Seminars in Cancer Biology 86 (2022) 396–419

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Dendrimers as nanoscale vectors: Unlocking the bars of cancer therapy

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ARTICLE INFO

Keywords:
Cancer
Chemotherapy
Dendrimers
Drug and gene delivery
Targeted therapy

ABSTRACT

Chemotherapy is the first choice in the treatment of cancer and is always preferred to other approaches such as radiation and surgery, but it has never met the need of patients for a safe and effective drug. Therefore, new advances in cancer treatment are now needed to reduce the side effects and burdens associated with chemotherapy for cancer patients. Targeted treatment using nanotechnology are now being actively explored as they could effectively deliver therapeutic agents to tumor cells without affecting normal cells. Dendrimers are promising nanocarriers with distinct physiochemical properties that have received considerable attention in cancer therapy studies, which is partly due to the numerous functional groups on their surface. In this review, we discuss the progress of different types of dendrimers as delivery systems in cancer therapy, focusing on the challenges, opportunities, and functionalities of the polymeric molecules. The paper also reviews the various role of dendrimers in their entry into cells via endocytosis, as well as the molecular and inflammatory pathways in

Abbreviations: P-gp, P-glycoprotein; HIF-1A, hypoxia inducible factor-1A; EMT, epithelial-to-mesenchymal transition; BBB, blood-brain barrier; BTB, blood-tumor barrier; EE, encapsulation efficiency; HA, hyaluronic acid; CDF, 3,4-diflourobenzylidene curcumin.

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https://doi.org/10.1016/j.semcancer.2022.06.003
Received 29 November 2021; Received in revised form 6 March 2022; Accepted 9 June 2022
Available online 11 June 2022

cancer. In addition, various dendrimers-based drug delivery (e.g., pH-responsive, enzyme-responsive, redox-responsive, thermo-responsive, etc.) and lipid-, amino acid-, polymer- and nanoparticle-based modifications for gene delivery, as well as co-delivery of drugs and genes in cancer therapy with dendrimers, are presented. Finally, biosafety concerns and issues hindering the transition of dendrimers from research to the clinic are discussed to shed light on their clinical applications.

1. Introduction

Cancer is one of the most life-threatening diseases, causing distress to humanity and the global healthcare system in terms of mortality, morbidity, and financial burden of cancer [1,2]. Despite numerous studies and novel cancer therapies discovered and developed over time, cancer remains the leading cause of death worldwide [3,4]. According to recent data, 1,898,160 new cancer cases and 608,570 cancer deaths are expected in the United States by 2021 [5]. Conventional treatment approaches mainly include the use of cancer therapies, chemotherapy, radiotherapy, and surgery [6]. Chemotherapy is the most common choice for almost all types of cancer, but its long-term treatment leads to the development of tumor resistance [7,8]. On the other hand, anticancer drugs lead to an unfavorable condition, resulting in further deterioration of healthy tissues and organs [9,10].

With the vision of overcoming the obstacles of current cancer therapies, cancer researchers have expanded their concept of research to include the applications of dendrimers in cancer therapies and diagnoses. The inclusion of dendrimers in cancer treatment has added a new dimension to cancer research, while changing the way the world views this dangerous disease [11]. Dendrimer molecules have a structure similar to the branched arrangement of trees. They are capable of binding to drugs and nucleic acids, and being transformed by target-specific ligands to effectively deliver them to target sites without affecting normal cells [12]. The overall molecular structures are built by covalent conjugation of synthons to the central core, and various drugs with different functional groups can be attached to their outer surface as capping agents [13,14]. There is a broad classification of dendrimers based on the functional moiety in the core and at the peripheral groups, such as polyamidoamine dendrimers, polypropyleneimine peptide dendrimer, poly(L-lysine) dendrimers, citric acid dendrimers, carbohydrate-based dendrimers, and various other functionalized and ligand-anchored dendrimers [13,15,16]. The dendrimers have a wide range of applications such as electrical conduction, ion channels, vaccines delivery, enzymes delivery, antibiotics delivery, gene delivery, and as an anticancer drug carrier [17–19]. Till date limited studies have been reviewed on functionalization and cell internalization of dendrimer without covering drug delivery, gene delivery and co-delivery using different dendrimers. On the contrary, the present review does not limit itself to the therapeutic potential of dendrimer but aims to provide a comprehensive picture of dendrimer starting from its types, functionalization, inflammatory and molecular signaling to drug and gene delivery. Thus, the current paper will open new possibilities for researchers around the globe to deal with dendrimer variations in clinical settings.

The present review addresses the chemistry, drug enhancement, classification, functionality, and contribution of dendrimers to different stages of inflammatory and molecular pathways. It outlines the various mechanisms of drug delivery, gene delivery, co-delivery of drugs, and nucleic acids, and their therapeutic potential in various types of cancers. It also introduces the delivery platforms using dendrimers, including stimuli-responsive, pH-responsive, enzyme-responsive, redox-responsive, thermo-responsive, and multifunctional external stimuli-responsive. In addition, the current article highlights the advantages of using dendrimer-based nanomaterials for drug delivery, targeting inflammatory and molecular pathways, thus exploring the success of this versatile therapeutic entity as a nascent approach to cancer treatment.

2. Types of dendrimers

Well-defined structures combined with controllable surface functionalities make dendrimers appealing carriers for drug delivery and nucleic acid-targeting. Dendrimers exist in various forms depending on the customizable ligands and cores. The most widely employed dendrimers used in anti-cancer drug delivery are as follows:

2.1. Polyamidoamine

Polyamidoamine has attracted the most attention and the first report goes back to 1985. The polyamidoamine molecule consists of a multitude of monomeric branched particles originated from the dendrimer molecule's central core (Fig. 1A) [20]. Ethylenediamine occupies the central core, which is repeatedly attached to methyl acrylate according to the desired generation. The G0, G1, G2, G3, G4, and so forth have been developed so far (Fig. 1B). Generation by generation, the molecular weight, count of atoms, and the number of primary amine moieties have dramatically risen. Notably, the radius of the molecule has undergone a huge increase, averaging approximately 10 Å [21]. The well-defined and monodisperse molecule of polyamidoamine possesses certain unique characteristics such as biological compatibility, size ranging in the nanoscale, multifunctionality in the periphery with interior chambers, size tunability, and multifarious operational capabilities. These features lead them to have diverse applications in a myriad of fields chiefly including gene therapy, drug delivery and medical sensing and imaging [22–25]. A major advantage of polyamidoamine dendrimer is the capability to encapsulate hydrophobic drug molecules within its internal cavities or pockets (Fig. 1C).

2.2. Polypropyleneimine dendrimer

Polypropyleneimine dendrimer is another group of nano-architectures that has gained vast popularity recently. First reported in 1978, this class of dendrimers is a highly branched macromolecule designed using the divergent method by placing amines in the terminal ends [26] and 1,4-Diaminobutane at the core [27] (Fig. 1D). *In situ* synthesis of PPI by the double Michael addition reaction results in dendrimers with other cores such as ethylenediamine and others. The appearance of multitudinous amine groups in the extremities of the molecule allows conjugation of various ligands effective in cancer targeting. Amino acids, folates, carbohydrates, peptides, and antibiotics are some of the ligands that are likely to be investigated for active targeting [28]. However, positively charged dendrimers reported to have a high toxicity level, which is a concern with dendrimer generation and an increasing number of cationic amino surface groups [29].

2.3. Carbosilane dendrimers

Carbosilane dendrimers are composed of a carbon-silicone framework and their multifunctional methyl silane units serve as branch sites between monomeric units. One advantage of using silicon for dendrimer synthesis is that the nucleophilic molecule readily binds to electrophilic silicon (Si^{+}). These dendrimers are composed of hydrophobic cores. However, they can be transformed into hydrophilic through cationic or anionic surface functionalization [30]. There are several methods for producing carbosilane dendrimers containing an integrated tetra-functional core such as tetra allyl silane, tetra vinyl silane [31] as

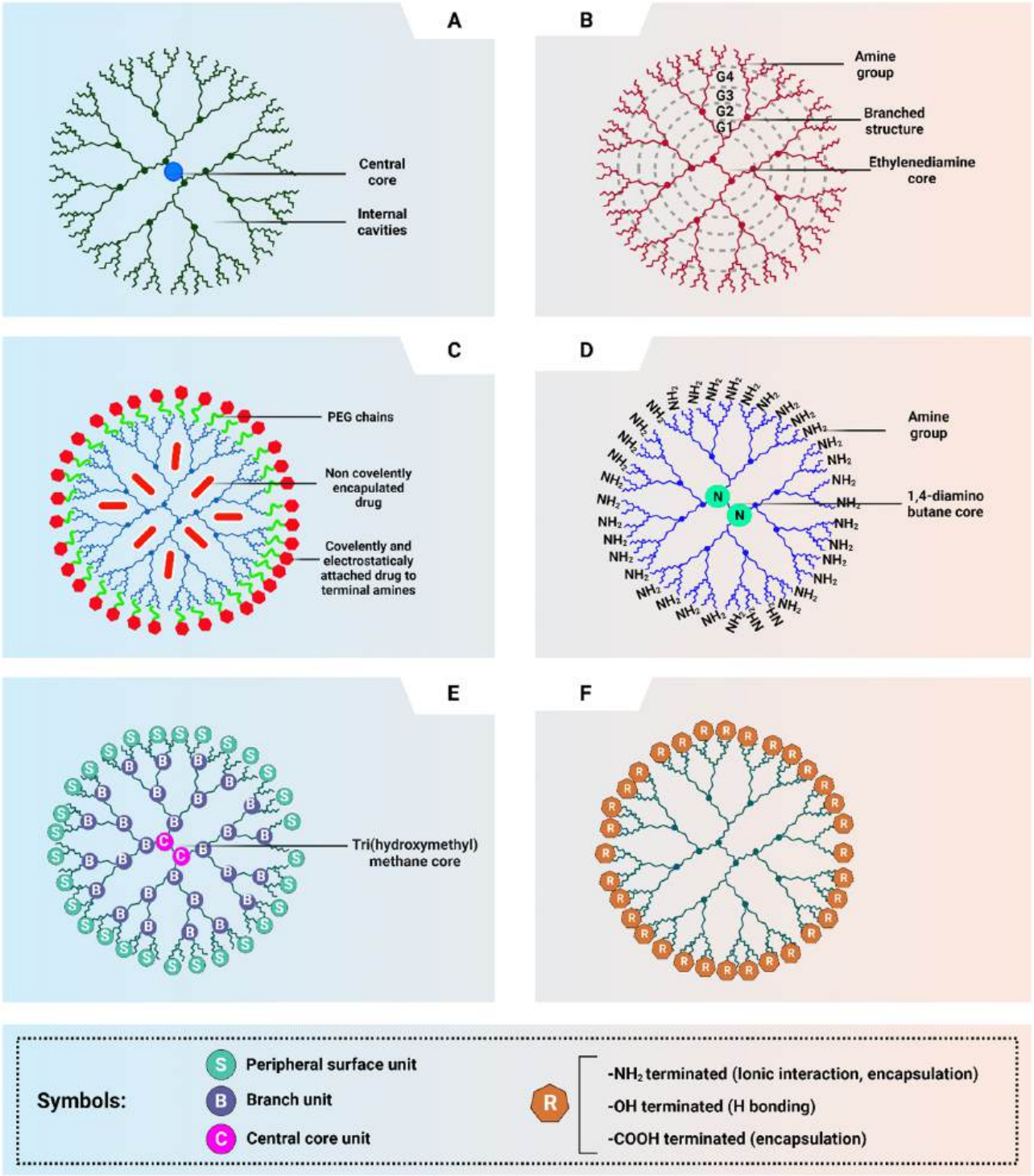

Fig. 1. General structure and morphological features of different dendrimers. (A) Morphology of dendrimer consists of three distinct components: a core, branches, and several terminal functional groups. Essentially the core is composed of a single atom or atomic group with at least a couple of similar chemical functions, while branches, extending from the core, consist of repeated elements with at least one branch node, which are repeated in a radially concentric layer resulting in different "generations". (B). A branching structure for polyamidoamine dendrimers with a comparison of the sizes of different generations. (C). Role of polyamidoamine as a drug delivery vehicle for encapsulating drugs in the cavities within them or by electrostatically attaching drug molecules to their surfaces. (D) polypropyleneimine dendrimers with branched macromolecules had amines at the terminal ends and DAB at the central core. (E) The chiral dendrimers with tri(hydroxymethyl)methane core combined with aromatic polyether dendrons. (F) Mechanism of drug loading of hydrophobic drugs via noncovalent association in PAMAM dendrimers containing different functional groups. **Abbreviations:** PAMAM: Polyamidoamine; PPI: Poly (propylene imine); DAB: 1,4-Diaminobutane.

well as a larger triple-function nucleus tri-oxybenzene [32]. The density of functional groups at the periphery of this class of dendrimers is high since the core is relatively small. Compared to other classes of dendrimers, carbosilane dendrimers are dramatically less toxic [33]. These agents are capable of penetrating through both cells of different origins and neurons and have been demonstrated to cross the blood-brain barrier [34].

