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PAMAM Dendrimers as Promising Nanocarriers for RNAi Therapeutics

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Therapeutics based on RNA interference mechanisms are highly promising for the management of several diseases including multi-drug resistant cancers. However, effective delivery of siRNAs and oligonucleotides still remains challenging. In this regard, hyper-branched, PAMAM dendrimers having unique three-dimensional architecture and nanoscale size, with cationic surface charge can potentially serve as siRNA condensing agents as well as robust nano-vectors for targeted delivery. In addition, their surface functionality permits conjugation of drugs and genes or development of hybrid systems for combination therapy. Thus far, *in vitro* cellular testing of dendrimer-mediated siRNA delivery has revealed great potential, with reports on their *in vivo* effectiveness starting to appear. These favorable outcomes portend a promising future for dendrimer mediated RNAi therapeutics.

Introduction

The use of nucleic acid constructs to treat or prevent certain types of disease is known as gene therapy. Gene therapy requires the introduction of foreign genes into targeted host cells to replace the mutated gene and/or initiate new functionalities [1]. Gene delivery has progressed rapidly over the past two decades [2,3]. In addition to DNA-based gene therapy, RNA interference (RNAi) offers another approach in which RNA is used to mediate gene knockdown. RNAi was first introduced in 1998, and quickly became a powerful strategy for both basic research and therapeutic development [4–8]. Although small interfering RNA (siRNA) therapy is promising, as shown in Fig. 1, successful delivery is challenging. Figure 2 shows that of all the reports that discuss RNA delivery, only a small percentage utilizes non-viral vector mediated transfection, indicating the vast unexplored potentials of nano-based siRNA delivery [9].

Several synthetic vectors have been examined for use as siRNA delivery vehicles including cationic polymers, cell-penetrating peptides, cationic lipids, dendrimers and surface modified carbon nanotubes (CNTs) – all of which can complex siRNA via electrostatic interactions. These carriers protect siRNA from degradation and facilitate uptake by target cells. Among these cationic vectors, dendrimers stand out because of their unique structure as well as tunable surface characteristics [7,10].

Dendrimers are highly symmetric, spherical, hyper-branched, nano-sized (1–100 nm), three-dimensional macromolecules with well-defined structure, molecular size and surface charge [11–15]. Their chemical homogeneity, the possibility of increasing their size by repeated addition of chemical moieties and a high density of surface groups that are suitable for ligand-attachment makes them an exceptional candidate for various

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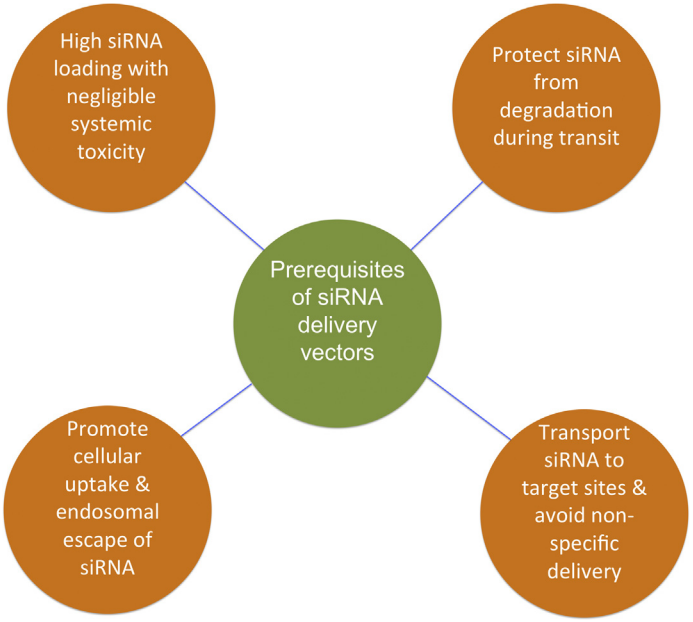

FIGURE 1

Key requirements for successful siRNA delivery.

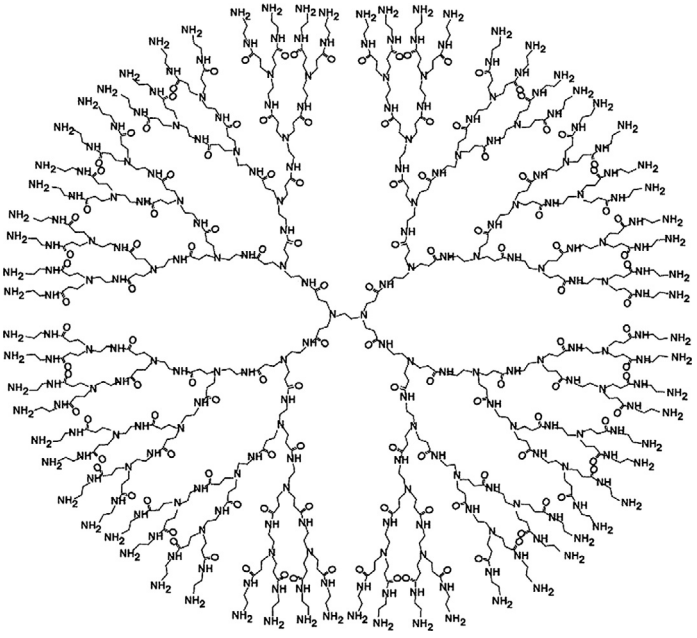

FIGURE 3

Typical structure of fourth-generation poly(amidoamine) dendrimer.

biomedical applications. Compared to traditional linear polymers, dendrimers also have significantly better physical and chemical properties [16].

