



This review exemplifies the latest progress in dendrimer-mediated targeted drug and gene delivery to cancers.



Recent advances in dendrimer-based nanovectors for tumor-targeted drug and gene delivery

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Advances in the application of nanotechnology in medicine have given rise to multifunctional smart nanocarriers that can be engineered with tunable physicochemical characteristics to deliver one or more therapeutic agent(s) safely and selectively to cancer cells, including intracellular organelle-specific targeting. Dendrimers having properties resembling biomolecules, with well-defined 3D nanopolymeric architectures, are emerging as a highly attractive class of drug and gene delivery vector. The presence of numerous peripheral functional groups on hyperbranched dendrimers affords efficient conjugation of targeting ligands and biomarkers that can recognize and bind to receptors overexpressed on cancer cells for tumor-cell-specific delivery. The present review compiles the recent advances in dendrimer-mediated drug and gene delivery to tumors by passive and active targeting principles with illustrative examples.

Margins of conventional anticancer therapy

Cancer is one of the world's most distressing diseases with no apparent cure in sight for several tumor types and millions of new cases reported every year [1]. Cancer is principally a disease of cells identified by the loss of normal cellular growth, maturation and multiplication leading to disturbance of homeostasis. The newly 'mutated' cancer cells begin multiplying uncontrollably; they can become parasitic and develop their own network of blood vessels to siphon nourishment away from the body's blood supply. The continuation of this process ultimately leads to the formation of a cancerous tumor, which in several cases becomes multidrug resistant (MDR) with the ability to proliferate and metastasize to distant organs and tissues within the body [2–4].

Cancer chemotherapy using conventional anticancer agents has been mired by several challenges such as unfavorable pharmacokinetic profiles, low aqueous solubility, narrow therapeutic index, poor membrane permeability, rapid clearance, instability, severe toxicity concerns and emergence of MDR phenotypes. These shortfalls call for exploration of advanced carrier systems that can, in part, mitigate some of the drawbacks associated with free-drug administration and

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facilitate tumor-targeted drug or gene delivery. In this regard, a wide range of organic and inorganic nanoconstructs such as polymeric nanoparticles, liposomes, polymeric micelles, dendrimers, solid-lipid nanoparticles, silica nanoparticles and carbon nanotubes with their massive structural diversity, tunable physicochemical properties and function can be utilized to enhance drug loading, protect the payload in transit and enable drug internalization in target cancer cells while limiting uptake in normal tissues and cells [5,6].

The development of smart cancer treatment approaches revolves around engineering such unique nanosystems carrying drug and gene payloads that can passively and/or actively target cancerous cells [7]. In this regard, a passive targeting approach is identified by accumulation of drug or drug-carrier system at a particular site as a result of the inherent pathophysiological, physicochemical or pharmacological factors [8–10]. However, an active targeting approach is identified by specific modification of drug or gene carriers with active ‘homing’ ligands that have high affinity for binding to a specific cell type, tissue or organ in the body [3,11–13] (Fig. 1). Several such delivery systems are currently under intense scrutiny and some have already made it into clinical phase trials, owing to their favorable preclinical outcomes [11,14]. In addition, unconventional alternatives are currently being explored for achieving better clinical responses [15–20]. In this review, we will focus our attention on the recent advances in dendrimer-based nanodelivery systems for targeted cancer therapy with examples.

Dendrimers as an emerging vista in anticancer therapy

In the current scenario, development of an ideal delivery system for cancer chemotherapy is extremely challenging for formulation

scientists as well as clinicians because of numerous limitations of anticancer agents, as noted above. In addition, gene therapy and newer molecular-target-based anticancer tactics involve use of potent but highly labile agents such as monoclonal antibodies, aptamers, siRNAs and miRNAs that are readily degraded and/or have limited stability *in vivo* [21]. More importantly, poor biodistribution and unfavorable pharmacokinetics of conventional anticancer agents lead to poor therapeutic response and adverse side effects involving healthy organs [22–24]. To overcome these limitations there is an urgent need for devising safe and effective carrier vectors that can protect the payload from degradation during transit, enhance targeting efficiency, optimize drug release profiles and reduce the adverse toxic effects caused by nontarget-organ accumulation of cytotoxic drugs. Such agents can also help tune the dosing regimen and ultimately improve patient compliance. Along these lines, several novel carrier systems are now available for anticancer therapy [25–30]. Among them, dendrimers are emerging as a favorable choice for delivery of a wide range of anticancer drugs and genes because of their unique properties such as high loading ability, appropriate nanosize, predictable release profile, favorable pharmacokinetics and targeting potentials [31] (Fig. 2).

Dendrimers are nanosized (1–100 nm) globular macromolecules with a unique architecture consisting of three distinct domains: a central core, a hyperbranched mantle and a corona with peripheral reactive functional groups [32]. Dendrimers can be conveniently fabricated by convergent or divergent synthesis [33,34]. The high level of control over the synthesis of dendritic architecture makes dendrimers a nearly perfect (spherical)