2.4. Chiral dendrimers

Chemically, these dendrimers are analogous to chiral cores but are synthesized using constitutionally diverse branches. Chirality occurs at the axis of the functional domains. Chiral dendrimers are further classified depending on the position where the chiral molecules are fixed (Fig. 1E). Therefore, five distinct types of chiral dendrimers have been synthesized, comprising: (1) Frechet-type poly (aryl ether) dendrimers containing chiral core with achiral branches; (2) tryptophan-substituted arborol at the peripheral end of chiral dendrimer; (3) chiral branches of trimesic acid in core and 1,2-diol branching component; (4) achiral core with at least 3 constitutionally different branches such as the dendrimer with pentaerythritol as core and Fréchet's aromatic ether as branches; (5) branches and core showing chirality and those dendrimers based on dihydroxy pyrrolidine [35].

2.5. Tecto dendrimers

Dendrimers of this type contain a central core surrounded by other dendrimers' shells. A core usually has a higher generation number than its surrounding dendrimers. A synthetic procedure governs the addition of extra shells, creating a nanoscale region of 1–100 nm in diameter. In biomedical applications, they exhibit outstanding properties analogous to that of single-generation dendrimers, while also overcoming some of the disadvantages of single-generation dendrimers like low loading capacity and improved permeability and retention owing to their small size [36].

Dendrimers have been shown to carry both hydrophilic and hydrophobic medicines, proving their adaptability. Various types of dendrimers with such promise include poly (propylene imine), polylysine, and poly(amidoamine) dendrimers, the latter being the first full dendrimer family that has been generated, described, and marketed [13, 15].

Furthermore, the internal hydrophobic and hydrophilic pockets of dendrimers made them ideal alternatives to unimolecular micelles for the encapsulating different types of bioactive moieties (Fig. 1F). Initial research on drug delivery potential of dendrimers was for noncovalent drug encapsulation as ''dendritic boxes''. Early research, for example, complexed DNA with PAMAM dendrimers for gene delivery applications [37], and hydrophobic medicines and dye compounds were integrated into different dendrimer cores [38–40].

The ability of PAMAM and PPI-based dendrimers to load hydrophobic agents has been extensively studied. These structures are appropriate for drug entrapment because their internal structure facilitates both hydrophobic/hydrophilic encapsulation. A major mechanism for drug solubilization in dendrimers comes from electron-transfer interactions with amines at the surface and tertiary nitrogen at the center [41]. In addition, the surface structure impacts the solubilization mechanisms. Amine-terminated dendrimers encase weakly acidic drugs by electrostatic interactions both with their interior amine groups and by hydrophobic interactions. However, hydroxy-terminated dendrimers solubilize drugs, for instance, methotrexate, primarily through weak hydrogen bonding, whereas ester-containing surfaces effectively encapsulate the drug [42]. There are also reports suggesting that encapsulating hydrophobic drugs via hydrogen bonding and electrostatic interactions with PEG chains on dendrimers can enhance drug encapsulation [43]. To achieve optimal drug solubilization, however, the chain size of the PEG chain must be considered. PEG chains longer than approximately 5000 Da can reduce drug encapsulation due to their greater rigidity. However, shorter PEG chains appear to have a greater capacity to solubilize more drug. As a result of the long PEG chains forming large structures inside the dendrimer, the volume available for drug encapsulation is reduced due to agglomerations [43].

3. Functionalization of dendrimers

The surface modification of particles is vital for improving their characteristics in cancer therapy. The biocompatibility and safety profile of nanostructures can be significantly improved via surface modification [44]. The surface modification with ligands can enhance the selectivity of nanoparticles towards tumor cells by binding to surface receptors [45]. This section focuses on surface modification of dendrimers for improving their selectivity towards cancer cells. Aptamers are synthetic short RNA or DNA molecules first discovered in 1990 and they are commonly utilized in the modification of nanoarchitecture due to their high affinity and specificity towards target [46,47]. Notably, multiple experiments have explored the potential of aptamers for surface modification of dendrimers in cancer therapy. The MUC1, AS1411, and ATP are among the aptamers utilized in dendrimer modification. The aptamer-modified dendrimers promote cytotoxicity of epirubicin against MCF-7 and C26 cells by selective targeting, while not being internalized by healthy cells, decreasing adverse effects. In addition to *in vitro* experiments, an *in vivo* study on xenograft mice revealed the potential of epirubicin-loaded aptamer-modified dendrimers in reducing tumor growth [48]. An experiment prepared PEGylated polyamidoamine dendrimers for delivery of camptothecin in colon cancer treatment (*in vitro* and *in vivo*). The encapsulation efficiency was 93.67% and AS1411-modified polyamidoamine dendrimers enhanced the internalization of camptothecin in colon cancer cells for suppressing progression and decreasing viability [49]. The surface modification of dendrimers with AS1411 aptamer promotes the potential of short hairpin RNA in gene silencing (reducing Bcl-xL expression up to 25%) and induces apoptosis in lung cancer cells (14% late apoptosis). Noteworthy, short hairpin RNA-loaded AS1411-modified dendrimers had a particle size of 128–230 nm with a zeta potential of 12.76–19.13 mV, showing their stability and capacity in internalization in tumor cells [50].

CD44 overexpression is found at the surface of many cancer types, especially pancreatic cancer cells. The surface modification of nanoparticles with hyaluronic acid selectively targets CD44-overexpressed cancer cells [51]. An experiment has prepared hyaluronic acid-modified polyamidoamine dendrimers for delivery of 3, 4-difluorobenzylidene curcumin in pancreatic cancer treatment. These 3, 4-difluorobenzylidene curcumin-loaded hyaluronic acid-modified selectively targeted CD44-overexpressed pancreatic cancer cells and blocking CD44 receptor promotes IC_{50} up to 1.71-fold. Therefore, this site-specific delivery is of importance for decreasing IC_{50} value [52].

Folate receptor is also an ideal candidate to promote selectivity of dendrimers in cancer therapy [53]. The surface modification of polyamidoamine dendrimers with folic acid selectively targets cervical and ovarian cancer cells (HeLa and SKOV3 cells) overexpressing the folate receptor. Then, 3, 4-difluorobenzylidene curcumin as an anti-tumor agent can be loaded and resulting polyamidoamine dendrimers with the particle size of 10–20 nm and zeta potential of 8.37–42.2 mV decrease tumor viability via apoptosis induction. Furthermore, folate-targeted 3, 4-difluorobenzylidene curcumin-loaded polyamidoamine dendrimers enhance the expression level of PTEN as a tumor-suppressor factor, while they inhibit NF-κB signaling in ovarian and cervical cancer therapy [54]. Taking everything together, increased internalization in cancer cells targeted delivery, and reduced side effects on normal cells are considered as results of surface modification of dendrimers with ligands (Table 1) [55–57].

Table 1
The surface modification of dendrimers for selective targeting of cancer cells.

Nanovehicle	Cancer type	*In vitro*/ *In vivo*	Cell line/Animal model	Particle size (nm) Zeta potential (mV)	Remarks	Refs
Aptamer-modified dendrimers	Breast and colon cancers	*In vitro*	MCF-7 and C26 cells	36.4 nm	Modification of dendrimers with MUC1, AS1411 and ATP as aptamers Selective targeting and uptake by tumor cells Promoting cytotoxicity of epirubicin against tumor cells	[1]
AS1411-modified dendrimers	Colon adenocarcinoma	*In vitro* *In vivo*	HT29 and C26 cells Xenograft model	14.2 and 18 nm 1.2 mV	Encapsulation efficiency as much as 93.67% for camptothecin Site-specific delivery of anti-tumor agent to cancer cells and increasing internalization	[2]
AS1411-modified dendrimers	Lung cancer	*In vitro*	A549 cells	128–230 nm 12.76–19.13 mV	Apoptosis induction (14%) Mediating targeted delivery of shRNA to cancer cells Reducing expression level of Bcl-xL up to 25%	[3]
Folate-functionalized dendrimers	Ovarian cancer	*In vitro*	OSC and OCCC cell lines	Not reported	Internalization in a dose-dependent manner Inducing cell death Promoting sensitivity of ovarian cancer cells to carboplatin Selective targeting of folate receptor	[4]
Folate-decorated PAMAM dendrimers	Ovarian cancer Cervical cancer	*In vitro*	SKOV3 and HeLa cells	10–20 nm 42.2 and 8.37 mV	Targeted delivery of flavonoid analogue to cancer cells overexpressing folate receptor Apoptosis induction Upregulating PTEN expression Inhibiting NF-κB signalling	[5]
Folic acid-conjugated PPI dendrimers	Breast cancer	*In vitro*	MCF-7 cells	Not reported	Encapsulation efficiency as much as 64.78% Low haemolysis High stability High drug release at acidic pH Enhanced cellular uptake in cancer cells due to surface modification	[6]
CXCR4-targeted dendrimer	Breast cancer	*In vitro*	BT-549-Luc and T47D cells	No reported	Encapsulation efficiency up to 97.25% Drug loading efficiency between 3.4% and 3.6% The surface modification with LFC131 peptide provides targeting CXCR4 receptors on breast cancer cells	[7]
Lactose-functionalized dendrimers	Different cancers	*In vitro*	A549, DU-145, and HT-1080 cells	Not reported	Inhibiting galectin-3-induced cancer cell aggregation via providing competitive binding site for galectin-3	[8]
SRL peptide coated PAMAM dendrimers	Glioma	*In vitro*	C6 cells	Not reported	Targeted delivery to tumor cells and enhancing transfection efficiency of DNA (GFP)	[9]
Sialic acid-, glucosamine-, and concanavalin A-modified PPI dendrimers	Glioma	*In vitro* *In vivo*	U373MG cells Rat	42.7 nm 18.2 mV	Drug loading efficiency as much as 29.6% for paclitaxel Increasing plasma levels of paclitaxel Selective targeting of tumor cells Modification of dendrimers with sialic acid is the best option compared to others	[10]

Abbreviation: CXCR4: chemokine receptor 4; PAMAM: poly(amidoamine); PPI: poly(propylene imine)

4. Dendrimer and cell internalization

Cells use endocytosis as a mechanism to internalize substances in their environment using vesicles formed by the plasma membrane [58,59]. The most well-known pathway for nanoparticle entry into cells is endocytosis. Overall, endocytosis is divided into two different types: phagocytosis and pinocytosis. The former is for the uptake of large particles, while the latter is responsible for the uptake of fluids and solutes [60]. Macrophages can entrap particles up to 20 μm in size via phagocytosis [61]. Although phagocytosis occurs only in some cell types such as macrophages, neutrophils, and dendritic cells, pinocytosis can occur in all cell types. The most well-known pathway of pinocytosis is endocytosis. Endocytosis is divided into two main pathways, clathrin-dependent and independent endocytosis [62]. Clathrin-independent endocytosis is more categorized and includes caveolae-mediated endocytosis, clathrin- and caveolae-independent endocytosis and micropinocytosis. Clathrin-mediated endocytosis relies on the binding of ligand receptors and the subsequent recruitment of coated pits in the cytoplasmic part of the cell membrane that form polygonal cages and mediate endocytosis. Nanoparticles and viruses enter cells mainly via clathrin-mediated endocytosis [63]. Caveolae-mediated endocytosis is similar and hairpin-like caveolin sheaths are formed in the cytoplasmic part of the cell membrane and assemble into flask-shaped caveolae with a size of 50–80 nm [64,65]. Micropinocytosis is mediated by actins, and a large amount of extracellular fluids and particles are engulfed during this process [66].