Dendrimers such as poly(amidoamine) [PAMAM] and poly(propylenimine) [PPI] possess cationic primary amine groups at the surface, which participate in binding with DNA and siRNAs and increase their intra-cellular uptake by forming nanoscale complexes called dendriplexes [7,17]. The synthesis and characterization of PAMAM dendrimers (Fig. 3) with ammonia or ethylenediamine (EDA) cores was first reported in 1985 by Donald Tomalia's group [18]. At present, PAMAM dendrimers are one of the most widely studied ones for their use as vectors for delivery of drugs and genes. In 1993, Haensler and Szoka first reported the gene transfection capabilities of PAMAM dendrimers [17]. PAMAM dendrimers interact with plasmid DNA (pDNA) via electrostatic interactions and condense it to form a dendriplex. Dendriplexes aids cellular uptake and the DNA is ultimately released from the endosomes after cellular entry by the well-known 'proton sponge effect' [19]. The excellent DNA complex forming ability of PAMAM dendrimers resulted in its widespread use as a commercial DNA transfection kits such as SuperFect® and PolyFect®. The success of PAMAM dendrimers in DNA-based gene delivery studies offers hope for siRNA delivery. In this regard, PAMAM based dendrimers are also the most widely used type of dendrimer for siRNA research. This review discusses the relevance and applications of PAMAM dendrimers in RNAi therapeutics, using siRNAs (Table 1).

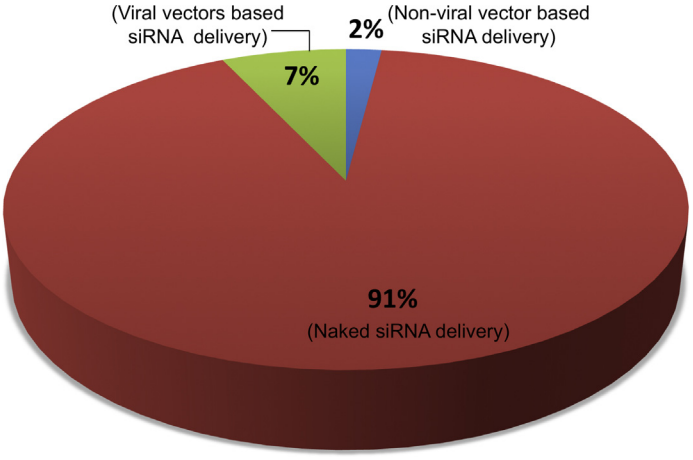

FIGURE 2

PubMed search analysis showing the percentage of published articles discussing viral and non-viral vector based siRNA delivery relative to the total number of articles reporting naked siRNA delivery [9].

Dendrimer toxicity

In spite of wide application of dendrimers in the drug delivery field, the use of dendrimers in biological system is constrained because of their intrinsic toxicity. The presence of surface cationic charge, which can interact with negatively charged biological membranes may result in membrane disruption via nano-hole formation, membrane thinning and erosion. It was documented extensively that dendrimers like PAMAM, PPI and poly-L-lysine (PLL) exert remarkable *in vitro* cytotoxicity because of these cationic surface groups [16,20,21]. There are reports on concentration and generation dependent toxicity of free amine groups present at their periphery [12,22,23]. Chen et al. reported the cytotoxicity of cationic melamine dendrimers having surface groups like amine, guanidine, carboxylate, sulfonate or phosphonate and concluded that cationic dendrimers were much more cytotoxic than their anionic counterparts [24]. Toxicity of dendrimers is generally considered to be species, dose, exposure duration and generation (size) dependent and to be influenced by the nature of the terminal groups. For instance cationic amine terminal groups for full generation dendrimers and anionic carboxylic acid terminal groups for half generation dendrimers are found to cause cellular toxicity.

In contrast, the positive charges of dendrimers are crucial for its interaction with negatively charged biological species leading to

TABLE 1

Summary of PAMAM dendrimer-mediated siRNA delivery.

Generation	Conjugated moiety	Drug	Hybridized with	siRNA type	Reference
G5	–	–	–	Cy5-labeled siRNA	[40]
G5	HA	DOX	–	MVP-siRNA	[44]
G5 & G7	PEG	–	–	Syn-4 siRNA	[37]
G5	$E_{16}G_6$RGDK peptide	–	–	Hsp27 dsiRNA	[55]
G4	PEG and cyclic RGD	–	–	Carboxyfluorescein-labeled siRNA	[58]
G3	α-cyclodextrin	–	–	pDNA/siRNA	[30]
G4	e-PAM-R	–	–	HMGB1 siRNA	[51]
G1–G4	E6 and E7 proteins	–	–	siE6/E7 s10siRNA	[52]
G4	PLL and PEG	–	PLL dendrimer	Bcl-2 siRNA	[63]
G4	PEG	DOX	DOPE	Cy-3-labeled GAPDH siRNA	[64]
G5	–	Cisplatin	SeNPs	mdr1 siRNA	[67]
G4 & G5	anti-CD71 Fab antibody	–	PEG-b-P(PrMA-co-MAA)	Bcl-2 siRNA	[68]
G4	–	–	Magnetic iron oxide NPs	EGFR siRNA or a GFP siRNA	[70]

Abbreviations: G, generation; HA, hyaluronic acid; PEG, polyethylene glycol; RGD, Arginine-Glycine-Aspartic Acid; PLL, poly-L-lysine; DOX, doxorubicin; e-PAM-R, biodegradable arginine ester of PAMAM dendrimer; DOPE, dioleoylphosphatidyl ethanolamine; NPs, nanoparticles; SeNPs, selenium nanoparticles; GFP, green fluorescent protein; siRNA, small interfering RNA; pDNA, plasmid DNA; dsiRNA, dicer substrate siRNA; PEG-b-P(PrMA-co-MAA), PEG-b-poly(propyl methacrylate-co-methacrylic acid); MVP, major vault protein.