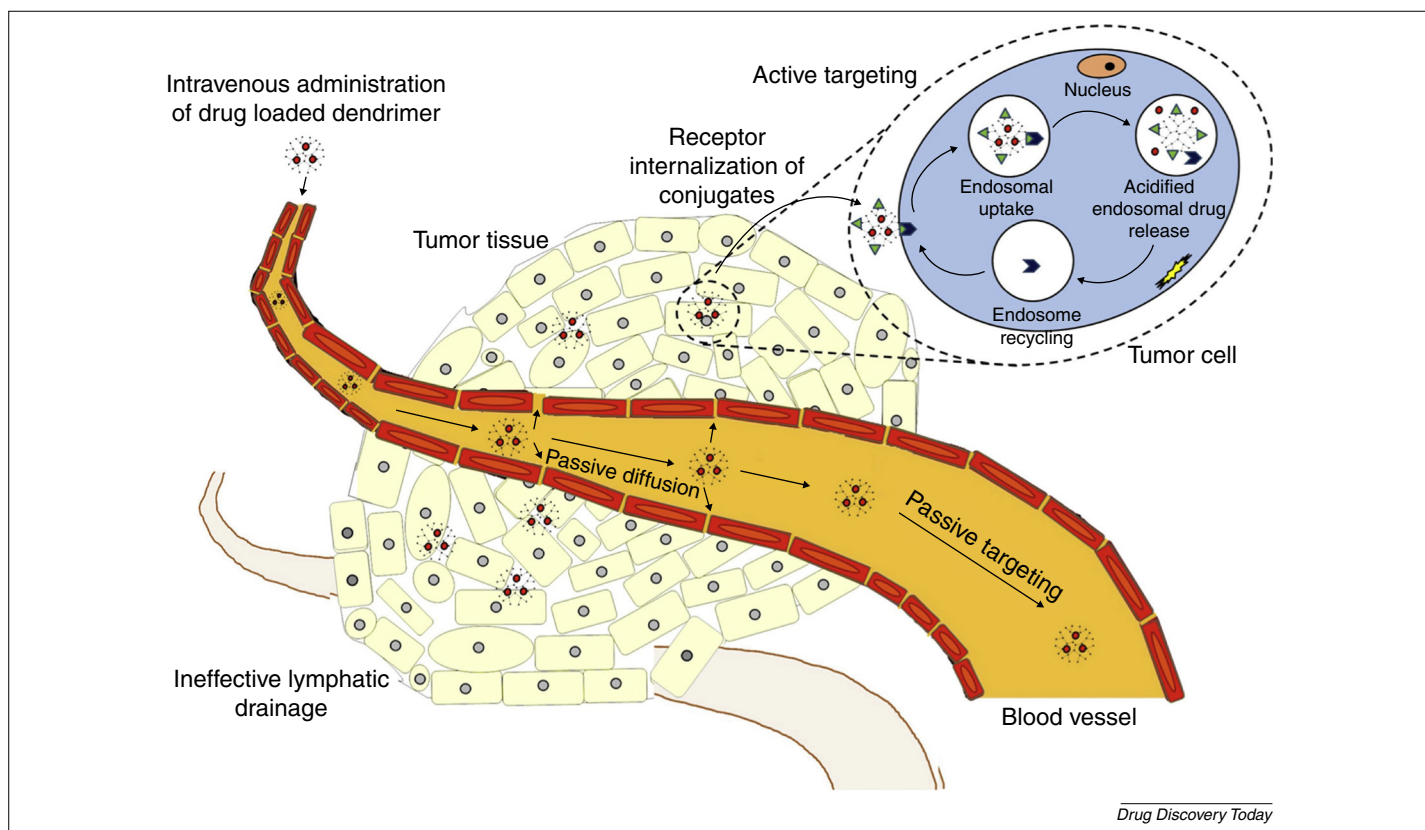


FIGURE 1

Dendrimer-mediated active and passive targeting approaches.

