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PAMAM dendrimers as efficient drug and gene delivery nanosystems for cancer therapy

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Abstract

Drug delivery systems for cancer chemotherapy are employed to improve the effectiveness and decrease the side-effects of highly toxic drugs. Most chemotherapy agents have indiscriminate cytotoxicity that affects normal, as well as cancer cells. To overcome these problems, new more efficient nanosystems for drug delivery are increasingly being investigated. Polyamidoamine (PAMAM) dendrimers are an example of a versatile and reproducible type of nanocarrier that can be loaded with drugs, and modified by attaching target-specific ligands that recognize receptors that are over-expressed on cancer cells. PAMAM dendrimers with a high density of cationic charges display electrostatic interactions with nucleic acids (DNA, siRNA, miRNA, etc.), creating dendriplexes that can preserve the nucleic acids from degradation. Dendrimers are prepared by conducting several successive "generations" of synthetic reactions so their size can be easily controlled and they have good uniformity. Dendrimers are particularly well-suited to co-delivery applications (simultaneous delivery of drugs and/or genes). In the current review, we discuss dendrimer-based targeted delivery of drugs/genes and co-delivery systems mainly for cancer therapy.

Keywords

PAMAM dendrimers; Targeted drug delivery; Gene delivery; Nanovehicles; Co-delivery

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1. Introduction

Cancer is one of the most serious diseases that affects mankind and is the second leading cause of mortality and morbidity in the world (after cardiovascular disease). The National Center for Health Statistics in 2013 reported that one in four deaths is caused by cancer in the United States [1]. Conventional strategies for cancer treatment mostly include chemotherapy, surgery and radiotherapy [2]. Chemotherapy is the most common choice for many types of cancers, but the long-term outcomes of most chemotherapy regimens are not usually very satisfactory due to the development of tumor resistance. Resistance develops, partly because the doses of cytotoxic drugs that can be used in humans are insufficient to kill all the cancer cells, and therefore resistant cells are selected for. Doses of chemotherapy are limited by unacceptable and indiscriminate toxicity to normal tissues and organs due to the non-specific drug distribution in the body. This sub-optimal efficacy of many chemotherapy agents has been attributed to the low water-solubility of many anti-cancer drugs, their poor stability, non-targeted biodistribution within the body, and pronounced side effects against healthy organs and tissues [3,4]. Drug-delivery vehicles are being developed to try to overcome the many deficiencies displayed by cancer chemotherapy agents [5–7]. The purpose of these drug delivery vehicles has been variously proposed to be: (a) protect their cargo from being degraded within the body before they reach their desired target; (b) protect the normal tissues from non-specific toxicity; (c) allow passive targeting due to the “enhanced permeability and retention effect”; (d) allow active targeting by attachment of ligands to their surface; (e) allow controlled drug release at predictable rates; (f) allow “smart” or on-demand release in response to some particular stimulus [6–16]. Combination therapy often uses several chemotherapy drugs co-administered at the same time, aiming to achieve a synergistic effect. Recently co-delivery systems have been developed, and investigated in order to deliver two or more therapeutic agents (both can be drugs or one can be a drug and the other can be a gene) into the same tumor simultaneously to improve treatment efficacy. Successful delivery of both chemotherapeutic drugs and genes to specific intracellular compartments of cancer cells, and with specificity for different tumors over normal tissues and organs is a desirable property of co-delivery systems. This approach can also help to overcome multi-drug resistance (MDR) of tumors [17,18]. There are a large variety of different nanostructures that have been used as nanocarriers, such as liposomes, polymeric nanoparticles, micelles, solid lipid nanoparticles, nanocages, gold nanoparticles, nanocrystals, graphene oxide, carbon nanotubes, and dendrimers. All of these have been investigated for delivery of various drugs and genes to target different diseases and cancer in particular [19–26]. Among these nanosystems, dendrimers represent a class of synthetic macromolecules and tree-like structures. Dendrimers are built up by repeating the assembly of the constituent layers (generations) by covalent conjugation of synthons to the central core, and eventually different functional groups can be attached to their outer surface as a “capping agent” [27,28]. Some advantages of dendrimers compared to traditional linear and branched polymers are as follows: (a) The well-defined tunable nanosize range, degree of uniformity, structure and molecular weight of dendrimers allow researchers to choose appropriate generation numbers for their specific purposes [29,30]. (b) The globular morphology of dendrimers and their abilities to pass through cell walls owing to their controllable size and lipophilicity make them a suitable drug delivery system [31]. (c)

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Dendrimers can be modified with different ligands such as targeting moieties, imaging probes and biocompatible ligands due to their high reactivity for specific attachment and good solubility [29]. (d) The excellent flexibility of dendrimers makes them a good gene delivery system, because they are able to enhance the endosomal escape of dendriplexes (dendrimers electrostatically bound to nucleic acids) via the "proton-sponge" effect [29,32]. There are many different types of dendrimers such as peptide dendrimers (PPI), poly(L-lysine) dendrimers, polyamidoamine (PAMAM) dendrimers, PAMAM-organosilicon dendrimers (PAMAMOS), etc. [33,34]. However, among these dendrimers, PAMAM has been widely investigated as a carrier for delivery of therapeutic molecules and genes [35]. Therefore, the goal of this review article is to provide a comprehensive overview of PAMAM dendrimer-based nanocarriers that have been used for the targeted delivery of drugs/genes as well as co-delivery systems.

2. PAMAM dendrimers

PAMAM dendrimers are a family of highly branched and mono-disperse synthetic macromolecules with well-defined structures and compositions. Synthesis of these nanocarriers can be carried out through the divergent route. Their core is usually ethylenediamine, to which methyl acrylate and ethylenediamine (EDTA) are repeatedly added according to the desired number of generations G0, G1, G2, G3, G4, etc. (Fig. 1). It is also possible to have generations called G0.5 by terminating the reaction sequence after addition of methyl acrylate that leads to terminal carboxylate groups (Fig. 2). Superficial branches PAMAM could be terminated by different functional groups including NH_2, OH, CHO, COOMe, Boc, COONa or CH_3 groups. The NH_2 group is typically employed to deliver genetic material into cells. The size and shape of G3, G4 and G5 PAMAM dendrimers are similar to insulin, cytochrome C and hemoglobin, respectively justifying the description of their properties as "artificial proteins". PAMAM dendrimers have internal cavities and peripheral functional groups that can be modified to encapsulate drugs or other cargos. Controlling interactions between PAMAM and drug is possible using this versatile nanomaterial. Additional properties of these nanomaterials include non-immunogenicity, water solubility, spherical structure, acceptable biodegradation, biocompatibility, minimal nonspecific blood-protein binding and controlled drug release that make them suitable carriers to deliver drugs and genes. Furthermore, they have been extensively used as co-delivery systems for the simultaneous delivery of genes and drugs [36–41].

2.1. Drug complexation to PAMAM dendrimers for controlled drug release

Hydrophobic drugs with low solubility are able to be physically encapsulated or entrapped inside the internal void or pockets of PAMAM dendrimers. This molecular encapsulation is responsible for the improvement in aqueous solubility and controlled release profile. It is crucial to note that these physical interactions between the PAMAM and drugs in the complexes in aqueous solution are able to be controlled through a range of noncovalent interactions such as hydrogen bonding, electrostatic interactions, steric hindrance and hydrophobic interactions. These interactions may have effect on the drug release profile. In the drug release process, the amine groups remain deprotonated, while the branches come together by contracting into the central core. This mechanism controls the drug release