complex formation for gene delivery. The overall toxicity of dendrimers can be mitigated by surface modification with agents like carbohydrates, PEG, acetate and so forth. Fortunately, the dendrimers toxicity can also be reduced to a large extent by complexation with oligonucleotides. Studies performed on PAMAM dendrimers aiming for gene delivery indicate that DNA complexed amino terminated PAMAM dendrimers of low generation (up to G3) do not possess any significant cytotoxicity *in vitro*, and that the toxicity of the dendrimers is reduced upon complexation with DNA [25,26]. Because of these observations, plethora of research is now available on the applicability of dendrimers in gene transfection, particularly amino terminated dendrimers like PAMAM, PPI, ornithine and arginine conjugated dendrimers. These amino terminated dendrimers enhance the transfection efficiency into nucleus by endocytosis [7,27]. Overall, care must be taken to weigh the toxicity of dendrimers versus its utility in gene transfection. As such with proper choice of dendrimer generation, the surface functional groups present, and its modification can result in arriving at the right choice for safe and effective gene delivery.

Suitability of PAMAM dendrimers for siRNA delivery

In order for a gene delivery vector to be successful, there are several requirements including optimum size, optimum charge with negligible cytotoxicity. The versatile nature of PAMAM dendrimers is because of their unique structural properties such as easily modifiable surface functionality, nanosize unimicellar nature and cationic surface charge because of the presence of several protonatable amine groups. The multiple cationic functional groups enable the formation of a strong, yet reversible, complex with siRNAs. Their small size allows delivery across cell membranes. The multiple surface functional groups also allow additional therapeutic agents to be incorporated into the nano-vector that make dendrimers a versatile platform for combination siRNA and drug delivery [28–32].

PAMAM dendrimer-mediated siRNA delivery

As shown in Fig. 4, PAMAM dendrimers condense siRNA to form dendriplexes. Vasumathi and Maiti examined the complexation between generation G3/G4 PAMAM and siRNA using atomistic molecular dynamics based on free energy calculations and inherent structure determination. The complexation behavior was also studied using different combinations of siRNA and vector, that is, one siRNA and two dendrimers (two G3 or two G4). A stable siRNA-dendrimer complex formed within nanoseconds. Increasing dendrimer generation from three to four increased the binding energy between siRNA and the dendrimer, possibly because of enhanced charge ratio. The authors concluded that one PAMAM dendrimer of G4 and two PAMAM dendrimers of G3 produced the smallest complexes and two G4 dendrimers had the strongest binding affinity (Fig. 5) [33].

To ensure that siRNA remains intact during delivery, it requires protection from enzymes such as RNAases. The ability of dendrimers to offer protection has been explored previously; for example, Abdelhady et al. used atomic force microscopy (AFM) to evaluate the ability of G4 (flexible) and G5 (rigid) dendrimers to shield siRNA payloads from degradation by RNase V1. Upon incubation with siRNA, the G4 and G5 dendrimers formed similar hexagonal complexes and, in both cases, the exposed RNA

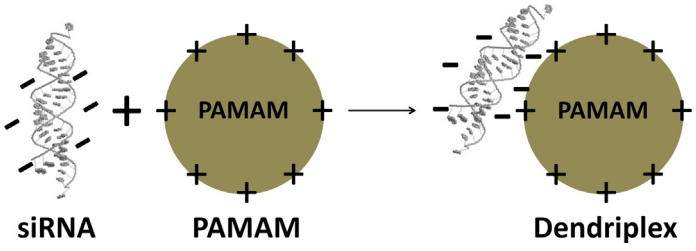

FIGURE 4

Mechanism of dendriplex formation.