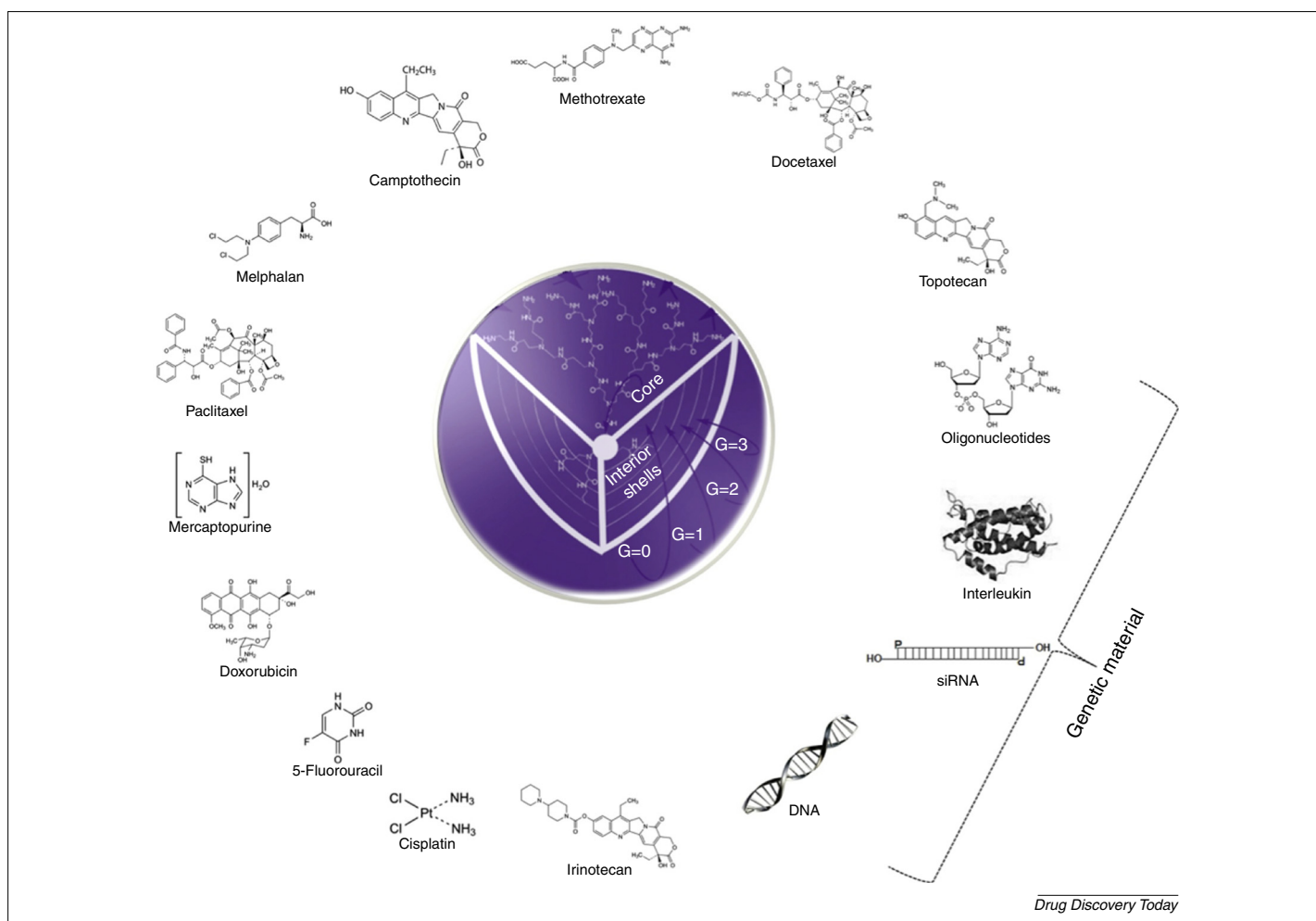


FIGURE 2

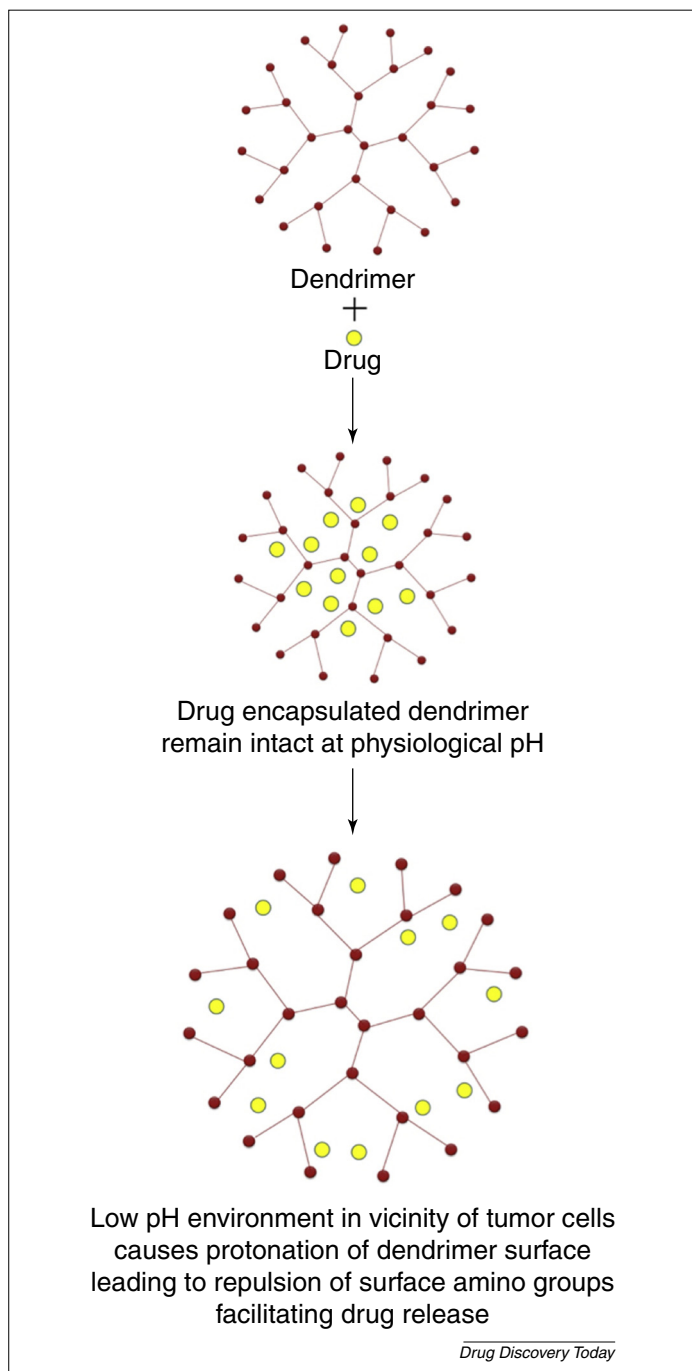
An overview of anticancer drug delivery based on the dendrimer platform.

nanocarrier with predictable properties. Numerous classes of dendrimers including polyamidoamine (PAMAM), polypropyleneimine (PPI), poly(glycerol-co-succinic acid), poly-L-lysine (PLL), melamine, triazine, poly(glycerol), poly[2,2-bis(hydroxymethyl)propionic acid] and poly(ethylene glycol) (PEG), as well as carbohydrate-based and citric-acid-based ones, have been developed for drug delivery [35–40]. Among them, PAMAM- and PPI-based dendrimers have been some of the most widely investigated vectors that have gained tremendous attention [41–43]. Importantly, amine-terminated dendrimers like PAMAM and PPI display stimuli-responsive (pH-dependent) drug release behavior. For instance, in the case of amine-terminated dendrimers, at high (alkaline) pH the tertiary amine groups are deprotonated, causing a collapse of the dendrimer on itself, which is known as ‘back folding’. Under these circumstances the dendrimers can trap large amounts of drug molecules within their cores, resulting in compaction of dendrimer architecture. However at low (acidic) pH the interior tertiary amine groups are protonated, leading to repulsion of charges. This charge repulsion results in an ‘extended conformation’, leading to apparent swelling of the dendrimer causing sustained and slow release of the entrapped drug. In the case of tumor delivery, it is important to note that the tumor microenvironment is known to be slightly acidic and thus targeted delivery

of dendrimers to the tumor tissues can be beneficial for sustained release of drugs for long-term cancer therapy [44] (Fig. 3).

Dendrimer-mediated passive targeting tactics

The utility of dendrimers can be appreciated by their ability to traverse several delivery barriers using two overarching principles; namely active and passive tumor targeting. The so-called passive targeting utilizes the inherent ability of macromolecules, liposomes and nanosized particles such as polymeric nanoparticles and dendrimers to extravasate and accumulate selectively in the tumor tissues based on a phenomenon called the enhanced permeability and retention (EPR) effect. The EPR phenomenon was discovered by Matsumura and Maeda more than three decades ago, while they were experimenting on the first polymer-conjugated anticancer drug SMANCS [9]. As discussed earlier, tumor cells divide and multiply at exponential rates and as a result develop complex networks of blood vessels that are highly disorganized, aberrant and ‘leaky’ toward blood flow. Furthermore, solid tumors in general have dysfunctional lymphatic clearance [45–48]. In addition, tumor cells secrete excessive levels of vascular permeability mediators that facilitate dilation of blood vessels [10,45,46,49]. The anatomical and pathophysiological abnormalities in tumor tissues in conjunction with overproduction

**FIGURE 3**

Mechanism of pH-dependent drug release via a dendritic platform.

of permeability mediators lead to extensive leakage of blood plasma components, macromolecules and nanoparticles including dendrimers into the tumor interstitium [10,45,48,50]. In general, it is observed that drugs conjugated to polymers (polymer-drug conjugates), as well as drugs and genes encapsulated in liposomes, polymeric nanoparticles, dendrimers and polymeric micelles, are all capable of exploiting this unique phenomenon to accumulate selectively in solid tumors [19–23]. As a consequence of the EPR effect, it is thus possible to attain very high local concentration of the drug-loaded dendrimers in the tumor tissues with negligible accumulation in nontarget-organs and -tissues.

The size range of all nanocarriers is crucial in dictating localization and retention. In this regard, it has been observed that nanoparticles in the range of tens of nanometers to a few hundred nanometers are capable of accumulating in tumor tissues via the EPR effect [51]. In general, dendrimers having a typical size range of 10–20 nm are favorable for passive tumor targeting [52]. In addition, binding of dendrimers to high molecular weight plasma proteins and biomolecules could possibly help prolong circulation half-life in the blood and avoid renal excretion. In effect, these nanosystems can reside in the blood for extended duration and accumulate in the tumor by the EPR effect, offering sustained and controlled delivery [53]. For example, in one study, it was observed that the tumor accumulation of cisplatin loaded in 3.5G PAMAM dendrimers could be enhanced as much as 50-times compared with free-drug administration. This enhanced tumor accumulation could be attributed to passive targeting by the EPR effect [54]. In the same way, another study reported enhancement and retention of a contrast agent in the tumor tissues when loaded in PEGylated lysine dendrimers as a result of the EPR effect [52,55].

