groups were conjugated with tri-iodophthalamide agents. This approach showed improved in vivo imaging with intravascular enhancement and a half-life of 35 min with G4 dendrimers (Fu et al. 2006).

Optical imaging has numerous advantages for tracking and detecting disease. For optical imaging, organic structure-based fluorescent molecules, fluorescein isothiocyanate (FITC), GFP, Cy3-fluorophores, Alexa Fluor 594, and so forth can be conjugated or load with different generations of dendrimer molecules that are utilized for cell uptake studies (Koyama et al. 2007; Waite and Roth 2009). Radioactive materials can also be conjugated with dendrimers for diagnosis and treatment. The conjugated radioactive dendrimer complex can be irradiated with gamma rays and imaged with single photon emission tomography (SPECT) and positron emission tomography (PET) (Xing et al. 2018; Zhao et al. 2017). The dendrimers can be encapsulated with metal-based nanoparticles that can be visualized inside the tissue

through transmission electron microscopy (TEM). High-density gold (Au) and silver (Ag) nanoparticles are easily visualized by TEM to locate dendrimer molecules inside the cells (Vasile et al. 2014; Kéki et al. 2000).

8.10 Conclusions

Dendrimers are demonstrated to provide an excellent platform for drug and gene delivery for cancer therapy. The unique and highly defined structure, characterized by the presence of many functional groups, allows dendrimers to incorporate or conjugate multiple agents, including chemotherapeutics, nucleic acid therapeutics, image contrast agents, and targeting ligands. Despite the promising use of dendrimers as nanoparticle drug delivery systems, the use of dendrimers in the clinic has not been successful. This is because cationic PAMAM dendrimers are more toxic than their anionic counterparts. Larger dendrimers are more toxic than smaller dendrimers of similar surface functionality. However, techniques such as PEG modification have been employed in masking cationic residues with neutral groups that improve the tolerability of PAMAM dendrimers and their uptake by the cells. With further understanding of dendrimers' molecular characteristics, interaction with biological membranes, toxicity profile, and improved synthesis, it is hoped that cancer therapy applications may be realized in the near future.

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