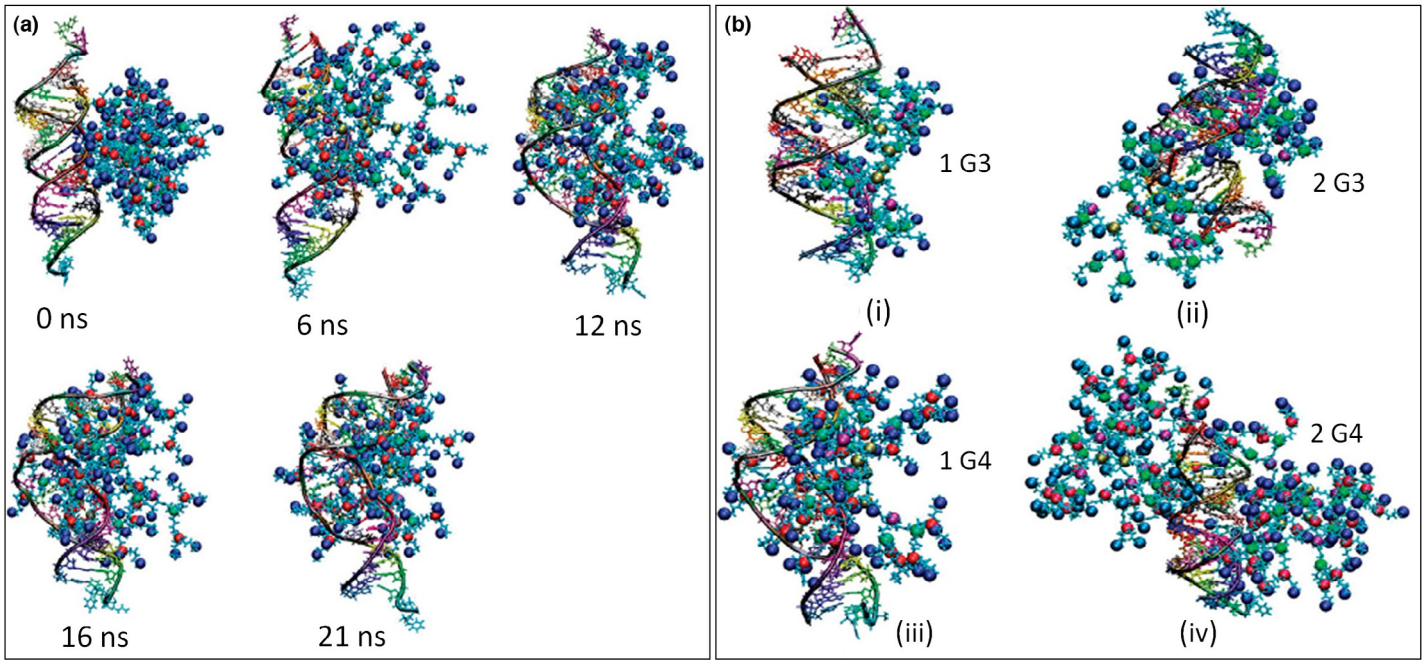

FIGURE 5

(a) Structure of siRNA–dendrimer complex formed between siRNA and a single G4 dendrimer during various stages of the binding process at the interval of few nanoseconds; **(b)** Structures of siRNA-dendrimer complexes: siRNA with (i) one dendrimer of generation 3 (1 G3), (ii) two dendrimers of generation 3 (2 G3), (iii) one dendrimer of generation 4 (1 G4) and (iv) two dendrimers of generation 4 (2 G4) are shown. Reproduced with permission from Ref. [33].

molecules were degraded by RNase V1 in a time-dependent manner (Fig. 6). However, the central core of the dendriplexes was able to protect the siRNA molecules from degradation for up to 60 min. In complexes formed at a low N/P ratio (the ratio of nitrogen atoms in the dendrimer to phosphorous atoms in the siRNA) of 2, the siRNA was able to resist degradation; the use of low N/P ratios also reduces the risk of non-specific toxicity when used *in vivo* [34].

Perez et al. investigated the influence of ionic strength of preparation media on the size, relative binding affinity and zeta potential of dendriplexes prepared using PAMAM dendrimers of different generations (G4–G7) with EDA cores. The effect of the medium on cellular uptake and silencing activity was also studied. The results suggested that the formulation strategy used to prepare dendriplexes could affect the complex in the same way as chemical structure modification. The authors concluded that the use of low ionic strength preparation media is crucial for obtaining small dendriplexes and those structural features other than size affected silencing activity [35]. The observed results were in accordance with previous reports, which indicated the importance of the charge ratio between the dendrimer and the RNA when preparing stable, uniform RNA–dendrimer complexes [36].

Shen and coworkers used flow cytometry to investigate the role of caveolin-1 and syndecan-4 in the internalization of G5 and G7 poly(ethylene glycol) [PEG]-functionalized (PEGylated) PAMAM dendriplexes in myoblast (C2C12) and hepatic (HepG2) cell lines. The results suggested that internalization of PEG-PAMAM dendriplexes by HepG2 cells was improved by downregulation of syndecan-4 and upregulation of caveolin-1; however, the internalization of dendriplexes into C2C12 cells was found to be unaffected by caveolin-1 and decreased by downregulation of syndecan-4. The results provide useful information for improvement of *in vivo* gene delivery using PEG-PAMAM dendriplexes [37].

In another study, three different types of cationic dendrimers (PAMAM, phosphorus and carbosilane) were complexed with anticancer siRNAs (siBcl-2, siMcl-1 and siBcl-xl), which silence anti-apoptotic genes. The transfection affinity of complexes in HeLa and HL-60 cells was analyzed using both single apoptotic siRNAs and a mixture (cocktail) of them. The results suggested that the cocktails were more effective than single siRNAs, permitting one to decrease siRNAs concentration in treating cells. A comparative study showed that phosphorus based dendrimers were most promising as siRNA carriers. However, they were also the most cytotoxic on their own. Additionally, it was found that siRNAs can easily form stable complexes with all the dendrimers tested and protect them against nucleolytic degradation, which is an important consideration for effective gene transfection [38,39].

Recently, Ziraksaz et al. developed siRNA vectors for stem cell modification that were based on PAMAM dendrimers (G5) and the cationic lipid *N*-[1-(2,3-dioleoyloxy)]-*N,N,N*-trimethyl ammonium propane methyl sulfate (DOTAP). The transfection of mouse embryonic stem cells (mESCs) was analyzed using flow cytometry and quantitative real-time polymerase chain reaction (qPCR). The authors concluded that DOTAP at higher molar ratios and PAMAM at lower molar ratios had the ability to knockdown octamer-binding transcription factor (OCT4) transcription more effectively than the commercially available reagent Arrest-In™ [40].