Dextran is an inert, biocompatible, biodegradable, nonimmunogenic macromolecule polysaccharide. After conjugation of dextran with dendrimers the nanoconstructs could be explored as a potential module for passive tumor delivery. In addition, the high molecular weight of dextran prevents its renal excretion and prolongs its circulation half-life. Along these lines, in a recently reported study, Agarwal *et al.* explored the potential of a dextran-conjugated fifth-generation PPI dendrimer for selective delivery of doxorubicin (DOX) against lung epithelial cancer cell lines (A549). Compared with free drug, the developed formulation was found to be more potent toward A549 lung cancer cells. This result might be caused by the extended circulation time (EPR effect) of the dextran-conjugated PPI dendrimers, which facilitated tumor-specific DOX delivery [56].

Dendrimer-mediated active targeting tactics

Passive tumor targeting in general has numerous limitations because of the anatomic and pathophysiological barriers presented by the *in vivo* biological environment. In addition, diffusion of several drugs and genes can be complex and inadequate. For instance, the passive diffusion of drugs and genes into cancer cells and intracellular delivery (without active transport) can be highly challenging. More importantly, cancer chemotherapy can fail or lead to further complications as a result of suboptimal drug dose reaching the target cancer cells, eventually leading to formation of MDR cancers [57]. Moreover, a passive targeting approach or the EPR effect is only applicable to highly permeable solid tumors. However permeability of several tumor types is very low or non-uniform throughout heterogeneous tumors and these impermeable or hypopermeable tumors might not exhibit the EPR effect [58]. These shortcomings can be resolved to some extent by utilizing active targeting; a phenomenon where conjugation of specific targeting ligands on nanocarrier surfaces can facilitate their selective binding to overexpressed receptors on specific tumor cells. In this regard, the periphery of dendrimers is rich in (terminal) reactive functional groups, which enables conjugation of a variety of cancer-targeting ligands. A wide selection of ligands such as biotin, folic acid, amino acids, peptides, aptamers and monoclonal antibodies has been successfully conjugated onto

dendrimer surfaces [14,41,42,44]. Some of the reported literature on such agents is recorded in Table 1.

PAMAM dendrimer-mediated cancer-targeting tactics

PAMAM dendrimers are arguably the most extensively studied prototype for biomedical applications, principally used as carriers for anticancer therapy. In one such reported study, phosphorylcholine-conjugated fifth-generation PAMAM dendrimers have

been developed for delivery of an anticancer drug: DOX. Cell morphology of the HepG2 cells suggested that the targeted dendrimers could internalize into cancer cells efficiently and inhibit their growth significantly [59,60]. In another study, Zhang *et al.* successfully delivered methotrexate (MTX) by folate-engineered third-generation PAMAM dendrimer [60]. Sharma *et al.* decorated 4.0G PAMAM dendrimers with gallic acid and

TABLE 1

Summary of dendrimer-mediated drug and gene delivery

| Dendrimer type | Generation | Ligand or conjugated moiety | Bioactive | Refs |
|----------------|-----------------|---|---|-------|
| PAMAM | 5.0 | Phosphorylcholine | DOX | [59] |
| | 3.0 | Folate | Methotrexate | [60] |
| | 4.0 | Gallic acid | NA | [61] |
| | 5.0 | Biotin and FITC | NA | [62] |
| | 3.0 | PDMA | Chlorambucil | [63] |
| | 5.0 | Folate | Methotrexate | [64] |
| | 4.0 | PEG | 5-Fluorouracil | [65] |
| | 4.0 | T _f and WGA | DOX | [66] |
| | 4.0 | Folate | Methotrexate and ATRA | [67] |
| | 2.0 | α-Cyclodextrins and galactose | Gene | [70] |
| | 4.0 | EGF and quantum dots | Nucleic acid | [72] |
| | 9.0 | pCF1CAT plasmid | Gene | [74] |
| | 2.0, 3.0, 4.0 | Arginine | Genetic materials | [75] |
| | 4.0 | Surface-acetylated internally quaternized | siRNA | [76] |
| | 4.0 | PEG | siRNA | [77] |
| | 5.0 | Selenium NPs | siRNA and cisplatin | [102] |
| | 5.0 | Lactobionic acid and PEG | DOX | [103] |
| | 3.0 | Glucuronylglucosyl-β-cyclodextrin | siRNA | [104] |
| | 5.0 | Pluronic F127 (PF127) | DOX | [105] |
| | 5.0 | Arginylglycylaspartic acid (RGD) and PEG | DOX | [106] |
| | 5.0 | Magnetic NPs | Gemcitabine and retinoic acid | [107] |
| | 4.0 | D-α-Tocopherol polyethylene glycol succinate (TPGS) | Docetaxel | [108] |
| 5.0 | Peptide | siRNA | [109] | |
| 5.0 | Hyaluronic acid | DNA | [110] | |
| 4.0 | PEG | DOX | [111] | |
| PPI | 3.0, 4.0, 5.0 | Folate | Melphalan | [44] |
| | 4.0 | Folate | Melphalan | [44] |
| | 4.0 | Folate | Paclitaxel | [78] |
| | 4.0 | Folate | Etoposide | [79] |
| | 5.0 | Folate | ATRA, DEXA, docetaxal | [80] |
| | 5.0 | Folate | DOX | [81] |
| | 5.0 | Azo-linkers | DOX | [82] |
| | 5.0 | Polysorbate 80 | Docetaxel | [83] |
| | 5.0 | Thiamine | Paclitaxel | [84] |
| | 5.0 | LHRH | siRNA | [89] |
| | 5.0 | Superparamagnetic iron oxide | siRNA | [90] |
| | 3.0 | NA | Gene | [91] |
| | 2.0, 3.0 | OEI | Gene | [92] |
| | 3.0, 4.0, 5.0 | Fluorinated | Gene | [93] |
| | 5.0 | Folate | Methotrexate | [112] |
| | 5.0 | LHRH | Paclitaxel and siRNA | [113] |
| | 4.0 | NA | Methotrexate, DOX and sodium deoxycholate | [114] |
| PLL | 5.0 | Folate | DOX | [95] |
| | 6.0 | PEG-linked hydrophobic penta-phenylalanine or penta-alanine | DOX | [96] |
| | 6.0 | DOX | DOX | [97] |
| | 5.0 | DOX | PEG | [98] |
| | 5.0, 6.0 | NA | Gene | [99] |
| | 3.0 | 6-Azido-β-cyclodextrin | DNA | [100] |
| | 3.0 | NA | pEGFP | [101] |
| | 1.0, 2.0, 3.0 | FITC | 5-Fluorouracil | [115] |