Various experiments have investigated the method of internalization of dendrimers by cancer cells. It has already been mentioned that surface modification of dendrimers promotes their selectivity towards tumor cells and enhances their cellular uptake. In a recent experiment, evaluated the internalization mechanism of aptamer-functionalized dendrimers in prostate cancer therapy was investigated. Modification with EpDT3 aptamer promoted internalization of dendrimers in PC-3 and DU-145 cells. To investigate cellular internalization of dendrimers, filipin (caveolae-dependent endocytosis inhibitor), phenyl arsine oxide (clathrin-mediated endocytosis inhibitor), and colchicine (macropinocytosis inhibitor) were used. The use of endocytosis inhibitors significantly reduces intensity of green fluorescence and cellular uptake of dendrimers. The strongest inhibitory effect was observed with the use of filipin and phenyl arsine oxide, whereas colchicine reduced the internalization of dendrimers to a lesser extent. This indicates that aptamer-modified dendrimers mainly utilize caveolae- and clathrin-mediated endocytosis

for penetration into prostate cancer cells [67]. In a comparative study, the internalization of dendrimers and surface-modified dendrimers in HeLa cells (cervical cancer) was investigated. Both conventional dendrimers and peptide (pHLIP)-modified dendrimers promoted internalization of doxorubicin in HeLa cells. However, polyamidoamine dendrimers do not penetrate HeLa cells via endocytosis, whereas peptide-modified dendrimers utilize endocytosis to promote internalization of doxorubicin into Hela cells and suppress their progression [68]. Based on various experiments, ligand-modified dendrimers show high cellular uptake into tumor cells via the endocytic pathway [69–73]. The surface charge of dendrimers is also a crucial factor for their penetration into tumor cells. In one experiment, anionic dendrimers were prepared for oligonucleotide delivery in cancer therapy (A431 cells, squamous cell carcinoma). Instead of liquid phase endocytosis, the dendrimers are taken up by A431 cells by adsorptive endocytosis, which increases internalization up to 100-fold. This process (adsorptive endocytosis) is mediated by the binding of negatively charged dendrimers to positively charged proteins on the surface of the cell membrane [74]. Transcytosis is also a form of endocytosis in which cargo is engulfed in membrane-bound vesicles and migrates through the cytoplasm [75]. A recent experiment has shown that internalization of the dendrimer-drug conjugate in cancer cells via transcytosis [76]. This mechanism allows deep internalization of dendrimers in tumors. The dendrimer-camptothecin conjugate can be internalized in pancreatic cancer cells via caveolae-mediated endocytosis followed by vesicle-mediated endocytosis, which increases deep internalization in pancreatic cancer cells [76]. These observations highlight the role of endocytosis and related mechanisms in the internalization of dendrimers in tumors (Fig. 2).

5. Inflammatory pathway and molecular signalling

Inflammation is an inherent component in oncogenesis that facilitates the supply of various bioactive molecules to the tumor microenvironment, leading to increased cell proliferation, viability, and angiogenic and metastatic potential [77,78]. More recently, this process has been shown to influence tumor response to chemotherapy [79]. Inflammation occurs when the immune system is stimulated by an external factor such as infection or tissue damage, during which various proteins, including but not limited to the nuclear factor kappa-light-chain-enhancer of activated B cells, cAMP response element-binding protein, CCAAT/enhancer-binding protein, and interferon regulatory transcription factors, are activated [80]. Subsequently, genes encoding enzymes, chemokines, cytokines, adhesion molecules,

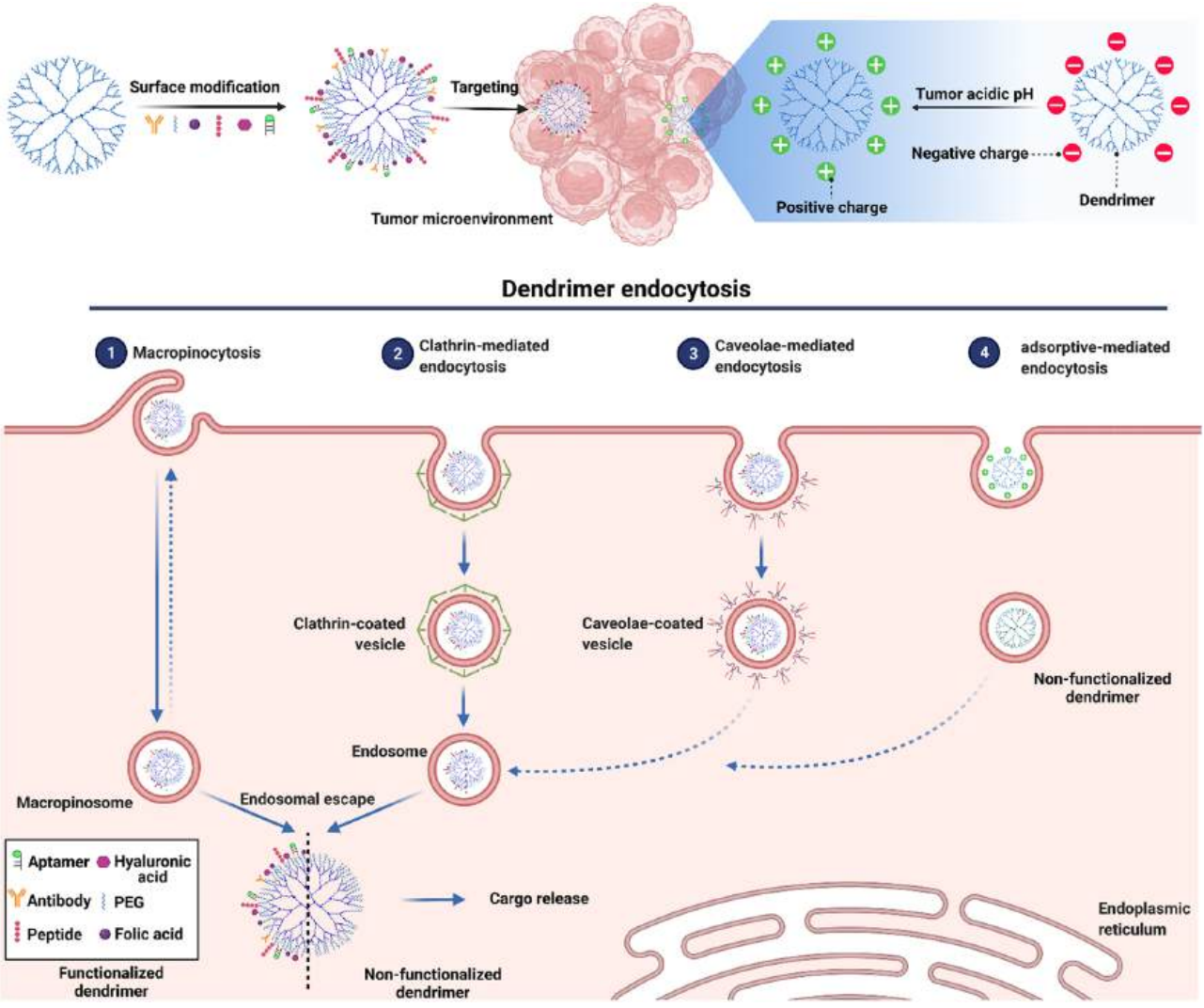

Fig. 2. The process of internalization of dendrimers in cancer cells by endocytosis. Surface modification of dendrimers leads them to enter cancer cells via receptor-mediated endocytosis. For example, hyaluronic acid-modified dendrimers bind to CD44 receptors to enter in cancer cells via endocytosis. The folic acid modification mediates endocytosis via folate receptors. In addition, anionic dendrimers can be converted to positive dendrimers in the mildly acidic pH of the tumor microenvironment. They can then interact with the positively charged cell membrane and enter cancer cells.

and extracellular matrix regulators are induced, which increases leukocyte activity and triggers inflammasome formation [81,82]. During the assembly of an inflammasome complex, caspase-1 is activated and cleaves off the propeptide sequences of IL-1β and IL-18 to activate them [82]. Activated IL-1β and IL-18 then initiate the adaptive Th1 and Th17 immune responses. Meanwhile, the chemokines produced stimulate G protein-coupled receptors, that enhance the transcription of pro-inflammatory factors. Allergens also form antibody complexes that stimulate Fc receptors on mast cells, which mediate phagocytosis or cytotoxicity toward the damaged or infected cells [82]. Throughout the process of inflammation, various chemicals, particularly reactive oxygen species, are released, which accelerate the accumulation of mutations leading to malignant transformation and chemoresistance [77].

Given the important role that inflammation plays in cancer and other diseases, several therapeutic agents have potent anti-inflammatory properties. To improve the delivery of these drugs, numerous attempts have been made to conjugate them with nanocarriers such as dendrimers (Table 2) [83]. The first report of *in vivo* delivery of anti-inflammatory drugs dates back to 2004, when two polyamidoamine dendrimers were injected into rabbits undergoing experimental glaucoma filtration to study their effects on scar tissue formation [84]. The first dendrimer was a generation 3.5 dendrimer that possessed anti-inflammatory properties. It ended up with 64 carboxylic acid groups, nine of which were conjugated with glucosamine residues. The second dendrimer with anti-angiogenic properties was based on the same skeleton as the first, but was conjugated with nine glucosamine-6-sulfate residues instead of glucosamine [84]. The use of these dendrimers significantly reduced hypercellular scarring and increased the success of surgery from 30% to 80% [84].

In another study using the rabbit model of shigellosis, glucosamine residues were found to prevent acute intestinal wall damage caused by severe inflammatory diarrhea by reducing the levels of the pro-inflammatory IL-6, IL-8, and TNF-α mRNA while increasing that of the anti-inflammatory IL-10 [85]. Similarly, a 1,2-diaminoethane-cored generation 4.5 glucosamine residues was found to suppress not only IL-6, IL-8, and TNF-α, but also macrophage inhibitory protein-1α and − 1β and IL-1β, in monocyte-derived macrophages and dendritic cells [86]. These reductions in gene expression were, at least in part, due to the polyamidoamine dendrimers rather than the conjugated glucosamine, as studies in three inflammatory mouse models (the subacute cotton pellet model, the acute model of carrageenan-induced paw edema, and the chronic model of adjuvant-induced arthritis) showed that polyamidoamine has inherent anti-inflammatory properties [87]. In the same study, polyamidoamine dendrimers with nine 6-O-sulfated glucosamine residues were found to exhibit only anti-angiogenic effects, whereas those containing NH_2 terminal (G4-NH_2), -OH (G4-OH), and -COOH (G4.5-CO_2H) reduced nitric oxide and cyclooxygenase-2 activity in mouse macrophages [87]. It is, however, important to note that dendrimers other than polyamidoamine are also known to regulate the inflammatory pathway. Poly propyl ether imine dendrimers, for example, are as effective as polyamidoamine in reversing the impact caused by severe inflammatory diarrhea in the same rabbit model of shigellosis, although it did not cause an elevation in the level of IL-10 mRNA [88]. Besides, 3- and 4-arm PEO 'stars' and second-generation dendrimers on the N_3P_3 core were found to have a similar anti-inflammatory effects to sulfated polysaccharides [89]. These dendrimers owe their anti-inflammatory properties to their hydroxylated lactose terminal groups, which block inflammatory mediators such as P- and L-selectins [86]. Thus, injection of these dendrimers into mouse models with acute inflammation resulted in a sharp decrease in the infiltration of neutrophils and macrophages [86].