TWIST1 transcription factor is a potential target for breast cancer, which is frequently overexpressed in aggressive triple-negative breast cancers and is a key regulator of cellular migration through epithelial-mesenchymal transition (EMT). In a reported study, siRNA-based TWIST1 silencing approach has been utilized using G3 PAMAM dendrimers. The study revealed that PAMAM–siRNA complexes can be efficiently taken up by SUM 1315 breast cancer cells and leads to significant knockdown of TWIST1 and

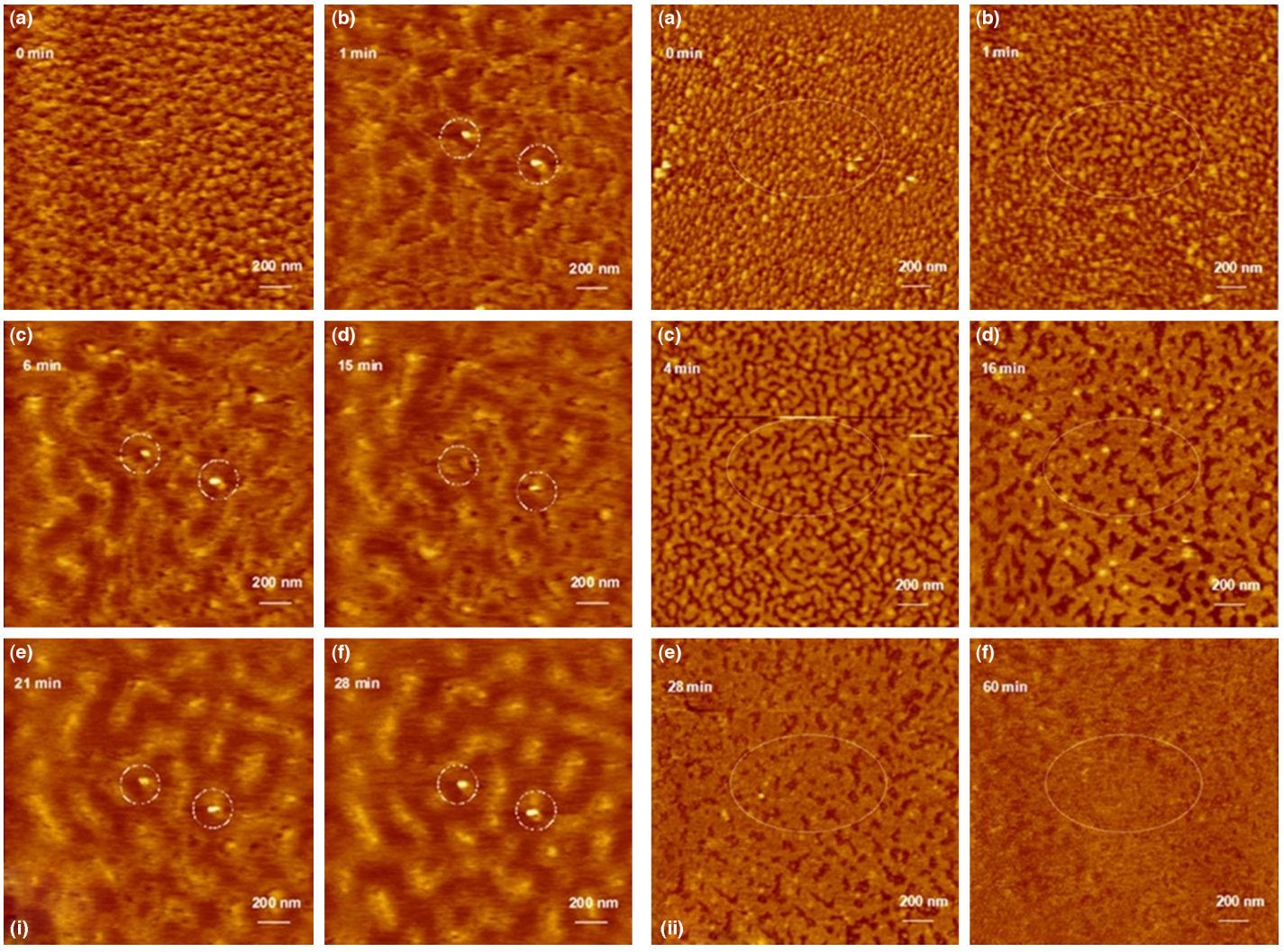

FIGURE 6

(a) AFM image of G4 (i) and G5 (ii) dendriplexes on a freshly cleaved mica surface. AFM images of G4 (i) and G5 (ii) dendriplexes after incubation with RNase V1 enzyme at different time points **(b–f)** showing separation of the adsorbed dendriplexes and degradation of siRNA (dark spots) over time. Two dendriplexes (dotted circles) remained intact throughout the experiment suggesting the formation of individual compact particles. Scale bar is 200 nm. Reproduced with permission from Ref. [34].

EMT-related target genes. Authors for the study also reported that the siRNA–PAMAM complex can delivered siRNA to xenograft orthotopic tumors. The siRNA remained in the tumor for at least 4 hours after treatment. These results indicate that PAMAM dendrimers mediated siRNA-based TWIST1 silencing approach could be a valuable adjunctive therapy for patients with triple-negative breast cancers [41].

Conjugates of the cyclic oligosaccharide α-cyclodextrin and PAMAM (α-CDE) mediate effective gene transfer activity into mammalian cells with very low cytotoxicity even at high charge ratios of α-CDE/pDNA. Tsutsumi and coworkers developed α-CDE–siRNA complex for use in a co-transfection system. Complexes formed between α-CDE or the commercial lipidic transfection reagents Lipofectamine™ 2000, TransFast™ and Lipofectin™ with pDNA and siRNA were developed, and evaluated. Ternary complexes of pGL3/siGL3/α-CDE were more effective siRNA vectors than the commercial vectors and showed negligible cytotoxicity in the various cell lines tested. Additionally, the binary complexes of siRNA/α-CDE mediated significant luciferase knockdown in NIH3T3 cells that transiently and stably expressed luciferase [30].