Abbreviations: ATRA, all-trans retinoic acid; DOX, doxorubicin; EGF, epidermal growth factor; FITC, fluorescein isothiocyanate; LHRH, luteinizing hormone releasing hormone; NP, nanoparticles; OEI, oligoethylenimine; PAMAM, polyamidoamine; PDMA, poly(*N,N*-dimethylaminoethyl methacrylate); PEG, poly(ethylene glycol); PLL, poly-L-lysine; PPI, polypropyleneimine; WGA, wheat germ agglutinin; NA, not applicable.

investigated their cytotoxicity against the MCF-7 cell line using an MTT assay. Fourfold higher cytotoxicity of the conjugates demonstrated the possibility that these dendrimers can be explored as a promising nanoplatform for cancer targeting [61]. Biotin and fluorescein isothiocyanate (FITC) conjugated to partially acetylated fifth-generation PAMAM dendrimers revealed much higher cellular uptake into HeLa cells compared with unmodified dendrimers. The proposed conjugate illustrated a promising potential for anticancer drug delivery [62]. In another reported study, third-generation PAMAM (core) and poly(*N,N*-dimethylaminoethyl methacrylate) (PDMA) conjugate (3.0G-PAMAM-g-PDMA) was synthesized via atom transfer radical polymerization (ATRP) and loaded with chlorambucil (CLB). The developed conjugates not only displayed pH-dependent drug release behavior but also offered a typical thermo-responsive character [63]. Baker and co-workers successfully delivered anticancer agents, MTX and paclitaxel (PTX) using PAMAM dendrimers as the drug delivery vehicle [64]. Using the concept of PEGylation, Bhadra *et al.* delivered 5-fluorouracil (5-FU) by PEGylated 4.0G PAMAM dendrimers and concluded that the nanoconstructs could act as long-circulatory sustained-released depot systems for anticancer drug delivery causing reduced blood dyscrasia compared with non-PEGylated systems [65].

It is well known that delivery of anticancer drugs to brain tumors still remains a challenging task owing to restriction caused by the blood–brain barrier (BBB). For such cases, development of a targeted drug delivery system by combining a single carrier and

single ligand is widely explored. Recently, an interesting approach has been proposed wherein 4.0G PAMAM dendrimers have been conjugated with two targeting ligands, namely transferrin (T_f) and wheat germ agglutinin (WGA), and utilized for the purpose of traversing the BBB and ingestion of drugs by brain tumor cells. The dual-targeting drug carrier system successfully delivered DOX inside the brain tumor and provided a potential application for brain cancer therapy [66].

In an alternative approach researchers have co-loaded MTX and all-*trans* retinoic acid (ATRA, tretinoin) using folate-conjugated 4.0G PAMAM dendrimers for treatment of solid tumors. The release kinetics of the developed conjugate were found to be dependent on the extent of dendrimer protonation and displayed significantly higher IC_{50} values compared with the simple drug combination [67].

Cationic dendrimers can be efficiently complexed with negatively charged biomolecules such as nucleic acids and genes. The complexes are termed ‘dendriplexes’. Dendriplexes exhibit good transfection efficiency and are hence utilized for delivery of genetic materials such as genes, oligonucleotides, siRNA and aptamers [68]. Dendrimers such as PAMAM, PPI, ornithine and arginine are broadly used as nonviral vectors for gene delivery owing to their high complexing ability [31,69] (Fig. 4). In a reported study, 3.0G-PAMAM-bearing α -cyclodextrins showed promising *in vitro* and *in vivo* transfection efficiency with low cytotoxicity [70,71]. In another study, Yuan *et al.* developed epidermal growth factor (EGF)-containing 4.0G PAMAM