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process in different environments. For instance, since the microenvironment of the tumor vasculature is acidic, the amine groups will be protonated and their conformation will be altered. Low pH can provide an appropriate stimulus for release of the drug. Therefore, drug release from PAMAM dendrimers always has a pH-sensitive property and tends to be faster in acidic environments [33,42–46]. In one report, tetramethyl scutellarein (TMScu) a poorly soluble flavonoid (with anticancer properties) was encapsulated by the EDTA core of PAMAM-G4 dendrimer to increase its solubility, encapsulation efficacy, drug loading and in vitro release profile [46]. Results of this research showed that PAMAM-G4 dendrimer was able to increase water solubility of the drug. The TMScu encapsulation efficacy and the drug loading rates were 77.8 ± 0.69% and 6.2 ± 0.06%, respectively. Also, the highest percentage of TMScu release from the TMScu/PAMAM-G4 complex was achieved under acidic conditions (pH 4.0). In another study, DOX (doxorubicin) was encapsulated into PAMAM-G5 dendrimers. The dendrimer was terminated with three different groups, including acetyl (PAMAM-G5-NHAc), glycidol hydroxyl (PAMAM-G5-NGlyOH) and carboxyl (PAMAM-G5-SAH). The influence of the terminal groups on the PAMAM-G5 drug release kinetics and inhibition of cancer cells was assessed. From the results of this study, all three types of functional groups could effectively help to encapsulate DOX. Furthermore, the DOX/PAMAM-G5 complex showed significant therapeutic effects in inhibition of cancer cell growth. All types of DOX/PAMAM complexes demonstrated variable rates of DOX release at two different pH values (neutral and acidic) at 37 °C. The rates of DOX release from PAMAM-G5-NHAc/DOX, PAMAM-G5-NGlyOH/DOX and PAMAM-G5-SAH/DOX complexes were 5.5%, 18.5% and 36.9% respectively under physiological pH conditions. However, these rates rose to 39.5%, 60.9%, and 68.6%, respectively under acidic condition (pH 5.5) [47]. In addition, it has been reported that the drug loading efficacy could be affected by different factors including the functional surface groups, size, chemical structural, generation numbers, the degree of the PAMAM PEGylation, molecular weight of the loaded drug, pH, type of solvent, and temperature. The generation number and attached polyethylene glycol (PEG) chain length on the surface of the PAMAM dendrimer could affect DOX encapsulation efficacy. For instance, conjugated PEG2000 on the surface of PAMAM-G4 could maximize the encapsulation of DOX molecules per single PAMAM dendrimer (almost 6.5 fold), while the same delivery system was able to encapsulate 26 molecules of methotrexate (MTX). It was concluded that the high MTX encapsulation efficacy was related to the electrostatic interactions among negative charges of MTX and positive charges of the PAMAM-G4 dendrimer [48]. It was proposed that one effective method to control the rate of drug release from delivery vehicles would be "encapsulation of the complexes through modulatory liposomal controlled release systems" (MLCRS). In 2005, Papagiannaros and coworkers [49] successfully encapsulated DOX into the PAMAM-G4 dendrimers, and then they encapsulated the formed DOX/PAMAM-G4 complex into the MLCRS system. This hybridation method increased the DOX loading efficacy and release time.

2.2. Drug conjugation to PAMAM dendrimers for controlled drug release

Covalent conjugation of drugs to the PAMAM dendrimers (peripheral groups) has been used to enhance therapeutic efficiency and solubility, reduce non-specific toxicity, and provide a sustained drug release profile [35,50]. The surface groups of the PAMAM dendrimers

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provide a versatile attachment point for conjugation of various therapeutic agents from anticancer drugs to imaging reporters without losing the spherical geometry of PAMAM dendrimers in solution. As shown in Fig. 3, anti-cancer drugs could be conjugated to the PAMAM surface via direct coupling or via cleavable linkers. Some chemotherapy drugs such as DOX, MTX, PTX and cisplatin have been conjugated to the PAMAM surface through direct coupling [51–53]. Alpha-tocopheryl succinate (a-TOS) a vitamin E derivative with anti-cancer activity has poor solubility in water. Therefore, in recent studies, a-TOS has been conjugated to the surface of PAMAM dendrimers through direct coupling [54–56]. In one of these studies, a new formulation was described containing a-TOS conjugated to PAMAM-NH_2 G5.0 through an amide bond. The major fraction of amines on the surface were acetylated and the remaining amines were covalently conjugated to fluorescein isothiocyanate (FI) and also to folic acid (FA). The final multifunctional formulation (PAMAM G5-$NHAc_n$–a-Tos–FI–FA) was water soluble and stable over the relevant pH range. In addition, this formulation could successfully inhibit growth of cancer cells [55]. In another study, Gurdag et al. in 2002 compared anti-cancer activities of two amide bonded PAMAM-MTX conjugates [57]. The amide bond in the first conjugate was formed between amine groups of MTX and the carboxylic acid terminated PAMAM-G2.5 dendrimer (conjugate A), while the second conjugate was formed between carboxylic acid groups of MTX and amine terminated PAMAM-G3 dendrimers (conjugate B). The results of this study showed that conjugate A was able to induce three-fold higher cytotoxicity on lymphoblastic leukemia cells compared to free MTX, while conjugate B revealed 10-fold lower cytotoxicity compared to free MTX. These results may be explained through differences in the intracellular MTX release profiles of the two conjugates A and B. Kurtoglua and coworkers in 2010 directly conjugated ibuprofen to PAMAM-NH_2-G4 and PAMAM-OH-G4 dendrimers by creating amide and ester bonds, respectively [58]. The results of this research indicated that the enzymes plasma esterase and cathepsin B were not able to hydrolyze amide or ester bonds. In addition, ibuprofen conjugated to the PAMAM-NH-G4 (amide bond) did not show any significant release at different pH ranges (1.2, 5.0, 7.4 and 8.5). By contrast, the direct ester conjugation of ibuprofen to PAMAM-OH-G4 was released at different pH values, and at pH 5 and 8.5 ibuprofen release was 3% to 38%, respectively. Furthermore, zero-order release kinetics for the ester conjugate were obtained. The stability of the ester bond to enzymatic hydrolysis suggested that the conjugated PAMAM-G4 dendrimers block the enzyme activity. It has been proven that steric crowding on the surface of the PAMAM by the conjugated drugs as well as the attaching bonds hinders the drug release process. However, the undesirable high stability of directly conjugated drugs under different conditions, especially amide bond conjugation, could be a limiting factor for this method [43,58]. To overcome the aforementioned problems, some cleavable linkers have been used as spacers to attach drugs to PAMAM dendrimers [59]. Further, the biological and chemical hydrolysis of these linkers has been examined in both exogenous and endogenous processes. For example, the cleavable ester linkers are highly sensitive to enzyme activated cleavage (alkaline phosphatase, carboxylesterase and hydrolases) as well as to chemical hydrolysis (high or low pH, and catalyzed by metal cations) [32,59,60]. In one study, poorly water soluble compounds such as ursolic acid (UA) and FA were covalently conjugated to PAMAM-G3 and G5 dendrimers via acid-labile ester linkers. This research found that the cytotoxicity of the drugs against Hela cells had been

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dramatically increased. Furthermore, since the linker was an ester type, the UA release from FA-PAMAM-G3-UA complex was sensitive to pH and the UA displayed a biphasic release pattern, an initial fast release followed by a longer sustained release phase [59].

Hydrazone hydrolysis is another mechanism for controlled drug release that depends on pH conditions. Hydrazine cleavable linkers such as acyl, alkoxycarbonyl and sulfonyl hydrazines are able to be hydrolyzed under acidic conditions (pH 5), and this property makes them an appropriate candidate for controlled drug release from drug delivery systems inside endosomal and lysosomal organelles [60–62]. In a report by Chang and colleagues, a newly developed complex was prepared from super paramagnetic iron oxide nanoparticle (IONPs), and modified PAMAM-G3.5 with an attached FA ligand, equipped with PEG and DOX compounds that were linked via acyl hydrazone cleavable linker [63]. The final complex made from core IONPs and shells including FA-PEG-PAMAM-DOX. This study indicated that the core-shell nano complex (FA-PEG-PAMAM-DOX@IONPs) could not induce significant cytotoxicity against MCF-7 cells, which may be explained by presence of PEG and FA ligands on the PAMAM dendrimer. In additions, the data obtained for drug release showed that around 75% of DOX was released from the core-shell complex at pH 5.03 after 15 h. In contrast, less than 5% DOX was released at pH 7.4 over the same period of time. [63].

Amide linkers are another type of linker that cannot be easily chemically hydrolyzed in physiological condition, while they are susceptible to some peptidase enzymes such as matrix metalloproteinase (MMP). However, the pH dependent cleavage of amide linkers including cis-aconityl, citraconyl and maleyl groups has been explored in controlled drug release [61]. The conjugation of DOX to PEGylated PAMAM-G4 dendrimers via cis-aconityl and succinimidyl linkers was reported by Zhu and coworkers [64]. The PEG-PAMAM-cis-aconityl-DOX (PPCD) complex showed increased cytotoxicity against SKOV3 cancer cells compared with the free drug. However, the cellar uptake was reduced in the presence of a high degree of PEG on the PAMAM-G4 dendrimers, while the drug release was increased with an increasing degree of PEG. The DOX release rate from the PPCD complex was both time and pH dependent. The designed PEG-PAMAM-succinic-DOX complex did not show drug release at different pH values [64]. In another study, PTX (paclitaxel) was attached to the PAMAM-G4 dendrimers via the cathepsin B-cleavable tetrapeptide, Gly-Phe-Leu-Gly (GFLG). It was noted that cathepsin B shows a high expression level in breast cancer cells [65]. Therefore, it could be useful to design a new drug delivery system based on cleavable GFLG linker giving controlled drug release after enzymatic hydrolysis. The results of this study revealed high cytotoxicity against breast cancer cells via PAMAM-GFLG-PTX complex. In addition, PTX release from PAMAM-GFLG-PTX complex was confirmed in the presence of papain (a homologue of cathepsin B enzyme) [65].