Use of targeting ligands in PAMAM–siRNA systems

Delivery alone is not enough for therapeutic activity; targeted delivery of siRNA to specific organs is required for therapeutic efficacy and low toxicity. Several PAMAM dendrimers incorporating one or more targeting ligands have been reported. Hyaluronic acid (HA), a natural linear polysaccharide is biologically inert with high aqueous solubility. HA receptors are expressed by several cancer cell lines pancreatic, lung, and breast cancer cells [42]. Decorating dendrimers with HA increases their residence time in the blood by decreasing the degree of opsonization by the reticuloendothelial system (RES) [43]. In a recent report, Han et al. described HA-modified G5 PAMAM dendrimers for the co-delivery of doxorubicin (DOX) and major vault protein (MVP)-targeted siRNA. It was envisaged that the downregulation of MVP would enhance the sensitivity of cancerous cells to DOX. The PAMAM formulation had higher cytotoxicity in MCF-7/ADR cells,

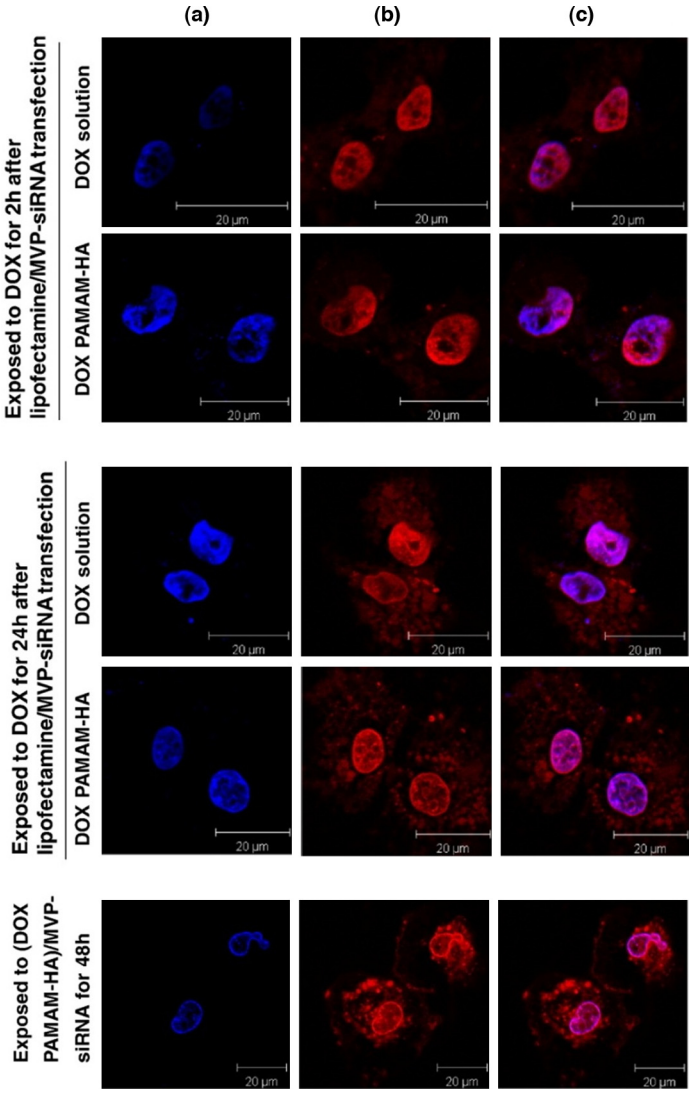

FIGURE 7

Intracellular distribution of DOX after MVP-siRNA transfection. After Lipofectamine 2000- or DOX PAMAM-HA-mediated siRNA transfection, MCF-7/ADR cells were exposed to DOX solution or DOX PAMAM-HA and observed under confocal laser scanning microscope. Nuclei were stained blue with Hoechst 33342 as a control **(a)**, and overlapped with DOX fluorescence **(b)**, as shown in panel **(c)** to show intracellular distribution of DOX. Reproduced with permission from Ref. [44].

tumor targeting affinity, intracellular accumulation and blood circulation time than DOX alone; the formulation also caused minimal *in vivo* toxicity. Confocal microscopy of MCF-7/ADR cells after transfection with MVP-siRNA showed that intracellular fluorescence was similar when using either DOX or DOX-containing dendriplexes (Fig. 7). The HA-modified dendriplexes were shown to be a promising vector for co-delivery of siRNA and an anticancer drug by reversing drug resistance by altering intracellular drug distribution [44].

Targeted drug delivery using arginine is an established concept because of numerous advantages. For instance, polyarginine peptides are most extensively used cell-penetration peptides for intracellular delivery [45,46] and arginine engineered carrier have been reported for their improved cellular uptake to transport various therapeutics, such as small molecular drugs, proteins and nucleic acids [47–49]. Motivated by these advantages, Liu et al. developed an arginine-decorated amphiphilic dendrimer composed of a hydrophobic alkyl chain and a hydrophilic PAMAM dendron bearing arginine terminals as non-viral vector for siRNA delivery. It was found that arginine modified dendrimers showed effective delivery of siRNAs in human prostate cancer PC-3 cells as well as human hematopoietic CD34+ stem cells. The vector showed improved gene silencing compared to the corresponding non-arginine modified dendrimer. Additionally these engineered dendrimers were stable and less cytotoxic than the plain dendrimers suggesting their utility in the delivery of bioactives [50].

e-PAM-R is a biodegradable arginine ester of PAMAM dendrimer, which degrades under physiological conditions. Kim et al. evaluated the effectiveness of e-PAM-R/siRNA complexes for siRNA delivery in primary cortical cultures and in rat brains. In primary cortical cultures, the complexes had a high transfection efficiency and low cytotoxicity. Examination of the rat brains suggested the successful delivery of high mobility group box-1 (HMGB1) siRNA by e-PAM-R. It was observed that the expression of HMGB1 was depleted in over 40% of neurons and astrocytes in the brain. These results strongly suggest that e-PAM-R is useful for probing and modulating gene functionality in primary neuronal cultures as well as in the brain [51].