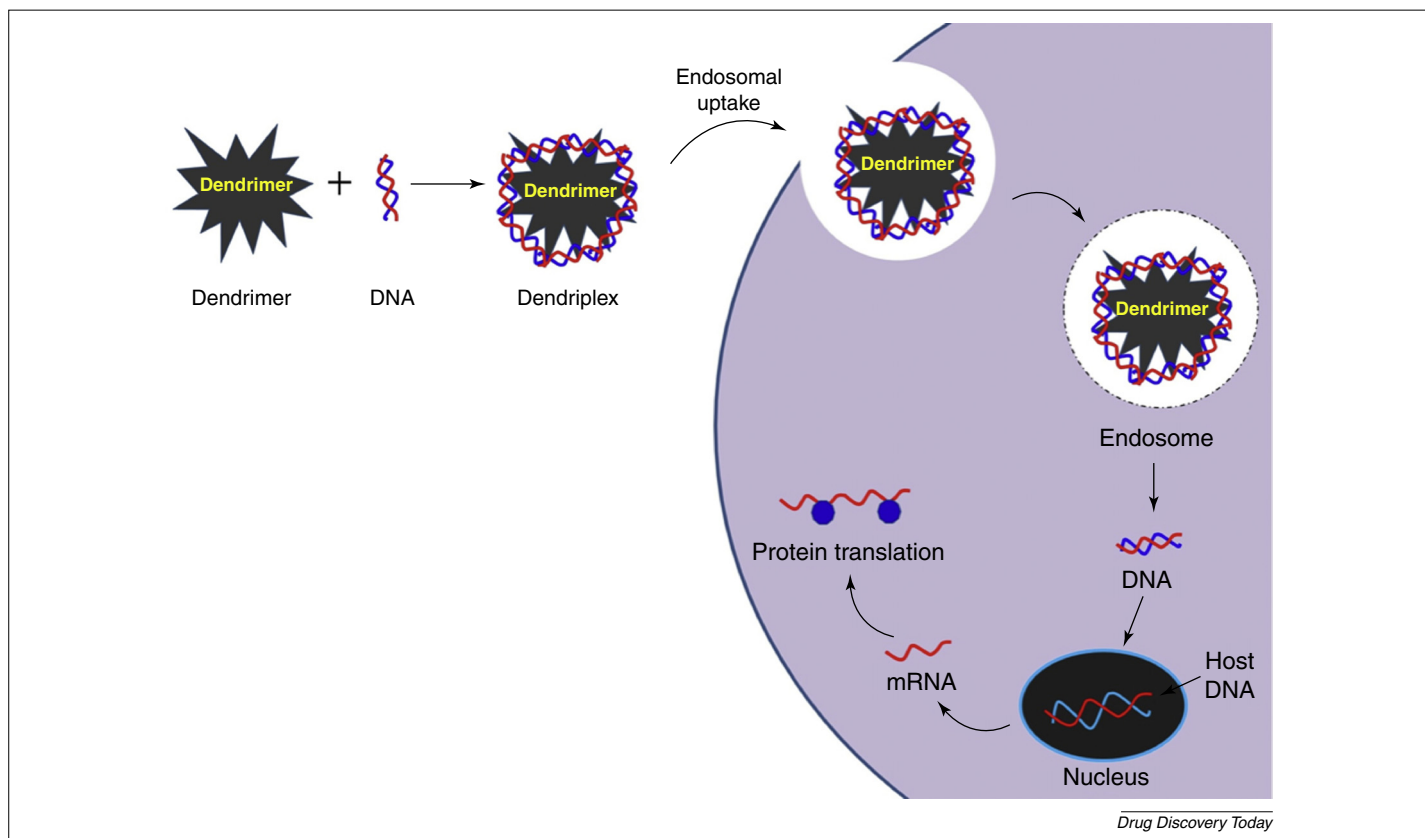


FIGURE 4

Dendriplex-assisted gene delivery.

dendrimer vector labeled with quantum dots for tumor imaging and targeted nucleic acid delivery. Researchers from the study revealed that the conjugates could localize intracellularly in an EGFR-dependent manner confirming the proposed approach for proficient delivery of nucleic acids such as shRNA plasmids and siRNAs [72]. Vincent *et al.* discovered the potential of nonviral gene transfer for cancer therapy using the antiangiogenic angiotatin (K1-3) and tissue inhibitors of metalloproteinase (TIMP) genes. Authors from the study suggested the possibility of PAMAM-dendrimer-like superfectant associated with 36-mer anionic oligomers (ON36) to inhibit tumor growth and angiogenesis by successfully delivering angiotatin and TIMP-2 genes [73]. In another reported study, Kukowska-Latallo *et al.* showed a high level of gene expression in the lung tissues of rats following intravenous administration of 9.0G PAMAM dendrimers complexed with pCF1CAT plasmid [74].

Nam *et al.* developed arginine-modified biodegradable PAMAM dendrimers of different generations for gene delivery. The transfection efficiency of fourth-generation-based dendrimer formulations was found to be higher as compared with second- and third-generation dendrimers. Interestingly, all these formulations have been found to be good options for the delivery of therapeutic genetic materials, including DNA, antisense oligonucleotides (ODNs) and siRNAs, according to which dendrimers were chosen [75].

Patil *et al.* developed surface-acetylated internally quaternized 4.0G PAMAM dendrimers for siRNA delivery. The cell cytotoxicity assay on A2780 ovarian cancer cells demonstrated the crucial role of modification of surface amine groups of PAMAM dendrimers to amide and internal quaternization in reduction of cytotoxicity and competent cell permeability of the dendrimer-siRNA complex [76]. Similarly, in another report, 4.0G-PAMAM-based novel triblock complex (PAMAM-PEG-PLL) efficiently delivered siRNA and exhibited exceptional stability in human plasma [77].

PPI dendrimer-mediated cancer-targeting tactics

PPI is another class of widely explored dendrimers extensively utilized for drug delivery. PPI dendrimers are amine-terminated hyperbranched macromolecules that are mainly synthesized by a divergent method (Fig. 5) [41]. The presence of numerous amine groups on the periphery enables conjugation with various cancer-targeting ligands such as folate, amino acids, carbohydrates, antibodies, peptides or tuftsin that can be explored for active targeting.

Kesharwani *et al.* evaluated the delivery of various anticancer drugs using different generations of PPI dendrimer [42,44]. Authors from the same group compared the cancer-targeting potential of three different ligand-conjugated dendrimers including folate, dextran and galactose. It was observed that the receptor-conjugated PPI dendrimers not only demonstrated enhanced tumor-targeting potentials but also significantly diminished hemolytic toxicity as compared with plain (nontargeted) PPI dendrimers. In addition, the release pattern of PTX was found to be sustained with surface-modified dendrimers as compared with plain PPI dendrimers. The results could be attributed to encapsulation of the PTX in the hydrophobic cavities of the targeted dendrimers that act as a sink to preserve the PTX for a prolonged duration [78].

On the basis of better performance of folate-mediated dendrimers for anticancer drug delivery, PEGylated diaminobutane 4.0G