Another mechanism to control the rate of drug release is called "intracellular disulfide exchange". Disulfide bonds are not affected by hydrolysis, while they are sensitive to disulfide exchange or reduction reactions. Therefore, the drug release process could be triggered via glutathione (GSH) that is localized in the mitochondria (30%) and cytoplasm (85%) [66,67]. To address this mechanism, a GSH-sensitive nanocarrier (PAMAM-G4-

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DOX-Angiopep-PEG) was prepared by covalent conjugation of Angiopep-2 as a dual-targeting group and DOX as a cytotoxic drug onto the peripheral groups of PAMAM-G4 dendrimer in order to improve transportation across the blood-brain barrier (BBB) and DOX accumulation in glioma cells. It was shown that around 44% of DOX was released from the PAMAM-G4-DOX-Angiopep-PEG complex in the presence of GSH (10 mM), while DOX could not release more than 3% in the absence of GSH at physiological condition [68].

A drug release mechanism has been assessed using optical techniques (photochemistry) for probing the drug release rate and time as well [69]. In this method linkers that absorb light (photocages or photocleavable) can be used to evaluate drug release light sensitive drug delivery systems by varying the wavelength and intensity of light radiation. Photocleavable linkers such as o-nitrobenzyl (ONB), benzophenone, coumarin, xanthene, and quinolone are used as UV-light responsive linkers [29,61]. Choi and coworkers in 2011 investigated the effect of light radiation on conjugated MTX to PAMAM-G5-FA via o-nitrobenzyl (ONB) linkers. From the result of this study, it was shown that the drug release rate was dependent on time, wavelength, and types of linker [70].

2.3. PAMAM based targeted delivery system

2.3.1. Passive targeting—Nanomaterial-based targeted drug delivery is a strategy for delivery to a patient of a therapeutic drug in a manner that increases accumulation at the desired tissue site. The rationale of using passive targeting depends on the specific features of the delivery system, and the particular pathogenesis of the disease to be treated. This strategy not only is effective in drug and gene delivery to specific cells/tissues but also has beneficial effects on maximizing efficacy and minimizing side effects. An important passive targeting method was introduced by Maeda and Matsumura [71,72] and became known as the "enhanced permeability and retention" (EPR) effect. The EPR been widely used by researchers not only to design nanomedicines for comating cancer, but also to target drugs to some other diseases like infections and chronic inflammatory conditions. Some of the unique properties of solid tumors, including the hyper-proliferative vasculature, defects in vascular architecture leading to leaky capillary blood vessels, and absent or non-functional lymphatic drainage are together responsible for the EPR effect, whereby nano-vehicle delivery systems accumulate specifically in tumors after intravenous injection. Tumor treatment by the systemic administration of polymer-based drug delivery systems can produce 10–100-fold higher drug concentrations in tumors due to the EPR effect [73]. Therefore, researchers have often used polymer-based nanosystems for passive drug delivery.

2.3.1.1. Active targeting: Active targeting is based on covalent attachment of specific ligands onto drug carriers that can be recognized by receptors, antigens or other molecules that are over-expressed on the target tissue or cells (compared to non-target surrounding healthy cells or tissues). Recognition of the ligand by its cognate receptor, results in the gradual accumulation of the nanocarrier at the pathological site [74,75]. Active targeting is mainly carried out by strong ligand-receptor binding affinity, and in addition this binding can also increase internalization of the cargo by enhancing cellular uptake. Tumor cells are characterized by a much faster proliferation rate (doubling time about 24 h) compared to

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normal cells (doubling times of days to weeks). This fast growth rate means the tumor cells require much more of many nutrients and vital molecular components compared to normal cells. Therefore, tumor cells upregulate the expression of several different types of receptors in an attempt to obtain these requirements. These receptors have been used as tumor-specific targets, especially folate receptors [76], transferrin receptors [77], low density lipoprotein receptors [78], as well as other molecules such as integrin receptors and adhesion molecules [79]. Therefore, functionalized nanocarriers that have specific ligands attached on their surface can be recognized and internalized by specific cells and tissues, and provided that the nanovector can pass through the blood vessels and capillaries supplying the tumor [75,80].

2.3.2. Targeted drug delivery using PAMAM-dendrimer—Targeted drug delivery using nanomaterials such as PAMAM dendrimer bearing ligands has several advantages: (a) it can protect normal cells from cytotoxic agents; (b) it can reduce the dose-dependent side-effects of drugs; (c) it can overcome drug resistance of cancer cells [81]. Monoclonal antibodies are probably the most useful ligand to target cancer cells by binding to a specific cognate antigen over-expressed on the cancer cells [82]. Conjugation of an antibody onto the dendrimer surface has been often used for tumor targeting, while the high molecular weight of antibodies (150 kDa) is the main disadvantage. Another well-studied cancer targeting ligand is folic acid (FA). It has been widely used to target FA receptors (FAR) due to the high rates of FAR over-expression in many different types of cancer such as breast, kidney, head, brain and lung [79]. Zhang et al. in 2011 successfully delivered methotrexate (MTX) via a folate-functionalized 3.0G PAMAM dendrimer (G3-MTX) into KB oral squamous carcinoma cells. They showed high dose-dependency cytotoxicity of G3-MTX in this model. They proposed that enhanced uptake of the G3-MTX by the cells via folate receptor-mediated endocytosis was the main explanation for the high cytotoxicity [83].

In an earlier study by Behrooz and colleagues in 2016, they used a single-strand AS1411 aptamer (APTAS1411) for targeting MKN45 gastric cancer cells. They successfully conjugated APT (aptamer) to PAMAM-PEG and loaded it with 5-FU. Use of this complex led to an increase in 5-FU uptake by the MKN45 cells. The cytotoxic effects of several complex structures were assessed by MTT assays in two different cell lines: HEK293 (human embryonic kidney cells) and MKN45. PAMAM-PEG-APTAS1411-5-FU treated HEK293 and MKN45 cells showed a viability decrease to around 18% and 11% survival, respectively [84]. Peptides have been explored as a way to target cancer cells that over-express certain specific receptors that can recognize the peptide. One well-known example is the Arg-Gly-Asp (RGD) peptide, which has a strong affinity to αvβ3 integrin, which is expressed at high levels on the surface of tumor microvasculature as well as some cancer cells [85]. In 2016 [86], Ma and co-workers investigated a peptide called RGD-TAT (RGD attached to amino acids 49–57 of the HIV TAT protein that functions as a “cell penetrating peptide”) and attached it to G4 PAMAM to produce a new RGD-TAT-PEG-PAMAM (RTPP) nanocarrier that could encapsulate methotrexate (MTX) as a model drug. In vitro targeting ability of the complexes was studied in HepG2 and MCF7 cells (expressing αvβ3 integrin at high and low levels respectively) was studied using a fluorescent indicator. Their results showed that HepG2 cells (over-expressing αvβ3 compared to MCF7cells) showed higher

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cytotoxicity due to a combination of RGD recognition and the TAT cell-penetrating ability [86]. Effective delivery of anticancer drugs to brain tumors is difficult because of limited penetration through the "blood-brain-barrier" (BBB). Kesharwani and co-workers decorated 4.0G PAMAM loaded with doxorubicin (DOX) with two different targeting ligands, comprising the iron-binding protein, transferrin, and the lectin, wheat germ agglutinin (WGA). Transferrin binds to transferrin receptors and allows translocation through the tight junctions of the blood vessels, while WGA binds to specific carbohydrate residues on the tumor cells especially in brain tumors. By using this dual-targeted drug delivery system, they successfully delivered DOX into brain tumors [87,88]. Almost 20% of breast cancers are highly positive for the human epidermal growth factor receptor type 2 (HER2). High expression levels of HER2 can stimulate growth of breast cancer cells, and the tumors are more aggressive and more likely to metastasize early, compared to HER2 negative breast cancers [88]. Trastuzumab (TZ) is a clinically applied monoclonal antibody that recognizes HER2. Kulhari and colleagues synthesized dendrimers (4.0G PAMAM) linked to TZ as a targeting agent, in order to improve docetaxel (DTX) delivery to HER2-positive breast cancer (Fig. 4). DTX-PAMAM, TZ-PAMAM-DTX and TZ-PAMAM effect on HER2-positive and HER2-negative negative breast cancer cells was evaluated using MTT assays and flow cytometry analysis. This study clearly showed that the total percentages of apoptotic cells for DTX alone, DTX-PAMAM and TZ-PAMAM-DTX were 16.5%, 32.7% and 42.6%, respectively [89]. Some of the targeted drug delivery systems based on PAMAM dendrimers that have been used to deliver chemotherapeutic drugs to cancer cells are summarized in Table 1.