Cervical cancer is associated with certain types of the human papilloma virus (HPV). The entry of HPV into cells and the subsequent translation of the HPV oncoproteins, E6 and E7, are responsible for transforming normal cervical keratinocytes into malignant cells. For this reason, Dutta et al. proposed the use of dendriplexes for the delivery of siRNA targeting proteins, E6 and E7, to cervical cancer cells. The authors optimized the dendrimer generation and N/P ratio of the complexes for effective encapsulation of siRNA targeting green fluorescent protein (siGFP). The efficiency of targeting resulted in 49.76% effectively mediated knockdown. The system was found to be non-toxic to the human cervical cancer cell line HeLa [52].

Patil and coworkers reported the synthesis of a novel, internally quaternized, surface-acetylated G4 PAMAM dendrimer (QPAMAM-NHAc) that interacted with siRNA via positive charges on the inside of the dendrimer. The authors suggested that the conversion of the surface amine groups to amides drastically diminished cytotoxicity, and QPAMAM-NHAc had the lowest toxicity in A2780 ovarian cancer cells among all tested formulations. Confocal microscopy confirmed the enhanced cellular uptake and homogeneous intracellular siRNA distribution mediated by QPAMAM-NHAc. The work clearly illustrated the value of converting dendrimer surface amine groups to amides and quaternizing the interior amines [53]. In another investigation, Waite et al. studied primary amine acetylation of G5 PAMAM dendrimers for optimizing siRNA delivery to U87 malignant glioma cells. Authors from this study found that increasing the degree of amine acetylation reduced the vector's cytotoxicity and increased the dissociation of dendriplexes. The acetylation of dendrimers reduced the cellular delivery of siRNA and this was found to be associated with a reduction in buffering ability. It was concluded that acetylating only a modest fraction (approximately 20%) of the primary amines of PAMAM preserves its siRNA delivery efficiency; however, higher degrees of amine neutralization reduce it transfection ability [54].

Recently Liu et al. developed structurally flexible G5 PAMAM dendrimers with a flexible core for the targeted delivery of Hsp27 Dicer-substrate siRNA (dsiRNA) in prostate cancer models *in vitro* and *in vivo*. The cancer cell targeting efficiency of dsiRNA/dendrimer complexes was enhanced by conjugating a targeting peptide with the amino acid sequence $E_{16}G_6$RGDK. The RGDK peptide has recently been shown to target the tumor endothelium through the interaction of the RGD sequence with the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins that are over-expressed on many cancer cells including prostate cancer. The peptide also enhances cell penetration via the binding of RGDK to the neuropilin-1 receptor. The enhanced targeting efficiency was found to be because of the interaction of the novel peptide conjugates with integrins and improved cell penetration via the interaction with neuropilin-1 receptors [55]. Luteinizing hormone-releasing hormone (LHRH) peptide is another novel targeting ligand that is widely used to bind to LHRH receptors that are over-expressed in the plasma membrane of several types of cancer cells and not expressed in healthy visceral organs. A novel G4 PAMAM-OH dendrimer (containing 64 hydroxyl end groups) modified with LHRH was developed for intracellular delivery of siRNA via receptor-mediated endocytosis. The cellular uptake of siRNA by A2780 human ovarian cancer cells was investigated to explore the effect of degree of quaternization and targeting ligand on dendrimer mediated delivery. The results suggested that the cellular uptake of siRNA when using LHRH-conjugated PAMAM-OH dendrimers was higher than when using non-conjugated PAMAM-OH dendrimers, even when the degree of quaternization of the conjugates was high [53]. These findings clearly demonstrated the importance of quaternization and the use of targeting ligands for intracellular delivery of siRNA [56]. Tat peptide is another cell penetrating peptide that is widely utilized for siRNA and antisense oligonucleotide delivery. Kang et al. conjugated G5-PAMAM dendrimers to Tat ligands to enhance the delivery of oligonucleotides. In one study, authors attached fluorophores (BOD-IPY) and Tat to evaluate the inhibition of MDR1 gene expression in MDR NIH 3T3 cells. Using non-toxic doses of Tat-PAMAM conjugate (BPT) MDR1 gene expression was partially inhibited by antisense oligonucleotide-containing complexes, and weakly inhibited by siRNA-containing complexes. It was concluded that conjugation with Tat peptide did not enhance the effectiveness of the dendrimer [57].

An approach based on a triblock dendritic nanocarrier, PAMAM (G4)-PEG-cyclic Arginine-Glycine-Aspartic Acid (RGD) has been proposed for *in vitro* delivery of siRNA targeting the human ether-à-go-go-related gene (hERG) in human anaplastic thyroid carcinoma (ATC) cells. It was noted that PEG-conjugated dendrimers had lower cytotoxicity than unmodified G4 PAMAM dendrimers. Flow cytometry experiments indicated 68% transfection efficiency at an N/P ratio of 3.5. Reverse transcription polymerase chain reaction (RT-PCR) experiments indicated that the expression of hERG was downregulated by 26.3% as compared to controls, suggesting that triblock nano-carriers can be a promising vector for siRNA delivery [58].