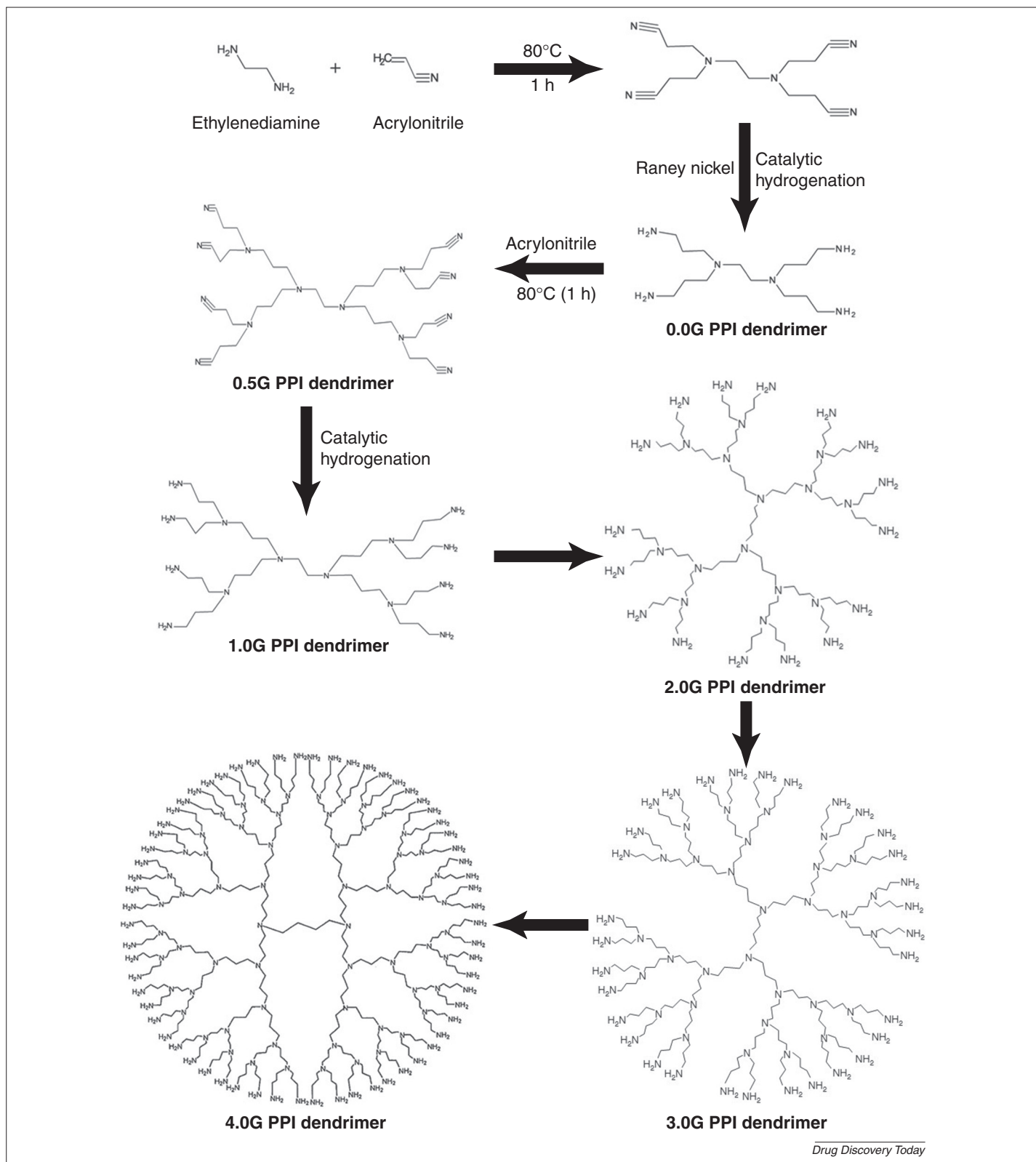
PPI dendrimers have been developed to deliver etoposide, an anticancer hydrophobic drug. The enhanced solubility and sustained release behavior of etoposide have been found favorable in addition to low toxicity and better targeting ability [79]. In another report, Dhakad *et al.* compared the cancer-targeting potentials of two folate receptor upregulators [ATRA and dexamethasone (DEXA)] on folate-decorated 5.0G PPI dendrimers using docetaxel (DTX) as the model drug. Authors concluded that, compared with DEXA, ATRA was found to be a superior folate receptor upregulator as well as being an adjunct bioactive in folate-based targeting [80]. Along similar lines, Jain and co-workers also delivered DOX using folate-conjugated fifth-generation PPI dendrimers [81].

In another interesting anticancer tactic against hepatic cancer delivery, nanoconjugates of DOX were prepared by coupling with fifth-generation PPI dendrimers using unique aromatic azo-linkers (L1-L4). The azo-linkers could be selectively recognized and cleaved via an NADPH-dependent mechanism using azoreductase enzymes present in the cytoplasm of hepatic cancer cells. This approach could be successfully explored for controlled delivery of drugs to hepatic cancer cells [82].

5.0G PPI dendrimers, similar to PAMAM dendrimers, also possessed the ability to deliver anticancer drugs to brain tumors. In a reported study, polysorbate-80-conjugated PPI dendrimers were explored for targeted delivery of DTX to the brain tumor. The study revealed that within one week of treatment the tumor volume reduced more than 50% in the case of developed formulations owing to higher BBB permeability of polysorbate-80-anchored dendrimers [83]. In another report, thiamine-conjugated 5.0G PPI dendrimers exhibited increased delivery of PTX across the BBB. The authors reported that the preferential brain uptake of PTX by the nanoconjugates might be attributed to the association with the thiamine transporters or increased passive diffusion secondary to an improved concentration gradient of the dendrimers situated at the BBB interface [84].

Recent therapeutic interventions using siRNAs are based on RNAi, holding promising potential in terms of targeted action [85-87]. siRNAs are duplexes of 21-23 nucleotides, approximately 7.5 nm long and 2 nm in diameter. The delivery of siRNAs for anticancer therapy is highly beneficial; however, 'unprotected' siRNAs suffer from low penetration ability across the cellular plasma membrane and are prone to rapid degradation by enzymes and nucleases present in the blood that make their systemic delivery even more daunting [11,88]. Taking account of the challenges associated with free-siRNA delivery, a novel approach was devised wherein a 5.0G PPI dendrimer was complexed with siRNA molecules. The complex was then caged with dithiol-containing cross-linker molecules followed by its coating with PEG to provide lateral and steric stability. The distal end of PEG was subsequently conjugated with a synthetic analog of luteinizing hormone releasing hormone (LHRH) peptide for targeting to cancer cells. The developed conjugates delivered significant amounts of siRNAs in the cytoplasm of cancer cells demonstrating their potential as an efficient gene delivery agent [89]. In another report, superparamagnetic-iron-oxide-nanoparticle-dendrimer complexes have also been reported for siRNA delivery specifically to cancer cells, exhibiting promising potential [90].

Dufes *et al.* developed third-generation PPI dendrimer complexes for gene delivery. Authors from this study concluded that

**FIGURE 5**

Synthesis of different generations of polypropyleneimine (PPI) dendrimers by a divergent method. Reproduced, with permission, from [42].

intravenous administration of the developed complex resulted in intratumoral transgene expression and regression of tumors [91]. Similarly, Russ *et al.* demonstrated that oligoethylenimine (OEI)-grafted PPI dendrimers (2.0G and 3.0G) displayed degradable

characteristics useful for gene delivery. The transfection efficiency of these systems could be enhanced by conjugation with OEI; however, it was found that increasing the dendrimer generation did not alter the transfection efficiency [92].

Liu *et al.* researched fluorinated PPI dendrimers of different generations (3.0, 4.0 and 5.0G) to improve the transfection efficiencies and safety profiles of PPI-based gene delivery vectors. The study revealed that fluorinated systems indeed showed better transfection efficiency compared with six representative transfection reagents: PolyFect[®], SuperFect[®], Lipofectamine[®] 2000, jet-PEI[®], branched poly(ethyleneimine) and arginine-modified dendrimer, on HEK293 and HeLa cells [93]. Further studies on these fluorinated dendrimers might thus yield promising alternatives to existing modes of delivering genes.