Attempts were also made to compare the cyclooxygenase-2 inhibitory effects of dendrimers with different terminals. It was found that aminoethyl ethanolamine-coated dendrimers and hydroxyl-terminated dendrimers (G4-OH) have a high inhibitory effect whereas dendrimers with tris (hydroxymethyl) aminomethane (G4-Tris) terminals, *N*-(3-carbomethoxy) pyrrolidone terminals (G4-Pyr), or polyethylene glycol (G4-PEG) terminals have a lower inhibitory effect [86]. This reflects the importance of terminal groups in influencing the anti-inflammatory activity of dendrimers.

Besides, aza-bis-phosphonate dendrimers are known to have anti-inflammatory and immunomodulatory properties [90]. Treatment with these dendrimers resulted in enhanced phagocytotoxicity in human T cells, and an increased natural killer cell proliferation in peripheral blood mononuclear cells [86]. In addition, dendrimers in human monocytes caused increased gene and protein expression of anti-inflammatory molecules such as MNC1 and decreased proinflammatory CD64 and CD13 [91]. Similarly, in mice studies, aza-bis-phosphonate dendrimers decreased monocyte and dendritic cell function and enhanced differentiation of IL-10-producing CD4 + T lymphocytes [92,93]. The dendrimers also contributed to the inhibition of pro-inflammatory mediator secretion along with increased IL-10 production in mice with rheumatoid arthritis. In addition, aza-bis-phosphonate dendrimers also reduced the level of matrix metalloproteinases and inhibited the differentiation of monocytes, which are effective in cartilage destruction and bone resorption, respectively [14,15].

More recently, fourth generation polypropyleneimine glycodendrimers coated with maltotriose particles were synthesized and patented (patent number: WO 2014/088434 Al). These glycodendrimers, named PPI-G4-OS-Mal-III, target genes involved in multiple pathways that drive carcinogenesis, such as inflammation and apoptosis, to achieve an effective treatment response [77,94]. Maltotriose-modified glycodendrimers have been shown to stimulate apoptosis in B lymphocytes by modulating the expression of multiple genes, resulting in inhibition of clonal expansion (Fig. 3) [95]. These fourth-generation polypropyleneimine glycodendrimers show promise for the treatment of a variety of B-cell lymphoproliferative disorders, such as B-lymphoma or chronic lymphocytic leukemia, and their applicability can potentially be extended to other inflammatory diseases [96].

Table 2
Dendrimer drug carriers in preclinical development stage.

Dendrimer Formulation	Drug	Indication	Ref
PEGylated lysine peptide dendrimer	Gemcitabine	Breast cancer	[11]
Amino capped PAMAM dendrimer	5-Fluorouracil	Pancreatic cancer	[12]
N-acetyl-D-glucosamine-labelled dendrimers	Camptothecin	Lung cancer	[13]
Poly (glycerol-succinic acid)	Camptothecin	Various cancers	[14]
Folic acid- Poly amido amine	Methotrexate	Epithelial cancers	[15]
Poly amido amine	Cisplatin	Solid tumors	
Poly amido amine - Polyethylene glycol	Doxorubicin	Acute myeloid leukemia, soft tissue sarcoma, and multiple myeloma, Hodgkin's lymphoma, cancers of the bladder, breast, stomach, lung, ovaries and thyroid, acute lymphoblastic leukemia	[16]
Peptide dendrimer	Doxorubicin, Gemcitabine	Pancreatic cancer	[17]
Hyaluronic acid modified PAMAM dendrimer	Cisplatin, Doxorubicin	Breast cancers	[18]
PEG modified PAMAM dendrimer	Camptothecin	Colorectal cancer	[19]
PEG-cored PAMAM dendrimers	Gemcitabine	Pancreatic cancer	[20]
Phosphoramidate dendrimers	Doxorubicin	Breast cancer	[21]

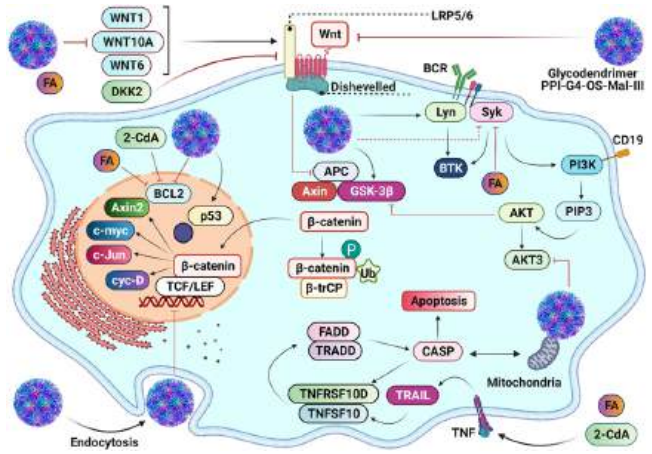

Fig. 3. The mechanism of action of the fourth-generation maltotriose-coated polypropyleneimine glycodendrimers, PPI-G4-OS-Mal-III, in B lymphocytes. PPI-G4-OS-Mal-III exerts its anticancer effect through multiple interconnected pathways. The dendrimer can block Wnt signaling pathway either by direct inhibition of WNT1, WNT10A, and WNT6 proteins or by binding to Wnt receptors, LRP5/6. Alternatively, it inhibits the expression of TCF/LEF transcription factors in the nucleus after entering the cell by endocytosis. As a result of these inhibitions, the cytoplasmic level of beta-catenin is kept very low through the activity of the destruction complex comprising APC, Axin, and GSK-3β. This prevents transcriptional activation of several oncogenes, such as c-myc and cyc-D. In addition, PPI-G4-OS-Mal-III can also activate GSK-3β and p53, inhibit BCL2, interact with mitochondria and inhibit AKT3, which collectively contribute to cellular apoptosis. Abbreviations: WNT1: Wnt family member 1; WNT10A: Wnt family member 10A; WNT6: WNT family member 6; DKK2: Dickkopf wnt signaling pathway inhibitor 2; BCR: B cell receptor; Syk: Spleen tyrosine kinase; APC: Antigen-presenting cell; BTK: Bruton's tyrosine kinase; PI3K: Phosphoinositide 3-kinases; Gsk-3β: Glycogen synthase kinase 3β; PIP3: phosphatidylinositol 3,4,5-triphosphate; AKT: Protein kinase B; β-trCP: β-transducin repeat-containing protein; CASP: Caspase; TNF: Tumor necrosis factor; 2-CdA: 2-chlorodeoxyadenosine; FADD: Fas-associated death domain; TRADD: TNFR1-associated death domain protein; TNFRSF10D: TNF receptor superfamily member 10d; TNFSF10: Tumor necrosis factor (ligand) superfamily, member 10; BCL2: B-cell lymphoma 2; cyc-D: Cyclin D; TCF/LEF: T cell factor proteins/lymphoid enhancer factor.

6. Fundamentals and mechanisms of drug loading and dendrimers

Based on the type of interaction occurs between drug molecules and the dendrimer, the release rate can be altered. For instance, in the case of anticancer drugs, there are mainly two types of bonding—covalent and non-covalent interactions including electrostatic, hydrogen, steric hindrance, and Van der Waals [97]. When it comes to non-covalent interactions or physical encapsulation of drug molecules, there is an interior cavity in which the drug molecules get directly encapsulated. This cavity is naturally hydrophobic making it suitable to interact with poorly soluble drug molecules [98]. Besides hydrophobic interactions, the existence of oxygen and nitrogen atoms in the cavity provides the formation of a hydrogen bond between the host and drug molecules [99]. On the upside, various types of anticancer, anti-HIV, and anti-inflammatory drugs can be quickly and easily encapsulated throughout the dendrimers. However, there are some drawbacks mainly related to the low stability of drug-loaded molecules plus premature liberation of them without inducing the intended therapeutic action [100].

The presence of different functional carboxylic acid and amino groups on the surface of dendrimers provides electrostatic interactions resulting in better lipophilic drugs solubility [101]. Many ionized drugs including ibuprofen, naproxen, diflunisal, ketoprofen, etc. have —COOH groups ready to make an electrostatic interaction with the ionized surface terminal groups of dendrimers [102].

In the case of covalent bonding, the drug molecules, which are encapsulated through this type of bonding, are released through enzymatic or chemical cleavage of the bonds. It is noteworthy that the conjugation of drug molecules covalently to the dendrimers can be achieved through adopting some spacers like PEG, lauryl chains, etc., or amide and ester bonds [103]. It is well-known that covalent bonding affects the stability of loaded drugs significantly followed by making the release kinetic more controlled. Besides many drugs including penicillin V, naproxen, venlafaxine, etc. which have been conjugated to the dendrimers through covalent bonding, numerous anticancer drugs have been anchored to the dendrimers among which cisplatin, methotrexate, doxorubicin, paclitaxel, etc. can be enumerated and they have indicated targeting potential [104]. Epirubicin is another anticancer drug anchored on the PEG-modified dendrimer covalently and showed an improved blood circulation time and therapeutic efficiency. A study revealed that the epirubicin stability was well preserved and just after chemical degradation the conjugated molecules were gradually released [102].

7. Drug and gene delivery

7.1. Drug delivery: overview on stimuli-responsive dendrimers

7.1.1. Internal-stimuli-responsive dendrimers

One of the issues with dendrimer-based drug delivery systems is non-specific drug release from these carriers, which limits therapeutic effectiveness and has significant adverse effects[105]. Although targeted dendrimers are well-known for their ability to reach a specific place in the body based on the targeting agent, several challenges remain unresolved, such as the lack of precise control of drug release at the site of action.[106]. A potential solution to the mentioned problems is to develop delivery systems responsive to internal and external stimuli to liberate their cargo once they are exposed to the exact stimuli [107].

7.1.2. pH-responsive dendrimers

There is a hallmark related to cancer cells known as aerobic glycolysis; whereby cancer cells have a limitless desire to take glucose resulting in the production of lactic acid. The produced acid causes the pH of solid tumors extracellular medium lower than healthy cells and numerous researchers have taken advantage of such a phenomenon to come up with pH-sensitive drug delivery systems [108]. There are some acid-responsive groups reported for pH-sensitive dendrimers among which hydrazones, boronate ester, orthoesters, acetals/ketals, and cis-aconites can be mentioned [107]. Hydrazone group was utilized in conjugation with doxorubicin and the linkage showed a stable structure at physiological medium (pH=7.4) without premature burst release, while being cleaved under the acidic environment [109,110]. It is worth mentioning that after endocytosis and being in touch with the endosome environment (pH=5.0–6.0), the release profile got accelerated compared to the tumor environment (pH=6.5–6.8). This hydrazine linkage can also be applied in the case of other anticancer drugs containing ketone or aldehyde groups. Another linkage is the boronate ester bond by which bortezomib as an anticancer drug was conjugated to a catechol modified polyamidoamine dendrimer. When bortezomib is directly injected into the bloodstream, it causes cardiotoxicity and thrombocytopenia, while its combination with the dendrimer culminates in an 'off-on' release profile. One of the interesting features of this delivery system was its neutral surface charge which impeded its internalization by the cells and the drug's liberation did not occur through lysosomal acidity. Therefore, the delivery system was found to efficiently eradicate cancerous cells at acidic media (pH=6.5), whereas showed almost nontoxicity to different cell types at physiological conditions (pH=7.4) [111]. Among acid-labile groups, polyacetals have attracted considerable attention because of their great sensitivity to acidic pHs plus non-acidic metabolites [112]. A group has synthesized a

stimuli-responsive drug delivery system for cancer therapy by combining pH-sensitive polyacetal dendrimers with zwitterions. The existence of acetal linkers made the loaded doxorubicin stable at physiological conditions but a fast drug release in the tumor's medium. Notably, the addition of zwitterionic sulfobetaine to the dendrimer had some advantages as follows: the pH-responsivity of the system became charge reversal, the dendrimer resisted proteins absorption which is an added value to provide more blood circulation, and a multilevel release mode was achieved through this combination. Fluorescence pictures of free doxorubicin and drug-loaded dendrimer were captured using confocal laser scanning microscopy. After 1 h, red fluorescence appeared to show the internalization of nanoparticles followed by their accumulation in the endosomes. Compared to free doxorubicin, the fluorescence intensity of dendrimer was stronger rooting in the positively charged surface functional groups of dendrimers which increase the affinity of nanoparticles towards negatively charged cell membranes. The kinetics of doxorubicin-loaded dendrimer release were studied at various pH levels (2.0–9.0), and there was a clear association between acidic circumstances and quicker release. [113].