3. PAMAM based gene delivery system

3.1. Gene and PAMAM dendrimer binding mechanisms

PAMAM dendrimers are able to create stable PAMAM-nucleic acid complexes to prevent nucleases activities. These properties have been considered by the researchers to design nanocarriers based on PAMAM dendrimers for gene delivery purposes [81–85,90,91]. From the biophysical studies concerning PAMAM/DNA complexes, electrostatic interactions are the dominant force for the binding process. Furthermore, it is proposed that the positively charged amine groups on the dendrimer and phosphate groups of the DNA play the main role in these interactions. Since PAMAM dendrimers is responsible for inducing DNA condensation and different wrapping around, it needs further investigation for gene delivery purposes [86–88]. To address this phenomenon, different structural techniques including circular dichroism (CD), Fourier transform infrared (FTIR), UV–visible spectroscopic methods and atomic force microscopy (AFM) were used by Froehlich and coworkers to analyze the binding site between calf-thymus DNA and mPEG-PAMAM-G3, mPEG-PAMAM-G4 and PAMAM-G4 dendrimers as the models. The results of this study indicated that the PAMAM dendrimers could strongly interact with DNA by interactions with the major and minor grooves and the backbone of phosphate groups. In addition, measurements of the stability of the PAMAM/DNA complexes clearly indicated that PAMAM-G4 dendrimer was more stable than the others [89]. Simulation studies of the siRNA complexes with PAMAM (G4–G6) dendrimer showed that PAMAM dendrimers bind to siRNA mainly through electrostatic interactions and some hydrogen bonds. The mentioned hydrogen bonds

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occurred between H atoms of the amine groups (PAMAM dendrimers) and O atoms of the phosphate groups (siRNA). Also, it should be mentioned that the successful formation of the hydrogen bonds is dependency on the pH. Furthermore, it is valuable to consider that the core of the PAMAM-G (4–6) dendrimers (triethanolamine, TEA) showed higher affinity to bind to siRNA at different pH values compared with the NH_3-core [92]. As previously mentioned, the surface modification of the PAMAM dendrimer has been carried out for different purposes. For instance, PAMAM-G (4–5) dendrimers that has been modified with PEG and acetyl groups will lose the positive charges on its surface, and therefore the cytotoxicity of the dendrimers would be reduced. Also, these modifications are appropriate to design safe nanovectors due to increasing the biocompatibility of the PAMAM dendrimers, but the reduction in cationic charge is responsible for reducing the transfection efficacy of the DNA. Poor condensation of DNA could be explained by the decrease of the interactions between positive charges of the PAMAM dendrimers and negative charges of DNA. However, different surface modifications could increase the transfection efficacy via the improving nucleic acid condensation. In a study, PAMAM-G5 dendrimer was modified with guanidyl and phenyl groups [93]. The obtained results in this study revealed that the localization of guanidyl and phenyl groups on PAMAM dendrimer periphery led to synergistic effects in crossing extracellular and intracellular barriers. The guanidyl groups on the PAMAM dendrimer surface could increase DNA and siRNA condensation through the guanidinium-phosphate interaction, while the phenyl groups could induce the efficient endosomal escape [93].

3.2. PAMAM dendrimer based targeted gene delivery

Gene therapy requires special methods to deliver nucleic acids into the targeted cells and tissues. This is because nucleic acids have a pronounced negative charge due to the phosphate groups linking the sugar backbone that prevents efficient uptake into cells. Therapeutic nucleic acids are designed to trigger or suppress the expression of specific genes that are responsible for the biosynthesis of different proteins, and modification of which can play a vital role in combating a wide range of diseases such as cancer. The successful delivery of nucleic acid-based therapeutic agents, including antisense oligonucleotides, ribozymes, siRNA and plasmid DNA into human cells needs effective transfer agents that should have a good safety profile and should show specificity for target cells [58,82,94]. In other words, an important challenge for gene therapy is finding effective and safe vectors to help deliver nucleic acid-based therapeutic agents into target cells/tissues. Since naked nucleic acid molecules are easily degraded by serum nucleases, and therefore have only a short half-life in vivo, it is important to design carriers to preserve them from enzymatic degradation. Moreover, increasing their serum half-life leads to longer circulation times increasing the chances of uptake into the target cells or tissues. On the other hand, it is also important to direct the nucleic acids into the appropriate locations inside the cell, such as the cell nucleus, mitochondria, or cytoplasm. It is known that viral gene vectors including: adenoviruses, retroviruses and adeno-associated viruses have an intrinsically efficient capability for delivery of genetic-based therapeutics into the target cells. However, the use of viral based vectors also possesses undesirable effects such as immunogenic reactions, and the possibility to cause cancer by insertion into the host cell DNA [83,95,96]. These important limitations have led to a search for non-viral vectors that will not be immunogenic

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or carcinogenic [83]. The positive charges on the surface of the PAMAM and the negative charges on the DNA or siRNA phosphate backbones interact with each other, thus forming stable complexes such as PAMAM-DNA or PAMAM-siRNA These complexes are called "dendriplexes" and display a high efficiency of transfection and a powerful ability to preserve the DNA or siRNA from degradation [84,97]. Recent reports that have demonstrated the role of PAMAM dendrimers in the delivery of DNA, siRNA and miRNA into cancer cells are summarized in Table 2.

3.2.1. DNA delivery using PAMAM dendrimer—PAMAM dendrimers possess amine groups on their surface and their interior. The surface amine groups have essential roles in binding and compacting the negatively charged DNA into overall neutrally-charged nanoparticles, thus increasing the cellular uptake of DNA and other nucleic acids [83]. However, the buried tertiary amino groups (within the dendrimer interior) act as a "proton sponge" after these dendriplexes have been taken up by endocytosis into endosomes, and help to release the DNA out into the cytoplasm. It has been suggested that PAMAM dendrimers that had been partially degraded were more flexible than intact dendrimers, so they could interact better with DNA.

There are some studies about the transfer of DNA into the cells and tissues by conjugation of a specific targeting ligand onto the PAMAM surface in both in vitro and in vivo models. Li et al. [85] reported that 5.0G PAMAM dendrimers could bind to DNA to form complexes via electrostatic interactions between the positive groups on the PAMAM surface and the negatively-charged phosphate groups on the nucleic acids. These researchers selected EGFR as the target, which was over-expressed on HepG2 cells, and grafted the anti-EGFR monoclonal antibody h-R3 onto the dendriplexes to deliver DNA coding for the tumor suppressor gene p53 (Fig. 5). Their study showed that in contrast to non-targeted (dendriplexes), h-R3-dendriplexes displayed low cytotoxicity, more nuclear accumulation in HepG2 cells, and higher cellular uptake and transfection efficiency. In addition, the ex vivo biodistribution within the tumor showed that h-R3-dendriplexes performed better as gene delivery vehicles. Xu et al. reported another targeted delivery system by conjugating FA onto 4.0G PAMAM dendrimer to function as a DNA plasmid gene carrier for delivering gene into head and neck cancer cells. The G4-FA/plasmid polyplexes were taken up by receptor-mediated endocytosis [84]. Another plasmid DNA delivery system was developed by Huang et al. in 2011 [85] using PAMAM-PEG-angiopep-2 complexes. Angiopep-2 can bind to low-density lipoprotein receptor-related protein-1 (LRP1), which is over-expressed in brain capillary endothelial cells (BCECs) and also in glial cells, and can therefore be used as for targeting. According to cellular uptake studies, the DNA sequence was delivered into the nucleus after the PAMAM-PEG-angiopep-2-DNA plasmid dendriplex had been released from the endosomes and lysosomes by the proton sponge effect. The authors also found that the in vivo biodistribution of PAMAM-PEG-angiopep-2-DNA plasmid within the brain (and particularly within the tumor itself) was higher than control plasmid dendrimers PAMAM-PEG-DNA and PAMAM-DNA.