PAMAM-based hybrid systems

To design a 'perfect' siRNA vector it may be necessary to combine two or more delivery technologies to form a so-called 'hybrid system'. For example, PLL dendrimers have been explored in the past for gene delivery. However, their siRNA delivery efficiency has been impeded due lack of amino groups to achieve a pK_a of ~5–7 required for the 'proton sponge effect'. As a result, the surface of PLL has been modified with targeting ligands or endosomolytic agents such as chloroquine or fusogenic peptides [59,60]. A considerable enhancement of transfection efficiency was observed when histidine or imidazole moieties were attached to PLL [61,62]. Recently a novel triblock PAMAM-PEG-PLL hybrid nano-system was developed and investigated as siRNA vector. Every component in this system had a unique role: the tertiary amines in the PAMAM dendrimer worked as a proton sponge agent and played a significant role in the endosomal escape and cytoplasmic release of siRNA. The PLL segment enhances penetration and also provides the primary amines needed to form dendriplexes and the PEG chains acted as a linker between the PAMAM and PLL dendrimers. The system was found to protect siRNA from enzymatic degradation and the conjugation of PLL to PAMAM and PEG reduced its toxicity. The novel triblock systems displayed excellent stability in human plasma; were effectively taken up by cancer cells; and more importantly, induced knockdown of the target bcl2 gene [63].

In another report, a triblock copolymer nanocarrier based on PEG-dioleoylphosphatidyl ethanolamine (DOPE)-modified G4 PAMAM was developed for the co-delivery of DOX and siRNA. The mixed micellar system was prepared by mixing PEG-DOPE-PAMAM and PEG-DOPE in a ration of 1:1. The results of fluorescence microscopic analysis showed that PEG-DOPE-PAMAM displayed amplified gene silencing of GFP in C166-GFP cells. Overall, PEG-DOPE-PAMAM was able to overcome the challenges of drug and siRNA co-delivery because of the combined properties of dendrimers and polymeric micelles [64].

Selenium nanoparticles (SeNPs) have attracted interest as potential drug carriers because they exhibit excellent antioxidant activity, good biocompatibility and low toxicity [65,66]. However, their usefulness is limited by poor cellular uptake. In the context of gene delivery, SeNPs have low transfection efficiency and are untargeted – something that may lead to undesirable side effects. To overcome these obstacles, Zheng et al. synthesized a novel hybrid drug delivery system using G5 PAMAM-modified SeNPs for the systemic dual-delivery of MDR1 siRNA and cisplatin. The authors observed superior siRNA loading, release and gene-silencing efficiency with their system. A cytotoxicity assay indicated that A549/DDP cells were arrested at the G1 phase and the treatment had improved cytotoxicity. *In vivo* studies using nude mice further demonstrated improved antitumor effect, with no noticeable abnormalities caused in the major organs [67].

PAMAM dendrimers can also easily interact with PEG-b-poly(-propyl methacrylate-co-methacrylic acid) [PEG-b-P(PrMA-co-MAA)] in a reversible fashion and this resulted in the formation of pH-responsive core-shell-type poly-ion complex micelles (PICMs) with a discrete size of 50–60 nm. These complexes are thought to hold antisense oligonucleotides and siRNAs in their cores. Based on this approach, anti-CD71 Fab-conjugated PICMs have been developed and loaded with siRNAs. The targeted PICMs showed good stability under serum conditions, and shielded siRNA against enzymatic degradation. The cellular uptake of targeted PICMs by PC-3 cells increased as the proportion of ligand-functionalized polymer was increased and reached a plateau at

1.25 mol% Fab per anionic polymer chain. Overall, these targeted PICMs were show to effectively downregulated the expression of Bcl-2 mRNA and oncoprotein [68]. Numerous methods have been reported to prepare nanotechnology-based therapeutics for the treatment of malignant gliomas. However, none of them offers a single modality of detection or delivery, and they have proven to be toxic at functional concentrations [69]. To achieve siRNA delivery to a transgenic murine model of glioblastoma, magnetic nanoparticles bearing polyvalent G4 dendrimers with a cystamine core (called dendriworms) were developed [70]. Authors reported that no significant *in vitro* toxicity in HeLa cells was seen at different concentrations of dendriworm or siRNA. It was also reported that siRNA and dendriworm doses strongly influenced gene silencing. The dendriworms were found to promote cytosolic release of the endocytosed cargo more efficiently, resulting in effective siRNA delivery to the cell cytoplasm. Additionally, the non-covalent attachment of siRNA resulted in dendriworms with tunable siRNA loading without the need for reformulation. Dendriworms enabled significant suppression of epidermal growth factor receptor (EGFR) expression in glioblastoma *in vivo* [7,70,71].

Conclusions and future perspectives

Numerous fundamental mechanisms of tumor progression, metastasis and invasion have been explained at both the cellular and molecular levels. This has enabled the development of nanotechnology-based therapies when conventional chemotherapeutic approaches are ineffective. Several nanocarriers have been developed for siRNA delivery; however, these nanocarriers are not without problems. The ideal nanocarrier system should be about 10–50 nm in size, non-toxic, non-immunogenic, stable, capable of protecting the siRNA from degradation and more importantly promote efficient intracellular delivery of siRNAs to the cytoplasm. Dendrimers especially PAMAM based ones are a versatile platform for siRNA delivery because of the presence of peripheral positive charges which interact with siRNA and result in the formation of dendriplexes. The unique properties and well-established characterization protocols of dendriplexes make PAMAM dendrimers as an ideal carrier for siRNA transfection. This review describes the advantages of PAMAM dendrimer-mediated siRNA delivery using dendrimers alone or in a hybrid system. Further exploration of PAMAM hybrid systems will result in even more effective nanocarriers for RNAi therapy. The possibility of using lower generation dendrimers as well as dendrimer-siRNA conjugates represents another step forward in this rapidly growing arena of dendrimer mediated RNAi therapeutics.

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