One of the limiting factors of both stimuli and non-stimuli-responsive drug delivery systems in cancer therapy is their penetration and accumulation in a tumor [114]. The problem is addressed by designing a pH-responsive size-switchable platinum-conjugated polyamidoamine. The initial particle size of the dendrimer was around 80 nm at physiological fluids (pH=7.4), while once the dendrimers have reached the acidic medium, they have experienced a sharp decrease in the particle size (less than 10 nm) resulting in a consistent distribution of all over the tumor microenvironment. A comparative study was performed on the effect of pH-responsivity of dendrimer and both insensitive and pH-sensitive dendrimers were synthesized, tested through weakly permeable BxPC-3 pancreatic tumor models, and compared to each other in terms of efficiency. It turned out that the pH-sensitive drug-loaded dendrimer had better penetration throughout the tumor followed by showing improved therapeutic effects (Fig. 4**(A-C)**) [115].

7.1.3. *Enzyme-responsive dendrimers*

Overexpression and activity of enzymes, being a crucial element in all biological processes, are thought to be a marker of several illnesses. Cathepsin B and matrix metalloproteinases are well-known enzymes that are overexpressed in many tumor microenvironments. Some peptides can be particularly cleaved by those enzymes; for example, cathepsin B can cleave Gly-Phe-Leu-Gly oligopeptide under physiological circumstances, and matrix metalloproteinases can degrade collagen peptides[116–118]. As a result, using enzymes as a stimulant to create enzyme-responsive dendrimers is of great interest. Through using the Gly-Phe-Leu-Gly oligopeptide and collagen peptide linkers, different types of anticancer drugs like doxorubicin, gemcitabine, etc. can be conjugated to the dendrimers culminating in the delivery of those drugs to the site of the tumor followed by tumor growth suppression [119, 120]. A PEGylated lysine peptide dendrimer modified with Gly-Phe-Leu-Gly oligopeptide linker was synthesized to encapsulate gemcitabine for breast cancer therapy. The dendrimer showed an

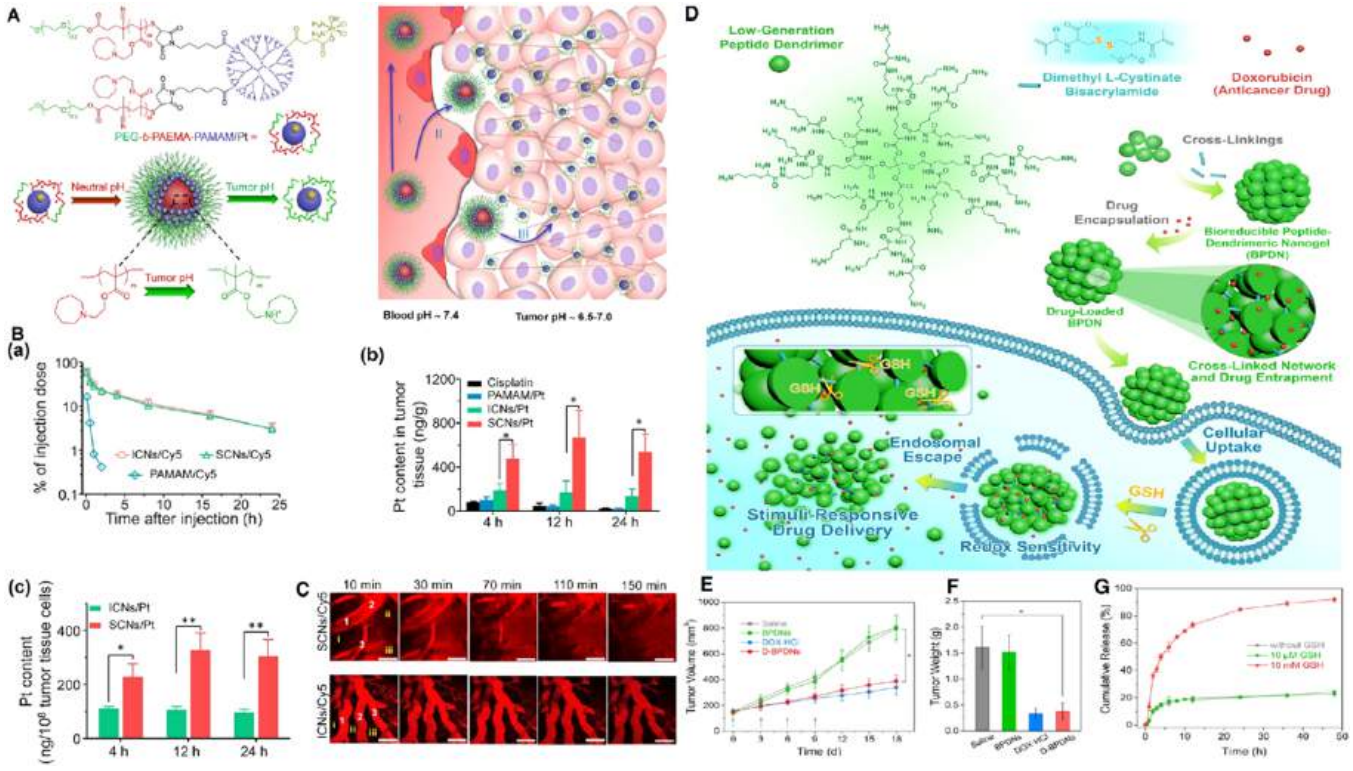

Fig. 4. pH- and redox-sensitive dendrimers for cancer therapy. (A) An illustration of the dendrimers synthesis, drug loading, and size-switchable property in the exposure of mild acidic medium of tumor environment. (B) *In vivo* blood circulation, penetration, and tumor accumulation of both pH-sensitive and insensitive dendrimers (ICNs/Pt and SCNs/Pt) as follows: (a) pharmacokinetic of different samples after injection; (b) cisplatin amount (ng/g) in the tumor tissue; (c) cisplatin content in the tumor tissue cells up to 24 h (* $p < 0.05$ and ** $p < 0.01$). (C) The real-time distribution of nanoparticles through weakly permeable BxPC-3 after injection up to 150 min (Scale bar = 100 μm). The numbers (1, 2, and 3) are related to three intravascular compartments, and the roman numbers (ɛ, ii, and iii) are attributed to extravascular compartments as shown. Abbreviations: ICNs/Pt: Cisplatin-conjugated pH-insensitive cluster nanostructures; SCNs/Pt: Cisplatin-conjugated pH-sensitive cluster nano bombs. (D) A schematic on the preparation of the peptide dendrimer and disulfide linker followed by the redox responsivity of dendrimer for site-specific drug delivery. (E) Tumor volume changes when treated with different samples. (F) The weights of tumor (mice bearing 4T1 tumor) after 18 days of treatment (* $p < 0.01$). (G) The drug release profiles of peptide dendrimer in PBS at physiological conditions in the exposure of different glutathione concentrations. **Abbreviations:** BPDNs: Bio-reducible peptide–dendrimeric nanogels; D-BPDNs: DOX-loaded bio-reducible peptide–dendrimeric nanogels.
(a) Reprinted from [115] with permission from ACS publication. (b) Reprinted from [130] with permission from ACS publication.

enzyme-responsive behavior and so the release rate was simply enhanced in the presence of cathepsin B. When compared to free gemcitabine, the dendrimer demonstrated lower toxicity with higher therapeutic indices [121]. In the case of colon cancer, there is an enzyme-responsive group capable of passing through the acidic medium of the stomach and releasing the cargo into the colon. Azoreductase is a high-content enzyme found in the colon that may be employed for azo bond breaking. An azo-containing linker was used to conjugate 5-aminosalicylic acid, an anti-inflammatory medication, to the polyamidoamine dendrimer. When the dendrimer was exposed to the medicine, the results indicated that the drug was stable in the stomach and that the drug was released quickly.[122].

Another method for synthesizing enzyme-responsive dendrimers is based on disrupting hydrophilic-lipophilic balance; the self-assembly of an enzyme-responsive dendrimer and polyethyleneglycol results in an amphiphilic co-polymer that disassembles once it comes in contact with targeted enzyme. It is noteworthy that the cargo plus the enzyme-responsive linker is entrapped into the hydrophobic part of the copolymer and the enzyme molecules are not small enough to penetrate the internal core of the micelle and cleave the bond. As a result, it is critical to maintain a balance between the micelle and the co-polymer contents [123]. The main concern revolving around the previous enzyme-sensitive dendrimers is their degradation upon releasing their cargo and so during *in vivo* drug delivery procedures, the remaining dendrimers may be of problem. The potential solution for that can be found through another category called self-immolating dendrimers. Through a single enzymatic stimulus, these dendrimers undergo a complete dissolution and reduce into building monomers. The self-immolating dendrimers include doxorubicin, naproxen, etoposide. etc. are designed and programmed to release the loaded drug upon a single enzymatic activation with less toxicity and better efficiency [124, 125].

7.1.4. Redox responsive dendrimers

It is known that cells have a high concentration of reducing thiols such as glutathione. Moreover, the concentration of glutathione inside the cells dominates its quantity outside the cells by a factor of two to three. Because of abundance of reactive oxygen species inside of cancer cells, these cells enhance glutathione secretion to confront the oxygen species, leading to a considerable rise (seven-folds) in the glutathione concentration inside of cancerous cells [126,127]. Through reduction disulfide exchange interactions, disulfide linkage is cleaved by glutathione and a rapid release inside of cancerous cells has occurred. Various anticancer drugs like doxorubicin, paclitaxel, etc. have been anchored to the dendrimers through disulfide bond and the results showed a significant decrease in the side-effects of free chemotherapeutic drugs plus an enhancement in their therapeutic index [128,129]. A bio-reducible redox-responsive dendrimer was developed for antitumor drug delivery. The delivery system was endowed with interior voids suitable for drug loading. The glutathione high concentration provided a situation in which the bond was broken and released guest molecules. The *in vivo* results implied that the growth of the 4T1 tumor was prevented and the adverse effects of free drug molecules decreased (Fig. 4(D-G)).