3.2.2. RNA delivery by PAMAM dendrimer—Small-interfering RNA (siRNA) has an important role in the inhibition or silencing of different cellular pathways via destroying

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mRNA molecules that code for the target genes. RNAi therapeutics use different siRNA molecules to inhibit signaling pathways related to cell proliferation and anti-apoptosis. In addition, siRNA has a remarkable potential to down-regulate the expression of multi-drug resistance genes to increase the activity of cytotoxic anticancer drugs/agents against tumors. Naked siRNAs can be rapidly eliminated or degraded (even faster than DNA plasmids) via serum ribonuclease enzymes, and their cell uptake is equally difficult due to their polyanionic nature and large molecular weight. Therefore, delivery of siRNA molecules alone is unlikely to have any therapeutic benefit in vivo. What is needed is a non-toxic delivery vehicle to guide and protect the siRNAs until they reach their intended target. Some recent studies have suggested that PAMAM dendrimers could fulfill this role, especially if one or more targeting ligands were to be attached to the exterior to enhance selectivity, and facilitate intracellular uptake.

Patil and co-workers in 2009 [84] successfully conjugated luteinizing hormone-releasing hormone (LHRH) peptide onto the surface of a quaternized dendrimer 4.0G QPAMAM-OH to transfer anti-Bcl2 siRNA into ovarian cancer cells by targeting the LHRH receptor. Bcl2 is an anti-apoptotic protein over-expressed in cancer, and destroying it can lead to induction of apoptosis without any other cytotoxic drug. The analysis of cytotoxicity by MTT assays indicated that the quaternized charged dendrimer QPAMAM-OH and QPAMAM-OH-LHRH (without any siRNA) gave only about 5–10% cell death even with as high a concentration as 12.5 μM. The cell uptake and intracellular localization of plain siRNA and siRNA-dendrimer complexes were compared. Confocal microscopy shows that plain siRNA failed to penetrate the cancer cells, while both the QPAMAM-OH dendrimer and QPAMAM-OH-siRNA were taken up well into the cancer cells, and had similar localization sites in the cytoplasm and the nucleus. Gene expression results also confirmed that QPAMAM-OH-LHRH complexed siRNA decreased the expression of Bcl2 better than the non-targeted QPAMAM-OH.

Ohyama et al. in 2015 successfully delivered siRNA against polo-like kinase 1(siPLK1) via conjugation of FA-PEG (as a targeting agent) onto 4.0G PAMAM dendrimers. They also conjugated FA-α-cyclodextrin onto the PAMAM dendrimer to increase the cargo-carrying ability and improve the siRNA efficiency and extend the blood circulation time. The siRNA delivery activity of this targeted complex was evaluated in cancer cells which over-expressed folate receptor-α (FR-α) compared to FR-α negative cells. The result of their study clearly revealed that the anti-cancer activity of the FA-PEG targeted system (carrier) was significantly higher than the non-targeted carrier. FA-PEG targeted dendriplexes improved uptake by endocytosis in FR-a positive cell. Moreover, their study showed lower cytotoxicity, high serum stability, increase endosomal escape and suppression of tumor growth in animal models [85]. In 2010, Yuan et al. functionalized 4.0G PAMAM dendrimers with epidermal growth factor (EGF) peptide and additionally conjugated quantum dots as an imaging agent to deliver siRNA into cells. The cells expressed EGFR on their surface and had been engineered to express yellow fluorescent protein (YFP). The results showed EGFR specific uptake leading to intracellular accumulation of 4.0G PAMAM-siRNA shown by QD red fluorescence and down-regulation of YFP yellow fluorescence [86].

Micro-RNAs (miRNA) are another new class of therapeutic agents that can play a role in regulation of multiple signaling pathways within the tumor microenvironment. MicroRNAs

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(as well as 17–25 nucleotide endogenous non-coding RNAs) regulate gene expression at the post-transcriptional level, and therefore can modulate cellular processes including proliferation, migration and differentiation. In recent years, studies have investigated the intracellular delivery of miRNAs into cancer cells as a possible anti-cancer therapy [87]. Wu et al. investigated methods to deliver two miRNAs (miR-15a and miR-16-1) into prostate cancer cells. In their studies, an aptamer (10-3.2) that recognized prostate-specific membrane antigen (PSMA) was conjugated to PEG-PAMAM, which was then used as a carrier for the miR-15a and miR-16-1 sequences (that had been selected to suppress PSMA expression). Results showed that the miRNA/PAMAM-PEG-aptamer was more effective in PSMA-positive cancer cells and reduced PSMA expression levels as shown by Western blot analysis [88].

3.3. Gene release from PAMAM dendrimer via endosomal escape mechanisms

Generally, the internalization of nanoparticles into cells strongly depends on their physicochemical characteristics, shape, overall charge and surface modification properties [98]. In a similar manner to other types of cationic nanoparticles, PAMAM dendrimers enter the cells by adsorption-mediated endocytosis. In this process, positively charged amino group of PAMAM dendrimers interact with negatively charged phospholipids on the cell membrane. Also, the surface of PAMAM dendrimers can be modified by different ligands (including antibodies, cell penetrating peptides (CPPs), targeting peptides, etc.). As these ligands recognize specific receptors on the cells, modified dendrimers can also be taken up into cells by receptor mediated endocytosis [99,100].

Endocytosis can occur by several different mechanisms such as, clathrin-dependent endocytosis and caveolin-dependent endocytosis, micropinocytosis, and a clathrin and caveolin independent endocytosis pathway [101,102]. Clathrin is a cytosolic protein that lines small (approx. 100 nm in diameter) vesicles formed by pinching off of coated pits. Caveolin is a cholesterol-binding protein (Vip21) located in a lipid bilayer enriched in cholesterol. Caveolae are small (approx. 50 nm in diameter) flask-shape pits in the membrane that resemble the shape of a cave. The specific internalization mechanism and modes of trafficking are dependent on the nanocarrier (nanoparticle) and cell types [103,104]. As mentioned above, the nanocarrier-cargo complex is taken up into the vesicle coated with caveolin, clathrin, or caveolin/clathrin-independent mechanism. This vesicle fuses with early endosomes existing inside the cell, and the pH drops to around 6.3. In the next step the early endosomes fuse to late endosomes and the pH drops further to around 5.5. Eventually, the contents of late endosomes are delivered to the lysosomes (pH around 4.7), where their contents are designed to be degraded via a range of different lysosomal enzymes that operate under acidic conditions. These include glycosidase, proteases, acid phosphatases, sulfatases, lipases and nucleases. Therefore, the major challenge for non-viral carriers in gene and drug delivery is engineering a vehicle that can be designed to quickly escape materials from the endo-lysosomal compartments and thus protect its cargo from degradation. Although there is still a lot of controversy about the different endosomal escape mechanisms, three mechanisms are generally proposed to explain endosomal escape. These mechanisms are (a) proton sponge effect, (b) membrane fusion and disruption, and (c) pore formation. The proton sponge effect was first proposed by Behr in 1997 [105]. Cationic