PLL dendrimer-mediated cancer-targeting tactics

Dendrimers based on PLL units represent another class of agents presently being explored as vectors for antiangiogenic therapy. In a significant development, a PLL-based dendrimer-enhanced version of DTX (Taxotere[®]) called DEP[™] docetaxel is being tested in Phase I clinical trials by Starpharma Holdings, located in Melbourne, Australia [94]. Starpharma reported that in preclinical trials DEP[™] docetaxel showed significant tumor targeting and superior anticancer effects across a range of cancer types when compared with Taxotere[®] [94]. In a recent study, folate-conjugated PLL dendrimers have been developed for delivery of an anticancer drug: DOX hydrochloride. Results of chorioallantoic membrane (CAM) assays, MTT assays and *in vivo* studies conclusively suggested superior antitumor activity of the developed systems [95]. In another approach, the hydrophobic cavity of 6.0G PLL dendrimers anchored with PEG-linked hydrophobic penta-phenylalanine or penta-alanine was utilized to deliver DOX. Intravenous administration of these nanosystems resulted in tumor accumulation by the EPR effect and ultimately led to significant suppression of tumor growth without loss of body weight [96]. Al-Jamal and colleagues prepared a complex of 6.0G PLL with DOX which resulted in improved anticancer activity in prostate 3D multicellular tumor spheroids (MTS) and solid tumors *in vivo*. These approaches may lead to newer options for combinatory antiangiogenic and/or anticancer therapeutics [97]. In yet another recent approach, PEGylated PLL dendrimers have been scrutinized for delivering DOX to lung metastatic breast cancer using a syngeneic MAT 13762 IIIB rat model. The outcomes of the study revealed that intratracheal instillation of PEGylated conjugates in tested rat models displayed better chemotherapeutic outcome than intravenous administration of DOX, indicating promising alternative routes of dendrimer administration for safe and effective cancer therapy [98] (Fig. 6).

Similar to PAMAM and PPI dendrimers, PLL dendrimers are also frequently used for gene delivery. In one report, a series of PLL-based dendritic nanoconstructs have been compared for their gene transfection efficiencies against linear and branched poly(lysine) polymers. The study revealed that PLL dendrimers containing 64 and 128 surface amino groups exhibited proficient gene transfection propensity in numerous cultivated cell lines [99]. In another study, cyclodextrin derivatives containing PLL dendrons were developed by Ma *et al.* for co-delivery of genes and anticancer drugs. The developed carrier was found to form colloiddally stable nanocomplexes with plasmid DNA in aqueous solution and showed high gene transfection efficiency [100]. In another approach, star-shaped porphyrin core PLL dendron (PP-PLLD) has been synthesized by click chemistry and demonstrated successful delivery of plasmid vector [101].

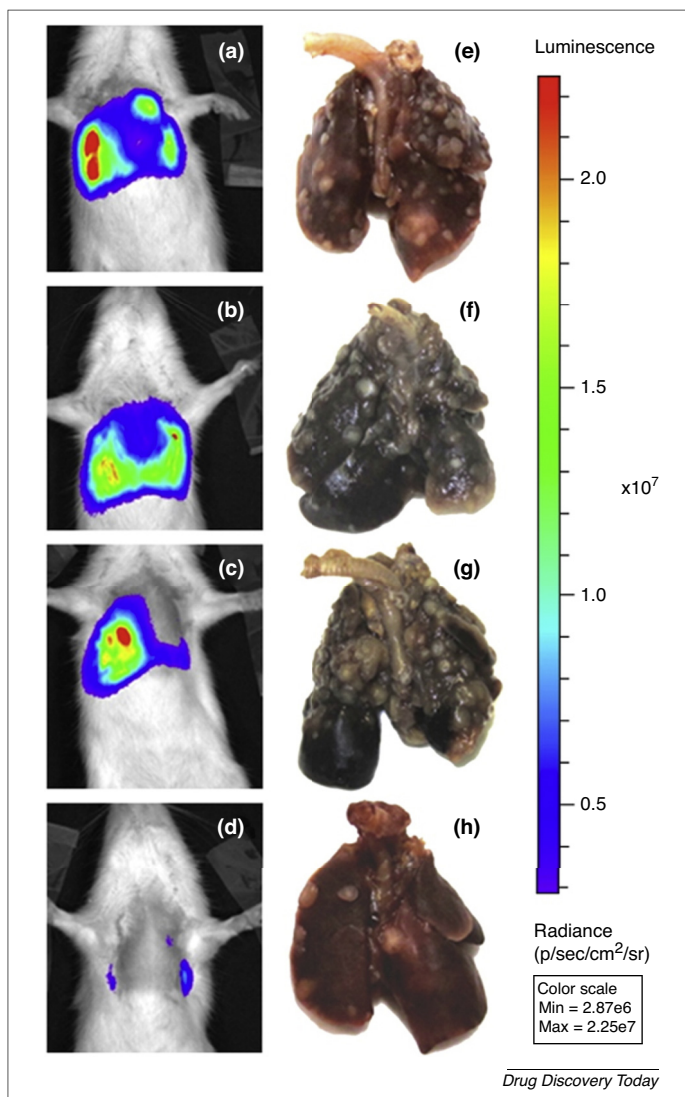


FIGURE 6

Representative images showing the reduction in lung tumor burden following intratracheal instillation of D-DOX in syngeneic F344 rats bearing lung metastases of firefly-luciferase-expressing MAT 13762 IIIB carcinoma. (a–d) Bioluminescent images of the lungs immediately before termination (18–21 days after injection of cells). (e–h) Images of fixed lung tissue showing lung regions and individual metastatic foci. Rats were treated with saline alone (a,e), IV DOX (b,f), IV D-DOX (c,g) or intratracheal D-DOX (d,h). The scale for bioluminescent images is depicted on the right. Reproduced, with permission, from [98].

These approaches using newer routes of polymer and dendrimer synthesis are likely to pave the way for future generations of advanced drug and gene delivery nanovectors for cancer therapy.

Concluding remarks

Late diagnosis and the margins of conventional chemotherapy have led to poor prognosis of cancer patients with development of MDR phenotypes. Even with the historical progress made in the understanding of the disease and development of newer targeted therapies, treatment of several forms of cancer remains a major challenge. Recent advances in dendrimer-mediated drug and gene

delivery have emerged as a superior option to overcome the shortcomings associated with conventional chemotherapy. The unique spherical architecture, tunable molecular size and multi-valent surfaces of dendrimers offer an exceptional opportunity for loading or conjugation of drugs, genes, targeting moieties and imaging agents for simultaneous cancer diagnosis and/or imaging and treatment. The current review presents an overview of dendrimer-mediated drug and gene delivery using passive and active targeting principles in the *in vitro* and *in vivo* settings. Thus far, these nanovectors portend to have promising potentials for targeted anticancer therapy. However, more-detailed studies are

warranted to ascertain their safety and utility for moving them forward from the bench to the bedside.

Conflicts of interest

There are no conflicts of interest or disclosures associated with this manuscript.

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