7.1.5. Thermoresponsive dendrimers

The temperature at which these dendrimers show a phase change is known as the lower critical solution temperature. The thermoresponsive dendrimers' hydrophilicity decreases significantly at this temperature. The following methods have been used to manufacture thermoresponsive dendrimers: (1) Direct incorporation of a thermosensitive polymer such as poly(N-isopropyl acrylamide) into the dendrimer's internal core or surface, (2) anchoring small thermosensitive moieties to the dendrimer's surface—N-isopropyl acrylamide, oligo(ethylene glycol), peptides, etc., and (3) use of amphiphilic polymers such as -amino ester and oligo(ethylene glycol) to construct temperature responsive dendrimers[131–133]. One of the most interesting aspect of thermoresponsive dendrimers is their internalization into cells in response to temperature change; a substantial cellular absorption occurs when a temperature rises above the lower critical solution temperature is applied [134]. For example, isobutyl amide-terminated dendron first assembles into capsules followed by being covered into micelles once it has been exposed to heat above its lower critical solution temperature [135]. This behavior implies that through changes in the applied temperature, the intracellular drug delivery efficacy of those dendrimers can be tailored. A thermosensitive hyperbranched polyethyleneimine modified with isobutyl amide groups was developed. Doxorubicin was encapsulated through the dendrimer and the formulation indicated an improved doxorubicin cellular uptake and cell compatibility at 40 °C. In the case of drug release, it was observed that during the first 10 min, a burst release occurred attributed to the loosely bonded doxorubicin molecules followed by a sustained release after applying the temperature. About 80% of the loaded doxorubicin was liberated at 40 °C within 3 h [136].

Although great efforts have been put into designing various types of thermoresponsive dendrimers, only some of them found their way through stimuli-responsive drug delivery applications. The main limitation factor may be the poor solubility of those dendrimers above their lower critical solution temperature leading to safety concerns when it comes to *in vivo* applications. The other problem relates to the activation of drug release; it is challenging to apply heat locally to tissue without damaging the adjacent tissues. The potential solution can be found through the addition of some photocatalytic agents into the dendrimer's structure capable of turning light into heat.

7.1.6. Multifunctional and external stimuli-responsive dendrimers

The term 'multifunctional' refers to dendrimers that have more than one specialized capacity for cancer treatment. It is well known that targeting of hypoxia microenvironment of tumor is a difficult task, so a multifunctional polyamidoamine system conjugated to macrophages was developed; the dendrimer could reach the hypoxia environment of a tumor thanks to the macrophages, followed by releasing the cargo in the presence of tumor acidity [137]. A doxorubicin-loaded dendrimer modified with collagen was produced via hydrazone linkages that were sensitive to enzyme and pH simultaneously [138]. Besides the internal stimuli-dendrimers, there is also an opportunity to combine both internal and external triggers including light, magnetic field, etc. [139, 140]. The inclusion of components that respond to external stimuli, such as magnetic and photocatalytic nanoparticles, would make the dendrimer appropriate for hyperthermia and imaging. Hyperthermia, also known as thermal therapy, is a form of cancer treatment that uses heat to raise the temperature of the tumor in order to kill the cancerous cells while leaving the healthy tissues unharmed [141,142]. Under the umbrella of phototherapy, there are two sub-categories: photodynamic and photothermal therapies. The former uses a photosensitizer material that becomes toxic to adjacent tissues after being exposed to light irradiation, while the latter raises the temperature of the targeted tissue after laser irradiation using a photocatalytic material (turning light into heat) [143]. However, the main concern about the clinical translation of phototherapy is its efficiency as the depth of laser penetration is limited [144]. Moreover, there are different regions in a solid tumor including normoxic and hypoxic exhibiting different responses to the cancer treatments and this is another problem that needs combinatorial therapy. Therefore, the combination of photodynamic and photothermal therapies is concurrently used to improve therapeutic outcomes. Because of the high levels of oxygen in this location, a size-switchable device was designed to alter the normoxic microenvironment through photodynamic treatment (Reactive oxygen species formation) and eliminate the hypoxic zone using photothermal therapy. The nanoparticles were made of poly(amidoamine) dendrimer and indocyanine green, which were then bonded to an amphiphilic polymer and loaded with the photosensitizer chlorin e6. The nanoparticles gathered in the perivascular parts of the tumor after being injected into the circulation.

Once the laser irradiation (660 nm) was applied the chlorin e6 started to produce singlet oxygen species affecting the normoxic environment and also breaking the linkage between the polymer and the dendrimer. Next, the small poly(amidoamine)-indocyanine green nanoparticles were liberated and penetrated through the hypoxic microenvironment. The laser irradiation (808 nm) this time caused an increase in the region's temperature and suppression of the tumor growth (Fig. 5(A-D)) [145].

Platforms with both therapeutic and diagnostic capabilities are particularly promising for cancer diagnosis and therapy. These platforms are known as theranostic; imaging agents through these systems highlight the tumor's location while the therapeutic agents, which can be a drug or any other sort of material, kill malignant cells [146]. Some well-known imaging agents for magnetic resonance imaging, photoacoustic imaging, and computed tomography such as magnetite (Fe_3O_4), gadolinium (III), and gold nanoparticles have been encapsulated into dendrimers and combined with radio, photothermal, and photoacoustic therapies [147–149]. A ternary multifunctional system composed of generation 5 polyamidoamine dendrimer, gold nanoflowers, and Fe_3O_4 nanoparticles was developed for theranostic applications. Thanks to the stabilizing role of the dendrimer, the gold nanoflowers embedded Fe_3O_4 nanoparticles were uniformly formed and a much higher r_1 reflexivity than the pure Fe_3O_4 nanoparticles was obtained culminating in improved photothermal efficiency (82.7 %). Moreover, the system was able to be used for diagnostic applications through adopting magnetic resonance imaging, computed tomography, and photoacoustic imaging [148]. Another ternary system comprising generation 5 polyamidoamine, gold nanoparticles, and gadolinium (III) was synthesized for imaging and targeting the hypoxia environment of tumors through radiotherapy. Upon X-ray irradiation, the nanohybrid system-generated excessive Reactive oxygen species promoting DNA damage to cancer cells [149]. An interesting recent study took advantage of photoacoustic properties as both imaging and therapeutic agent to cross the blood-brain barrier and turn laser energy to shockwave followed by mechanical damage to the tumor. Through imaging properties of this theranostic system, some valuable information including tumor size, depth, and morphology was obtained, and also the trend of therapy can be tracked carefully (Fig. 5E) [150].

7.2. Gene delivery

Gene therapy using dendrimers has received great interest for the treatment of a variety of disorders, including cancer [151]. Cationic dendrimers have been widely used to deliver nucleic acid therapeutic such as ribozymes, antisense oligonucleotides, plasmid DNA, and small interfering RNA [152]. They often features a significant number of amine groups at the end of branching sites, which allows nucleic acids to

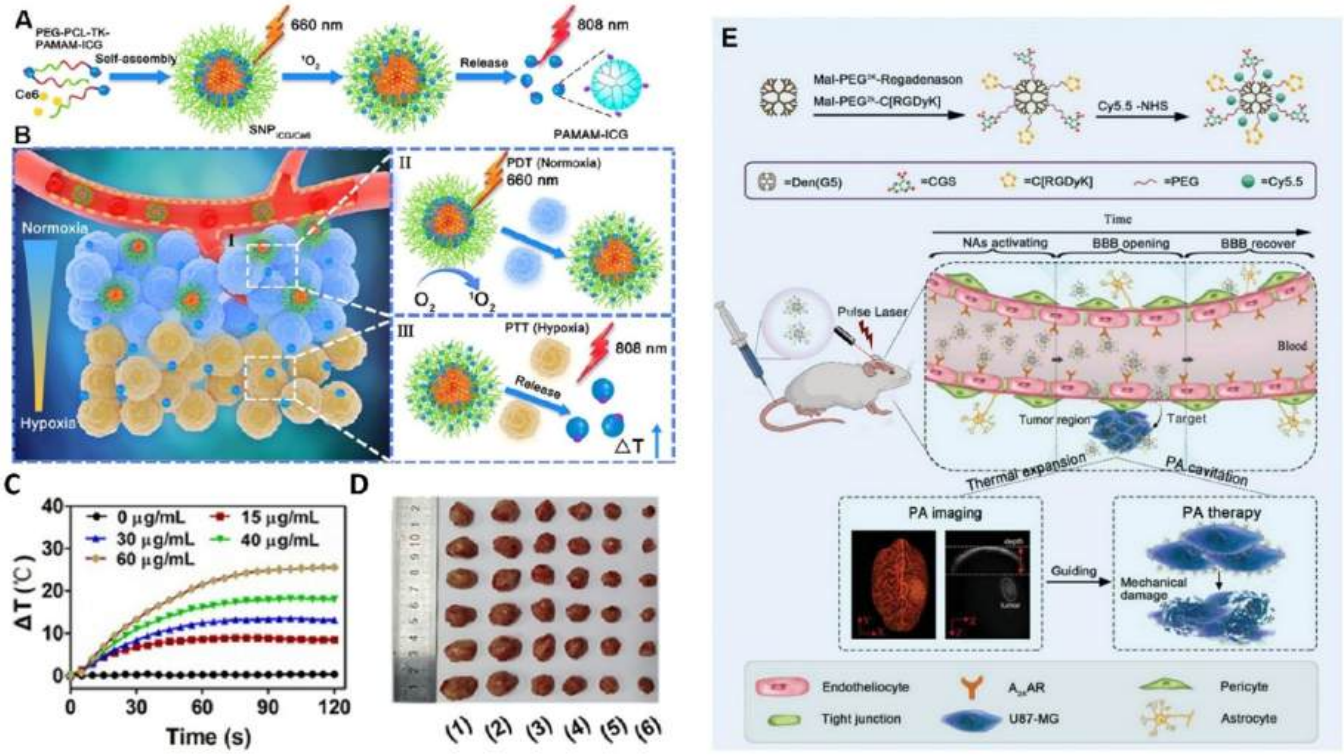

Fig. 5. Multifunctional external-responsive dendrimers. (A) An illustration of the preparation of PEG-b-PCL and PAMAM-ICG which linked together via thioketal bond (PEG-b-PCL-TK-PAMAM-ICG); in the exposure laser irradiation (660 nm), the linkage bond breaks and leads to the release of small PAMAM-ICG nanoparticles. (B) The applicability of nanoparticles towards normoxic and hypoxia microenvironments. (C) Infrared thermal images and (D) temperature alteration related to PEG-b-PCL-TK-PAMAM-ICG ($SNP_{ICG/Ce6}$) with different concentrations when exposed to 808 nm irradiation (1.0 W cm^{-2}). (E) Tumor inhibition ratio and (F) images of the tumors after being treated with different samples ((1) PBS, (2) SNP, (3) SNP+ 660 nm, (4) SNP+ 808 nm, (5) inSNP+ 660 nm + 808 nm, and (6) SNP+ 660 nm + 808 nm). NS ($P > 0.05$), * ($P < 0.05$), * *($P < 0.01$), * ** ($P < 0.001$). Abbreviations: PEG-b-*PCL*: poly(ethylene glycol)-b-poly(ε-caprolactone); PAMAM-ICG: poly(amidoamine) (PAMAM)-indocyanine green. (E) A schematic on the preparation of dendrimer (Den)-(Arg-Gly-Asp-D-Tyr-Lys) (RGD)/4-[2-[[6-Amino-9-(*N*-ethyl-β-D-ribofuranuronamidosyl)− 9 H-purin-2-yl]-amino] ethyl] benzene propanoic acid hydrochloride (CGS)/Cy5.5 nanoparticles and glioblastoma therapy through PA. After the injection of nanoparticles, they were directed to αvβ3 integrin, which is known as an over-expressed protein in the glioblastoma vessels. The connection between CGS. agonist and A2A adenosine receptor (A2AAR) were facilitated through the increase in nanoparticles concentration. The included CGS in the nanoparticles provides a situation by which more nanoparticles can cross the blood-brain barrier. Next, the nanoparticles could directly target the tumor as the αvβ3 integrin can be found high in content in the tumor's site. Upon the laser irradiation, PA shockwaves start to damage the cancerous cells and PA imaging can give important information regarding the tumor's size, depth, etc.
(a) Reprinted from [145] with permission from ACS publication. (b) Reprinted from [150] with permission from Wiley.

be easily condensed into dendrimer chains while preserving them from enzymatic degradation [153]. The electrostatic interactions between phosphate groups of the DNA and the positively charged amine groups of the dendrimer are crucial for the binding process [154]. Furthermore, hydrogen bonds can occur between O atoms of the phosphate groups of nucleic acids and H atoms of the amine groups of dendrimers, which is highly dependent on the pH condition. Subsequently, the formed dendrimer/nucleic acid complexes, known as "dendriplexes", are internalized by cells via different endocytic pathways, such as caveolin, clathrin, or caveolin/clathrin-independent mechanism [155].