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carriers containing a range of internal secondary and tertiary amino groups can prevent acidification of endosomes by capturing and absorbing protons, known as the "proton sponge effect". These carriers are able to neutralize endosome acidification and prevent the pH dropping below physiological levels (reducing H^+ influx from the external buffer) and reducing capture of H^+ ions. Any delay in the fusion between lysosome and endosome will help to prevent degradation of nucleic acids, by encouraging the influx counter-ions such as Cl^- and H_2O. Furthermore, vesicularswelling can permeabilize the endosomes allowing vector–nucleic acid complexes to efflux into the cytoplasm. This swelling and permeabilization results in a high osmotic pressure that eventually causes the endosome/ lysosome to rupture [106–108]. Some other more conventional gene-delivery vehicles such as polyethylenimine (PEI), poly(L-histidine), and imidazole-containing polymers can exert the proton-sponge effect as well, but it is thought that PAMAM dendrimers are particularly effective as gene delivery vehicles due to possessing a mixture of primary, secondary and tertiary amine groups. The primary NH_2 groups are located on the surface of the PAMAM dendrimers, while the tertiary amines are located inside. The tertiary amino groups are mainly responsible for the proton sponge effect, while the primary amino groups play an important role in DNA binding, enhancing the cellular uptake of DNA, and compacting the uncoiled DNA structure. Overall, it should be noted that the intracellular delivery of genetic materials and subsequently their therapeutic efficiency can be affected by these mechanisms [108,109]. To address this phenomenon, experimental studies and computerized simulations have been carried out. For instance, Ouyang and his coworkers demonstrated that the affinity of the nucleic acids for dendrimers under low pH conditions was higher than at neutral pH. They showed that genetic cargos strongly interacted with dendrimers at low pH after entrance of dendrimer/nucleic acid complex into the lysosomes and late endosomes, which protected the nucleic acids from degradation. Fig. 6 displays 1.0G PAMAM dendrimer complexation with siRNA at low pH. After the release of dendrimer-nucleic acid complex from the endo-lysosome and encounters the higher pH in the cytoplasm, the binding between the components decreases which leads to release of free nucleic acids into the cytoplasm [107]. A study on the ability of PAMAM dendrimer to induce endosomal escape was performed by Jin and coworkers [110]. They synthesized PAMAM dendrimer with 1,2-diaminoethane peripheral groups through an amidation reaction on 3.5G PAMAM dendrimer with diethylenetriamine (DET), and the final PAMAM-DET construct was used for delivery of DNA. Their results indicated that the PAMAM-DET had the best buffering capacity, and the membrane disruption ability was pH dependent (going from pH = 7.4 to pH = 5.5). They concluded that better transfection efficiency of PAMAM-DET was related to facilitation of endosomal escape along with endosome acidification.

4. PAMAM dendrimer based co-delivery system

Cancers typically develop resistance to any chemotherapeutic agent that is initially successful (even with recent pathway-targeted drugs) and this phenomenon predictably leads to treatment failures. Therefore, combination therapy approaches (drug + drug, or drug + gene) are being explored to avoid or lessen the development of resistance [111–114].

Hyaluronic acid (HA) is an inert biopolymer with high aqueous solubility and is now being explored in targeting systems [115]. Specific receptors for HA (CD44) are often expressed

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by several cancer cell lines such as pancreatic, breast and lung cancer. Conjugation of HA to the surface of the dendrimers leads to an increase in their blood circulation time by reducing the degree of opsonization and clearance via the reticuloendothelial system (RES). Han and coworkers functionalized 5.0G PAMAM with HA for effective delivery of DOX, as well as a small interfering RNA targeting major vault protein (MVP, a drug efflux pump). The targeted (MVP-siRNA) could reduce MVP expression and increase the cytotoxicity of DOX chemotherapy inMCF-7/ADR cells (Fig. 7). The results of their study revealed that the cytotoxicity, tumor targeting, higher accumulation, and blood circulation time were enhanced using the DOX-PAMAM-HA complex compared to DOX alone. In addition, co-delivery of MVP-siRNA with DOX-PAMAM-HA showed a good gene silencing effect, high stability, and efficient intracellular accumulation of siRNA that led to even higher cytotoxicity [116].

The amino acid sequence HAIYPRH (this peptide is called T7 and has an affinity to the transferrin receptor) has been investigated for targeted cancer therapy. The T7 peptide was linked to a PEGylated PAMAM dendrimer (PAMAM-PEG-T7) in order to co-deliver a therapeutic plasmid (encoding human tumor necrosis factor-related apoptosis-inducing ligand) called pORF-hTRAIL in combination with DOX (an anti-cancer drug) into cancer cells that over-expressed transferrin receptors. This co-delivery system showed good intracellular uptake and efficient gene suppression. Compared to DOX, and pORF-hTRAIL alone the T7 modified co-delivery system showed good induction of apoptosis in vitro and efficiently inhibited tumor growth in an in vivo tumor model [117].

Another co-delivery system was designed by Chen et al. in 2016 to overcome drug resistance in cancer cells. In their study, mesoporous silica nanoparticles (MSNs) were attached to 2.0G PA, to produce a hybrid nanocarrier with two different compartments. Nanocarrier 1 (MSN) was used to deliver the hydrophobic anticancer drug MTX, while nanocarrier II (dendrimer) was used for the hydrophilic drug DOX. HA was used as a tumor-targeting agent. Consequently, the final constructed complex included MSNs-PAMAM-HA containing both MTX and DOX. Confocal florescence microscopy indicated rapid binding of to tumor cells. This was attributed to binding between HA and over-expressed CD44 on solid tumor cells [118]. The cytotoxicity of DOX and MTX, which were loaded into the MSNs-PAMAM-HA hybrid carrier, were evaluated by MTT assays in vitro showing that DOX and MTX used alone had equally weak cytotoxicity effects against both normal cells and tumor cells. 40% of cells treated with DOX and MTX loaded into MSNs-PAMAM-HA were viable after 24 h incubation, and the viability was only 15% after 48 h incubation 15%. These results confirmed that the combination of two different anticancer drugs delivered by the MSN-dendrimer-HA nano-vector had major improvements in efficiency for cancer therapy [118].

5. The parameters that affected drug and gene releases from PAMAM dendrimers

It was reported that dendrimer macromolecules have a special tendency to be linearly increased in diameter parameter and adopted with globular shape by rising dendrimer

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generation number. For example, PAMAM dendrimer generations 2–8 contains the ranging size from 3 to 12 nm. Further, drug loading and release processes could be impacted by PAMAM dendrimers generations (size). As a report [119], Rouhollah et al. investigated the release profile of DOX from the different dendrimer generations (G2, G3, G4 and G7) that were coated with magnetic nanoparticle. This study showed that the DOX release was basically relied on PAMAM dendrimer generation number and the release procedure was declined with increasing of PAMAM generations. It was suggested that the increment of PAMAM dendrimers generations has a strong relationship with increasing the functional surface groups. That is the reason for compacting of the branches in PAMAM structure. This limited swelling property especially in lower pH conditions is responsible for delay in the drug release process [119]. In addition, the higher generations of PAMAM dendrimer are able to restrict the hydrolysis enzyme activities due to 3D spherical structure of the PAMAM dendrimers [58].

The stability of nucleic acid/PAMAM complexes is dependent on PAMAM dendrimer generations. It is reported that stability of the complexes would be increased with addition of the nanocarrier generations number [120]. It seems that the high generations of PAMAM dendrimer (>G7) have a low flexibility due to increasing the surface groups density and compacted spherical structures [121]. These drawbacks would lead to losing inducible characterization of the higher generations of PAMAM dendrimer in endosome.

The surface properties of the nanocarriers have a vital role in its different physicochemical activities including aggregation/agglomeration, biological interactions, dissolution, etc. [122]. Since the surface of PAMAM dendrimers could be decorated with various therapeutic molecules, targeting ligands, imaging agents and other polymers for different purposes, they could be used for improving drug delivery through three basic mechanisms: (1) the improvement of drug loading and release [123]; (2) the decrease of surface positive charges; (3) the enhancement of gene transfection efficacy [124]. For instance, modification of the surface of PAMAM-G4 dendrimers with heparin (HEP) and monomethoxypoly(ethylene glycol) (mPEG) which were loaded with DOX showed approximately 29.5% drug release at 8 h, while release was 79.2% without modification. It was proposed that inhibition of diffusion occurred due to the presence of the HEP polymer on the PAMAM dendrimer surface [125]. Also, surface modifications of PAMAM dendrimer could encourage intracellular release of nucleic acids. For instance, the TEA-core PAMAM-G4 dendrimer surface was modified with long peptide segments (arginine-rich) and then used for delivery of siRNA (against heat shock protein 27, Hsp27) into human prostate cancer PC-3 cells. It was shown that the localization of Arg-segments on the surface of PAMAM-G4 dendrimer could increase the cellular uptake rate. Furthermore, considering the proton sponge effects for the PAMAM-Arg complexes, it seems that 63% of the protonatable groups (guanidine and amine groups of arginine residues) would be protonated at the physiological pH 7.4, while this percentage could increase to 89% under acidic condition (endosomal, pH 5.0). From the results of this research, these modifications on the surface could increase the siRNA release rate from the PAMAM-Arg-siRNA complexes, which is attributed to the buffering capacity of the PAMAM-Arg complexes [126].