As gene vectors, cationic dendrimers such as polyamidoamine dendrimer, poly(ether imine) dendrimer, polypropyleneimine dendrimer, poly(L-lysine) dendrimer, carbosilane dendrimer, triazine dendrimer, phosphorus dendrimer, and viologen dendrimer have been studied. As gene delivery vectors, polyamidoamine dendrimers with a mix of primary, secondary, and tertiary amino groups hold the most promise. [37, 156]. The primary amino groups on the polyamidoamine surface are responsible for capturing nucleic acid, compacting the uncoiled nucleic acid structure, and increasing the cellular uptake of nucleic acid. The tertiary amino groups, on the other hand, are mainly responsible for the proton sponge effect [157]. Accordingly, these properties may alter the intracellular transport of genetic agents and, as a result, their therapeutic efficacy. *In silico* computational assessments have been performed to address this phenomenon. As an example, a research group found that the genetic agents had a higher affinity for dendrimers when the pH was acidic than when the pH was neutral [158]. They showed that after dendriplexes entered lysosomes and late endosomes, nucleic acids interacted significantly with dendrimers at acidic pH, preserving the nucleic acids from destruction [158]. Recent investigations have revealed that the structural flexibility, size, and charge density of dendrimers have major impacts on the nucleic acid binding as well as on their gene transfection activity. For instance, polypropyleneimine dendrimers as another type of dendrimers have been broadly investigated for potential gene delivery vectors *in vitro* and *in vivo* [154]. It was demonstrated that G2 generation of polypropyleneimine has high transfection efficiency in delivering DNA, but possesses poor cytotoxicity [159]. G4 generation of polypropyleneimine is preferred for delivering small nucleic acid molecules such as siRNAs [160]. A research group study also claimed that a higher generation of polypropyleneimine including G4 and G5 was efficient in delivering siRNA and improving the downregulation of targeted mRNA in the A549 cell line [161]. Highly branched poly(L-lysine) dendrimers, on the other hand, are offered for enhancing the intracellular accumulation of large nucleic acid molecules with less cyto/genotoxicity effects compared to polyamidoamine and polypropyleneimine dendrimers [162].

Nonetheless, cationic dendrimers perform poorly in gene transfection because they often induce severe cytotoxicity due to non-specific interactions with cellular membranes, mitochondrial damage, and the production of reactive oxygen species[163]. In dendrimer-based gene delivery vectors, there is a connection between transfection effectiveness and cationic dendrimer cytotoxicity. Poor-generation dendrimers have minimal transfection efficacy but low toxicity, whereas high-generation dendrimers have high transfection efficacy but severe cytotoxicity. In addition, polyplex instability, inadequate cellular uptake and endosomal escape, difficulties in DNA unpacking, and DNA destruction by cytosolic nuclease are all obstacles to successful gene transfection[164]. To address these disadvantages, cationic dendrimers should be changed with diverse functional ligands in order to build highly efficient gene vectors based on low-generation dendrimers. The introduced modifications are generally including lipids, amino acids, saccharides, polymers, nanoparticles, fluorous compounds, cationic moieties, and targeted ligand [165].

7.2.1. Lipid-based modification

Because lipids such as fatty acids and cholesterol have significant fusogenic qualities, lipid-based gene vectors like Lipofectamine, Lipofectin, and Lipofectam have high transfection efficiency in a range of cell types [166]. Polyamidoamine dendrimers treated with fatty acids such as lauric acid, myristic acid, and palmitic acid significantly increase gene transfection effectiveness in mesenchymal stem cells [167]. The inclusion of lipids enhances dendrimer/DNA polyplex cellular absorption and raises the degree of cellular uptake as the chain length of the modified lipids (lauric acid < myristic acid < palmitic acid) is rises [167]. Dendrimers with longer lipid modifications have difficulties in releasing intracellular DNA due to tight interaction between DNA and lipids. As a consequence, dendrimers modified with lauric acid (the shortest lipid) are the most successful at gene transfection. Similarly, the efficiency of polypropyleneimine dendrimer transfection with alkyl carboxylates is strongly dependent on the chain length of altered lipids [168]. When compared to unmodified polypropyleneimine dendrimers, C6- and C16-alkane-modified dendrimers show a negligible increase in transfection efficacy, whereas C10-alkane-modified dendrimers show a considerable increase in transfection efficacy. Another study found that a short lipid (C6 alkane) modification on a triazine dendrimer effectively increased siRNA transfection, with improved endosomal escape, better intracellular siRNA distribution, and smaller polyplex size [169]. Unsaturated C18-alkane-modified poly(L-lysine) dendrimers similarly enhance RNA transfection *in vivo* without significant toxicity [170]. Novel amphiphilic Carbosilane dendrons with generation 1–3 modified with two different fatty acids (e.g. hexanoic acid or palmitic acid groups) indicated potentially applicable as carriers for therapeutics [171]. The siRNA binding modified was similar between the two different fatty acids, however, relied on the dendrimer generation (G1 <<G2 <G3).

Another potential technique for enhancing gene transfection efficiency in low-generation dendrimers is to introduce several lipid moieties. Conjugation of several lipid moieties, including alkyl chains (C4, C12, saturated and unsaturated C18) and cholesterol, to G2 polyamidoamine dendrimers, for example, demonstrated significant transfection effectiveness[172]. However, because other important parameters like as dendrimer formation, dendrimer species, and even the linkage bond are involved, we cannot determine which chain length or amount of conjugated lipids is optimal for effective DNA or siRNA transport among the lipid-modified dendrimers.

7.2.2. Amino acid-based modification

Histone octamers cover the DNA during synthesis, and the phosphate backbone plays a key function in generating a salt bridge with a large basic residue. As a result, basic amino acids and oligopeptides can have a major impact on the development of biocompatible gene vectors [173]. Twenty commonly used amino acids are classified as anionic, cationic, or neutral amino acids, as well as hydrophilic or hydrophobic. These amino acids have the same fundamental structure, which includes amino groups, carboxyl groups, and amide bonds. [174]. Some amino acids have different residue groups such as guanidine, amine, imidazole, lipid, thiol, and aromatic ring, which are primarily employed because of their excellent structure-activity correlation. For example, numerous studies indicated that the conjugation of dendrimers with peptides rich in arginine, histidine, lysine, and phenylalanine could significantly promote the transfection efficacy compared to simple dendrimers [175].

Cationic amino acids such as lysine and arginine, contain two positively charged groups in their structures, and conjugating them with dendrimers improves the charge density on the dendrimer surface, allowing for better DNA condensation and polyplex stability easier [176]. In addition to the charge density effect, the positive charge of the guanidinium group in arginine is delocalized on three nitrogen atoms, allowing it to connect to phosphates in DNA more readily than localized cations such as ammonium. Furthermore, the guanidinium group in arginine has a strong affinity for cell membranes due to ionic pairing and hydrogen bonding [177]. As a result, lysine- and arginine-based amino acid-functionalized dendrimers have seen a lot of use as potential gene carrier. It is noteworthy to note that the efficacy of these modifications is also affected by dendrimer type, and production as well as dendrimer

and amino acid linkage [178]. For instance, polypropyleneimine dendrimers with lysine modifications are more efficient in gene transfection than those with arginine modifications, while polyamidoamine dendrimers with arginine modifications are more efficient than those with lysine modifications [179,180]. Degradable ester linkages in lysine-polyamidoamine dendrimer and arginine-polyamidoamine dendrimer conjugates, on the other hand, have been found to be more effective in gene transfection than nondegradable amide bonds [178]. A research group developed arginine modified generation 5 and 6 (G5 and G6) dendrimer via click chemistry to investigate the transfection activity, the biocompatibility, and generations of dendrimers as gene delivery vectors in both *in vitro* and *in vivo* models [181]. They demonstrated that high generation dendrimers could condense plasmid DNA and protect the loaded plasmid DNA from nuclease degradation. Breast tumor *in vitro* and *in vivo* models revealed that the transfection effectiveness of G5 dendrimer (17 kDa) was six-fold higher than branched polyethyleneimine, as well as higher than G6 dendrimer (46 kDa) along with good biocompatibility. Thus, the arginine modified dendrimer G5 could be a useful carrier for safe and efficient gene delivery [181]. Another interesting work developed novel modified lysine-based dendritic macromolecules including D3K2 (comprising two additional lysine residues (Lys-Lys) with charged NH_3^+ groups between each pair of neighboring branching points of standard poly(lysine) dendrimer of 3rd generation (D3)) and D3G2 (containing two additional glycine residues (Gly-Gly) [3]. In cervical adenocarcinoma (HeLa) and microvascular endothelial cell lines, the dendrimers were tested for cytotoxicity, DNA damage, and transfection capabilities. They claimed that the cationic D3K2 dendrimer could deliver large nucleic acid molecules such as plasmid DNA to tumor and normal cells. Also, the dendrimers showed specific cytotoxicity towards tumor cells without exhibiting high toxic effects on normal cell lines.

Another possible alteration for enhancing cationic dendrimer gene transfection is histidine amino acid[182]. Due to the pH-sensitivity nature of the imidazole group, the histidine-modified dendrimers are also serum-resistant, which reduces polyplex cytotoxicity. Hydrophobic amino acid modifications, such as phenylalanine and leucine, can also increase polyplex cellular internalization and consequently gene transfection efficiency. [183]. Nevertheless, a high concentration of hydrophobic amino acids on the dendrimer surface produces high cytotoxicity to the transfected cells. Thus, tailoring a dendritic gene vector's amino acid composition and cationic charge increases cellular absorption and polyplex biosafety. Many modifications of dendrimers with numerous amino acids are another potential option for overcoming the multiple extracellular and intracellular hurdles in gene delivery [184]. It was suggested, for example, that siRNA transport may be very successful utilizing a dendrimer comprising an endosomal escape moiety (e.g. histidine) and a hydrophobic moiety (e.g. aromatic amino acids such as phenylalanine and tyrosine)[177]. The optimal ratio of arginine, phenylalanine, and histidine on dendrimers also has a synergistic impact on gene transport, enhancing gene interference efficiency (Fig. 6A) [177]. A recent study similarly used a combination of guanidyl and phenyl groups on the surface of a cationic polyamidoamine dendrimer to generate a possible carrier for gene delivery [185]. The guanidyl group on the dendrimer promotes nucleic acid condensation by creating hydrogen bonds with phosphate groups on nucleic acids and phospholipids, whereas the phenyl group on the polymer is required for effective endosomal escape (Fig. 6B). As a result, the suggested carrier was substantially more effective than unmodified dendrimers and dendrimers modified just with guanidyl or phenyl groups in both siRNA and DNA delivery [185].