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In agreement with these results, surface modifications by acetylation (60%) of the primary amine groups of PAMAM-G5 dendrimers led to promotion of siRNA release from the complex in the presence of a competitive binding agent (heparin sulfate) [127]. It was described that PAMAM dendrimer size could be changed in the presence of solvents via the occurrence of swelling. PAMAM-G5 dendrimer in the presence of solvents exhibited 33% swelling compared to the absence of solvents [128]. Therefore, it could be concluded that solvents would be likely to affect the drug release rate. In a recent report, berberine (BBR) a natural alkaloid with considerable anticancer properties was conjugated to two different forms of PAMAM-G4 dendrimer [BBR conjugated PAMAM (PCB) and BBR encapsulated PAMAM (PEB)]. The results revealed that the BBR release rate from both of the complexes (PCB and PEB) in the presence of distilled water was slower than in the presence PBS (phosphate buffer saline) [129]. It was also reported that 70% of MTX could be released from MTX-PAMAM-G5 dendrimer complexes in the presence of PBS at physiological pH after 2.5 h, while this rate was significantly decreased in a water environment [130].

6. Conclusion

Chemotherapy drugs are the mainstay of cancer therapy, but their side effects are often dose-limiting in clinical practice. In an attempt to overcome these challenges, PAMAM dendrimers were introduced as a drug delivery vehicle with some unique properties. Their spherical architecture, modifiable cationic groups on their surface, and their tunable molecular size are attractive attributes. Many modern approaches to nanoscale drug-delivery take advantage of active targeting strategies by attaching specific ligands onto the surface that will be recognized by receptors that are over-expressed on cancer cells. The terminal primary-amino groups naturally present on the PAMAM surface provide a suitable handle to attach these ligands. Moreover, many receptor-ligand binding interactions stimulate cellular internalization by receptor-mediated endocytosis. However, the process of receptor-mediated endocytosis usually results in the cargo vehicle being taken up intact into endosomes and lysosomes. This is not the ideal location for most cytotoxic drugs and nucleic acid-based therapeutics. These moieties are usually most active when present in the nucleus of the cancer cells. However, it is possible for certain cationic delivery vehicles to escape from the endosomes and lysosomes by the process known as “endosomolysis”. This occurs when secondary or tertiary amine groups experience a drop-in pH from physiological levels (pH = 7.4), to more acidic conditions such as those found inside endosomes (pH ~ 5.5) or lysosomes (pH ~ 4.7). The amine groups become more or less protonated and single targeted delivery systems (drug or genes) cannot effectively overcome all obstacles related to cancer treatment. Thus, co-delivery system using PAMAM dendrimer was designed and applied to overcome these problems. The current paper has provided an overview of PAMAM-based drug and gene delivery or co-delivery systems using active targeting in both in vitro and in vivo conditions, and it can be concluded that this class of nanocarrier exhibits high potential for targeted cancer therapy. In addition, a co-delivery system was a more effective method in cancer therapy compared to either single drug or single gene delivery systems. However, because of safety problem associated with positive charges on the dendrimer surface, prolonged administration of dendrimers may result in undesirable effects or toxicity to organs like the liver, spleen, and kidney. But the possibility of tailoring the surface

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functionalization of dendrimers provides an opportunity to circumvent structural obstacles and provide an effective delivery system.

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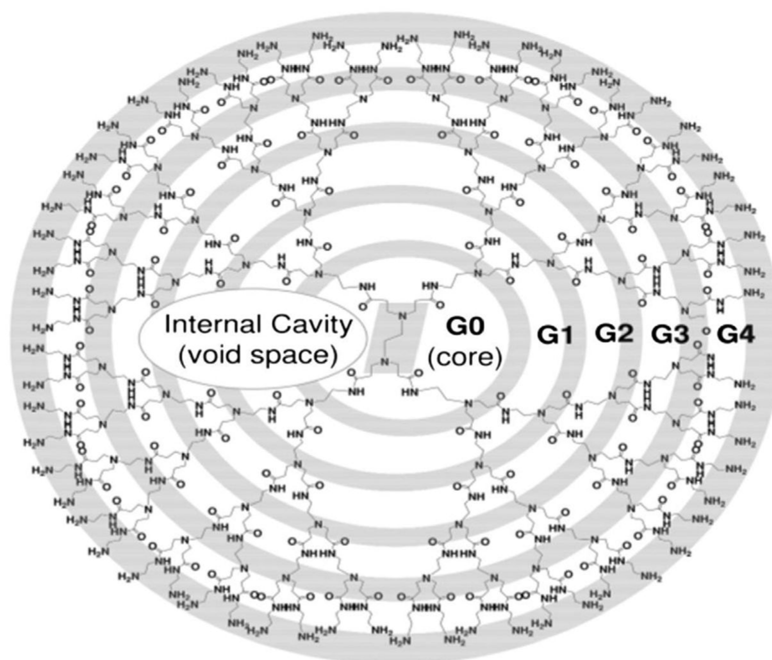

Fig. 1.

Schematic representation of PAMAM-NH_2 dendrimer G0 to G4. PAMAM-NH_2 dendrimer starts from an ethylene diamine core; the branches or arms were attached by exhaustive Michael addition to methyl acrylate followed by exhaustive aminolysis of the resulting methyl ester using ethylene diamine. Reprinted with permission from Ref. [11].

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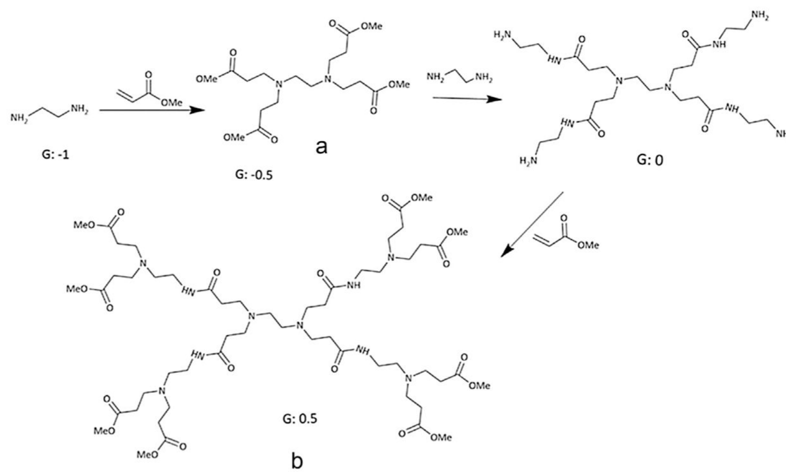

Fig. 2.

Representiation of G-0.5 PAMAM ended to (a) carboxylate group and (b) with carboxylate group surface.

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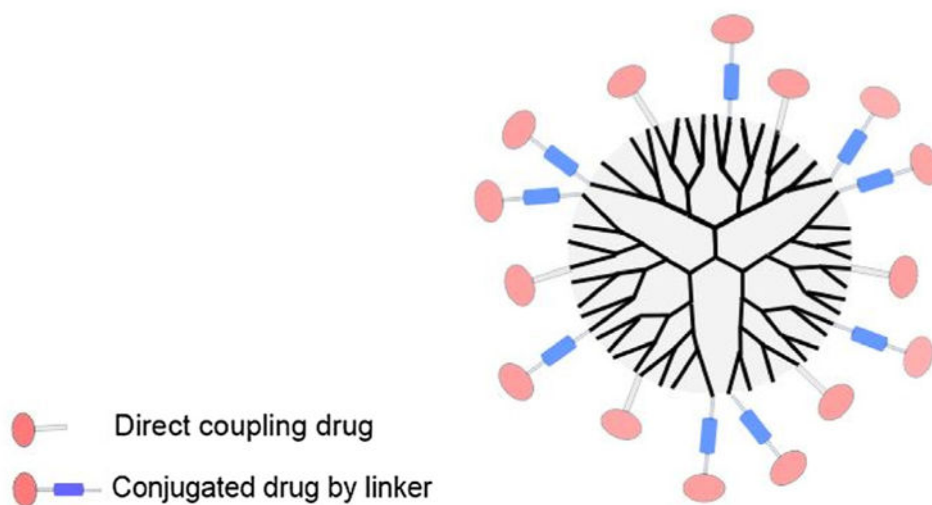

Fig. 3.
Shematic representation of PAMAM-drug conjugation in which direct and clevage linker conjugations have been shown.

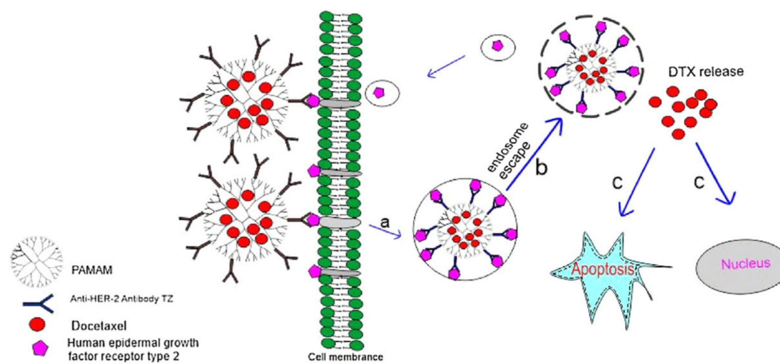

Fig. 4.

Conjugation of the anti-HER-2 antibody, trastuzomab (TZ) to PAMAM dendrimer loaded with docetaxel and FITC (fluorescent label). (a) Cell uptake by receptor-mediated endocytosis; (b) Endosomal escape of PAMAM was accomplised and endosome containing cargo-burst open; (c) The anti-cancer drug is released into the cytosol, and reaches the nucleus and induces apopotosis [83].

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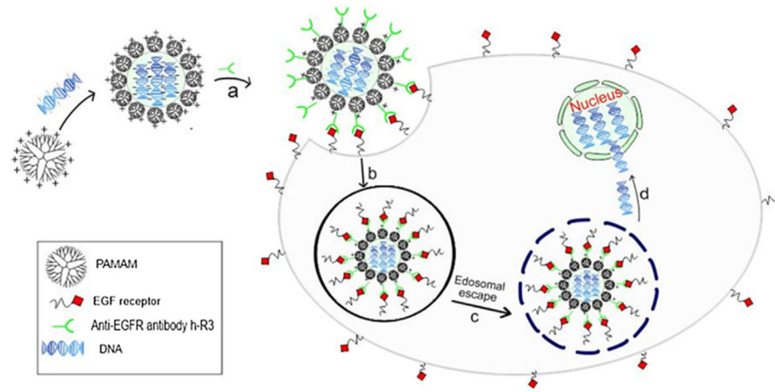

Fig. 5.

Targeted DNA delivery system based on PAMAM dendrimer. (a) Dendriplex formed by electrostatic interaction between PAMAM and DNA, and the anti-EGFR antibody h-R3 attached via electrostatic interaction; (b) receptor-ligand mediated endocytosis; (c) endosomal escape leads to lysosomal breakage; (d) DNA was released and transported into the nucleus [78].

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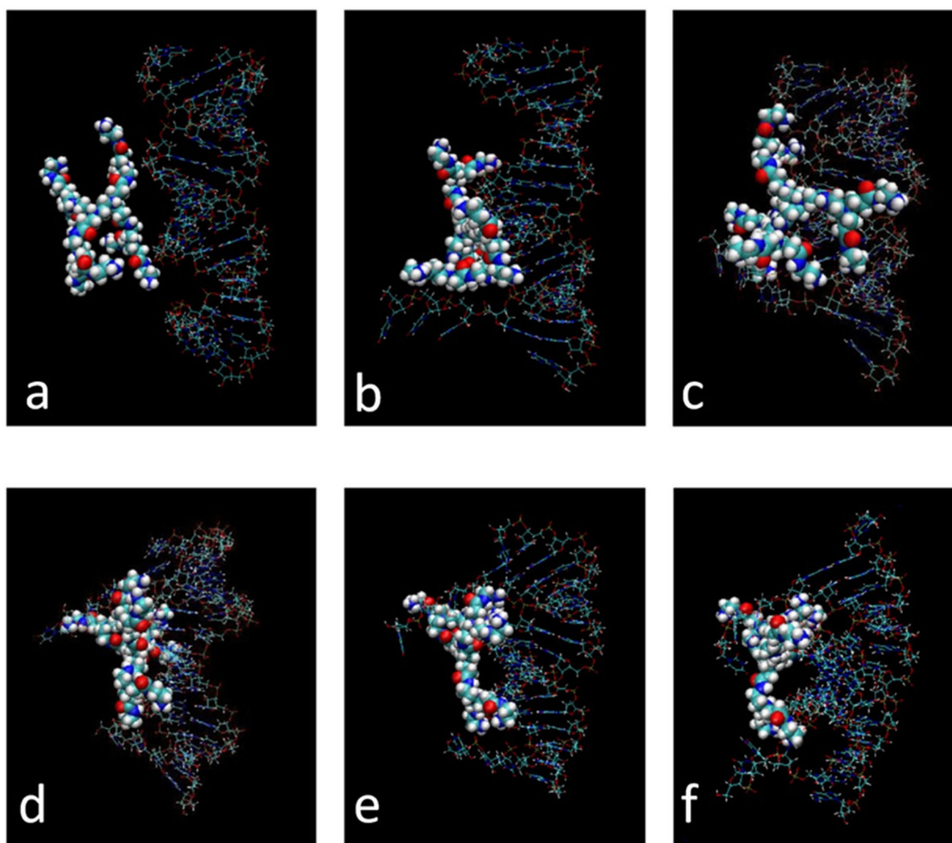

Fig. 6.
Molecular dynamics simulation of G1 PAMAM complexation with siRNA. After (a) 0 ns (nanoseconds); (b) 4 ns; (c) 8 ns; (d) 12 ns; (e) 16 ns; and (f) 20 ns. The endosomal pH in this simulation was almost 5, and this simulation was performed to determine the relationship between endosomal escape and the proton sponge effect. At low pH siRNA was more compact than at pH = 7.
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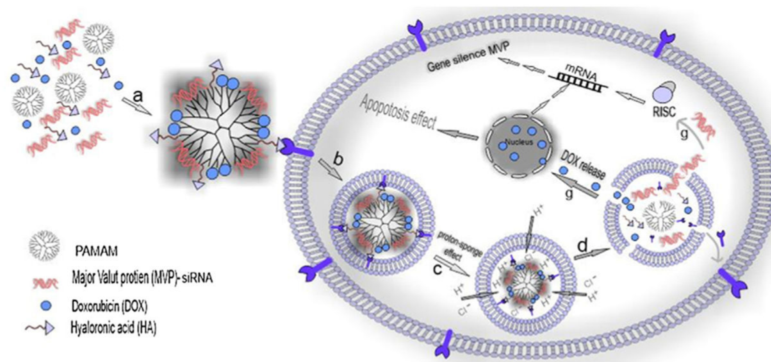

Fig. 7.

Targeted PAMAM dendrimer for co-delivery of a drug and siRNA. (a) Doxorubicin and siRNA against major vault protein (MVP), plus hyaluronic acid (as a targeting agent) were conjugated to PAMAM dendrimer. (b) Hyaluronic acid interacted with its receptor CD44. (c) Complex was taken up by receptor-mediated endcytosis. (d) Strong buffering capacity allows proton and chloride influx. (e) Resulting in endosomal membrane rupture. (f) Release of cargo (DOX and siRNA) into the cytosol, inducing apoptosis and MVP gene silencing respectively.

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Table 1

Summary of PAMAM-based dendrimer-mediated drug delivery.

PAMAM/generation	Ligand	Target receptor	Target cell types	Drug moiety	Ref.
G4	Flt-1	VEGF	Pancreatic cancer	Gemcitabine	[131]
G5	FA	FA receptor	KB cell line	Paclitaxel	[132]
G4	RGD	αvβ3	Glioma cell	Doxorubicin	[133]
G4	HA	CD44	MiaPaCa-2 cancer cell lines	Curcumin	[134]
G4	Peptide YLFFVFER	RGD	SKBR3 and 293T cells	Doxorubicin	[135]
G4	Octreotide	Somatostatin receptors (SSTRs)	MCF-7	Methotrexate	[136]
G5	FA	FA receptor	KB tumor cells	Methotrexate	[137]

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Table 2

Summary of targeted dendrimer-based gene delivery systems.

PAMAM/generation	Ligand	Targeted receptor	Targeted cancer cells/tissues	Gene agent	Ref.
G5	RGD	$\alpha v\beta 3$	U87 malignant glioma cells	siRNA	[138]
G4	FA	FA receptor	HN12 and HN12-YFP cells	siRNA-VEGFA	[139]
G5	FA	FA receptor	KB cells	DNA	[140]
G5	Thioaptamer (TA)	CD44	breast cancer	miRNA	[141]