7.2.3. Polymer-based modification

Polymer modification can also boost transfection efficiency while reducing cationic dendrimer cytotoxicity. PEG is an exceptionally beneficial polymer for increasing dendrimer effectiveness in gene delivery, because of its exceptional features such as high biocompatibility, non-immunogenicity, and antifouling activity (Fig. 7A) [186]. Furthermore, PEGylated dendrimers can improve the blood circulation time of the polyplexes and serum stability. It also enhance permeability and retention effect, which leads to increased tumor accumulation [187]. An increase in the amount of PEG on the dendrimer surface, decreases the number of positive charges for DNA compacting. Polyplex sizes may be rather large due to inadequate DNA condensation, resulting in poor preservation of the attached nucleic acids[188]. As a result, PEGylation

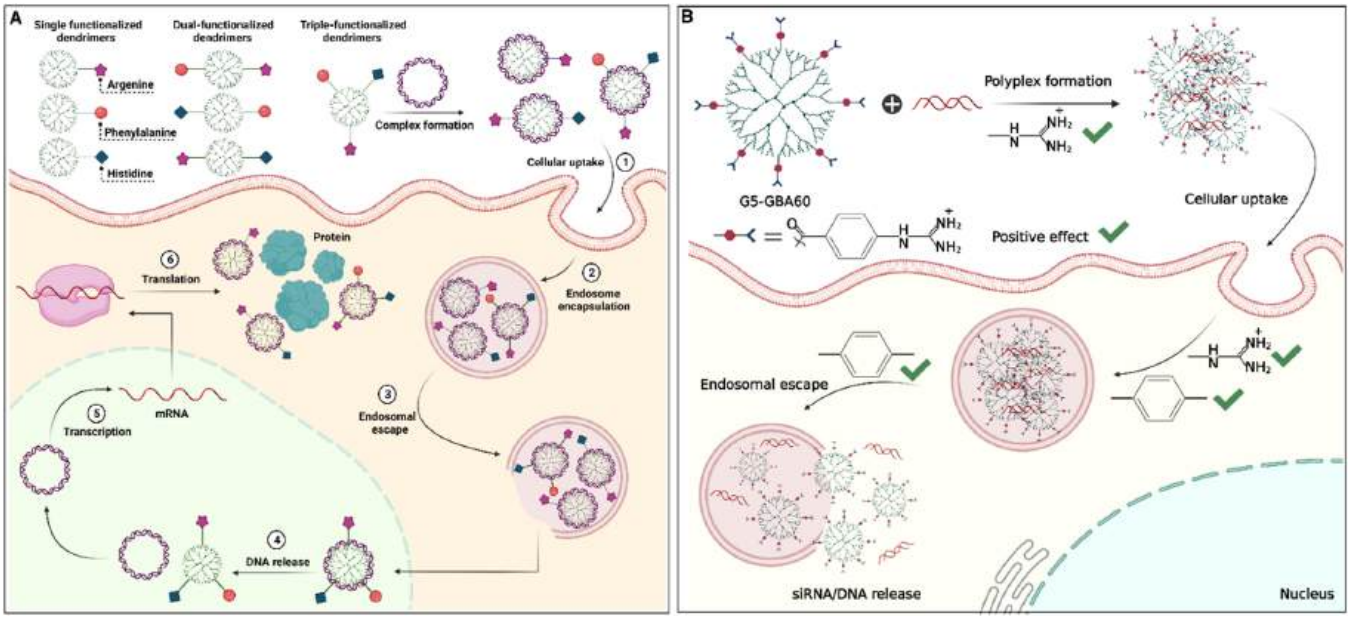

Fig. 6. Transfection mechanisms of functionalized dendrimers. (A) Schematic illustration of transfection mechanisms of single-, dual- and triple-functionalized dendrimers. (B) Proposed mechanism for Generation 5 (G5)-Guanidinobenzoic acid (GBA60) G5-GBA60in efficient gene delivery, the dendrimer's guanidyl group enhances nucleic acid condensation by creating hydrogen bonds with phosphate groups on nucleic acids and phospholipids, but the polymer's phenyl group is necessary for endosomal escape.
(A) Reprinted from [177] with permission from Elsevier. (B) Reprinted from [185] with permission from ACS.

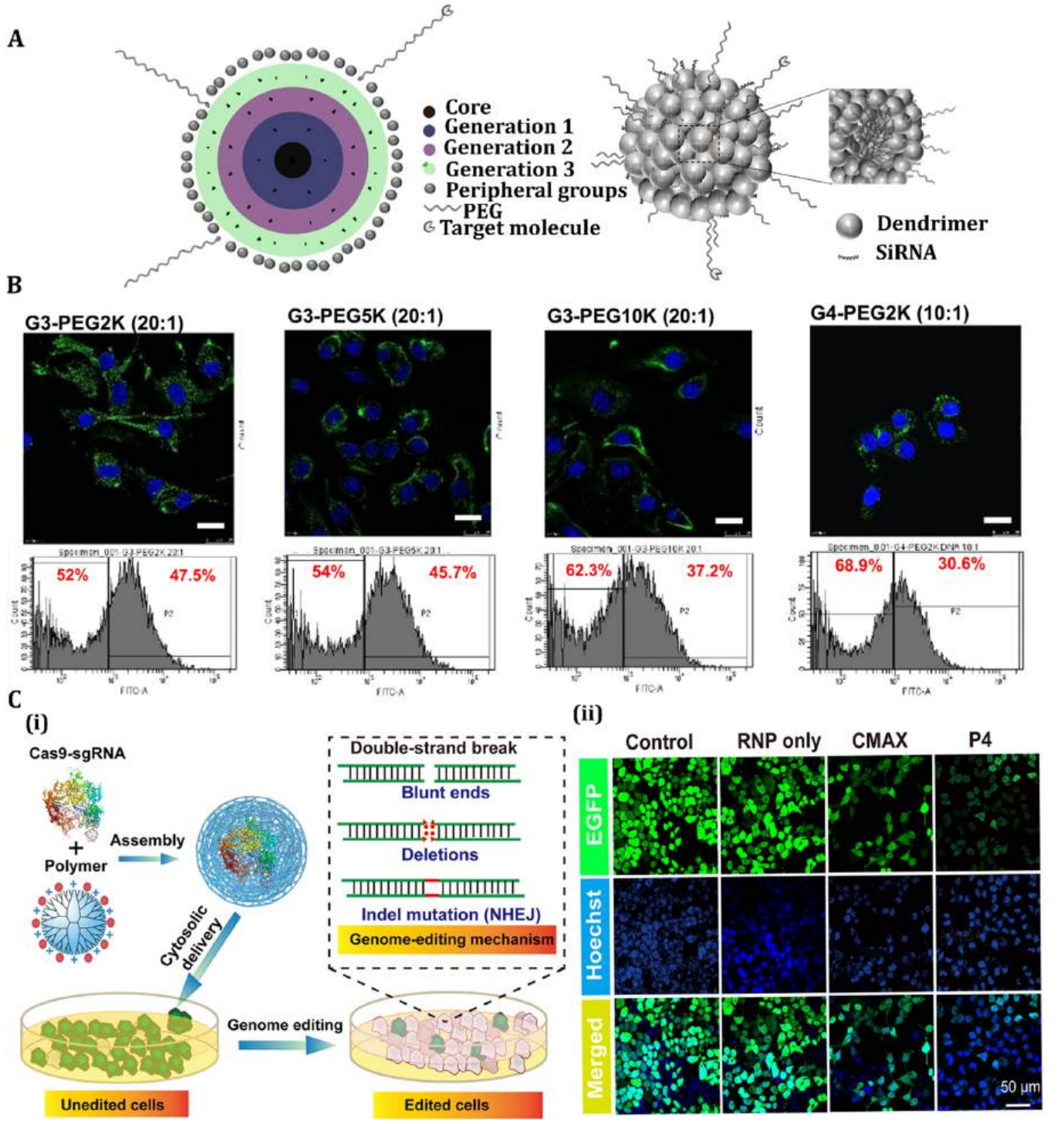

Fig. 7. Targeting of siRNA molecules using dendrimer complex. (A) Schematic illustration of a dendrimer with modified chains of poly(ethylene glycol) (PEG) and a target molecule siRNA/dendrimer complex (dendriplexes), (B) Confocal microscopy imaging and flow cytometry quantification of fluorescein-labeled DNA (2.5 g/well) complexed with G3-PEG2K, G3-PEG5K, G3-PEG10K (dendrimer: DNA weight ratios: 20:1), and G4-PEG2K (dendrimer: DNA weight ratios: 10:1) after 1 h incubation with B16F10-Luc cells (Bar: 10 μm). (C) Boronic acid-rich dendrimer with robust efficiency in cytosolic protein delivery, **(i)** P4-mediated Cas9/sgEGFP delivery for efficient EGFP genome editing, **(ii)** Confocal images of 293 T-EGFP cells treated with P4/RNP for 4 h. Scale bar, 50 μm,
(A) Reprinted from [196] with permission from Bentham Science Publishers B.V. (B) Reprinted from [190] with permission from Nature. (C) Reprinted from [195] with permission from the American Association for the Advancement of Science.

may impair the cellular uptake and transfection efficacy of cationic dendrimers. For example, *in vitro,* and *in vivo* studies revealed that 8 % PEG-conjugated polyamidoamine dendrimers resulted in the most effective gene transfection, hemolytic activity, and decreased toxicity when compared to 4%, 8%, and 15% PEGylation on G5 or G6 polyamidoamine dendrimer [189]. Another study likewise showed that G3 diamino butyric polypropyleneimine dendrimers conjugated to low molecular weight PEG (2 kDa) dramatically reduced the cytotoxicity of amine-terminated dendrimers (Fig. 7B) [190]. G4-DAB conjugated to 2 and 5 kDa PEG similarly reduced cytotoxicity, however, only at low

concentrations (less than 20 μg/mL). These dendrimers also exhibited high DNA transfection as compared with the unmodified dendrimers on different cell lines including DU145, B16F10-Luc, PC3-Luc, A431 cells.

Besides PEG, other polymers, such as chitosan, polyethyleneimine, polyethylene glycol- polyethyleneimine, poly(D, L-lactide-co-glycolide) acid, polylactic glycolic acid- polyethyleneimine, etc. were also conjugated on cationic dendrimers to improve the efficiency of dendrimers in gene delivery [191–193]. For instance, functionalizing the carbosilane dendrimers (G2 and G3) with polylactic glycolic acid can condense antisense oligonucleotides at low complexation ratios, 0.25/1, and 1/1 ratio res polyethyleneimine actively for G2 and G3 dendrimers [194]. The best results were also obtained utilizing the G2 dendronized complex, which achieved gene silencing efficiencies up to 90% with minimal cytotoxicity on the transfected cells. In an interesting recent work, the phenylboronic acid-based polymer was grafted onto G5 polyamidoamine dendrimers for cytosolic delivery of native proteins [195]. Phenylboronic acid-rich polymer provides strong binding affinity with various types of proteins through cation-π and ionic interactions, and a combination of nitrogen-boronate complexation, resulting in efficiently delivered 13 cargo proteins into cells' cytoplasm (Fig. 7**C**). This dendrimer also enables the efficient transport of Cas9 ribonucleoprotein to numerous genomic loci in a variety of cell lines, indicating that it could be a valuable material for the delivery of genome editing tools in different biomedical applications [195].

7.2.4. *Nanoparticle-based modification*

Carbon nanotubes, graphene, quantum dots, magnetic nanoparticles, gold nanoparticles, and silicon are among the nanomaterials being studied for cancer gene delivery [197]. These nanostructures can be readily grafted onto dendrimers to create multifunctional carriers with lower cytotoxicity and higher gene uptake and transfection efficiency than unmodified dendrimers. For example, it has been demonstrated that dendrimer modification with multiwalled carbon nanotubes, carbon nano horns, and carbon dots may be used for successful siRNA, plasmid DNA, and antisense oligonucleotide delivery [198,199]. Recently, a research group created anionic fluorescent carbon dots with a round shape and an average size of 4 nm that emit intrinsic fluorescence in three regions (blue, green, or orange/red region) [200]. Three generations of fluorescent carbon dots were then used to self-assemble the stable fluorescent carbon dots. The nanohybrids were readily

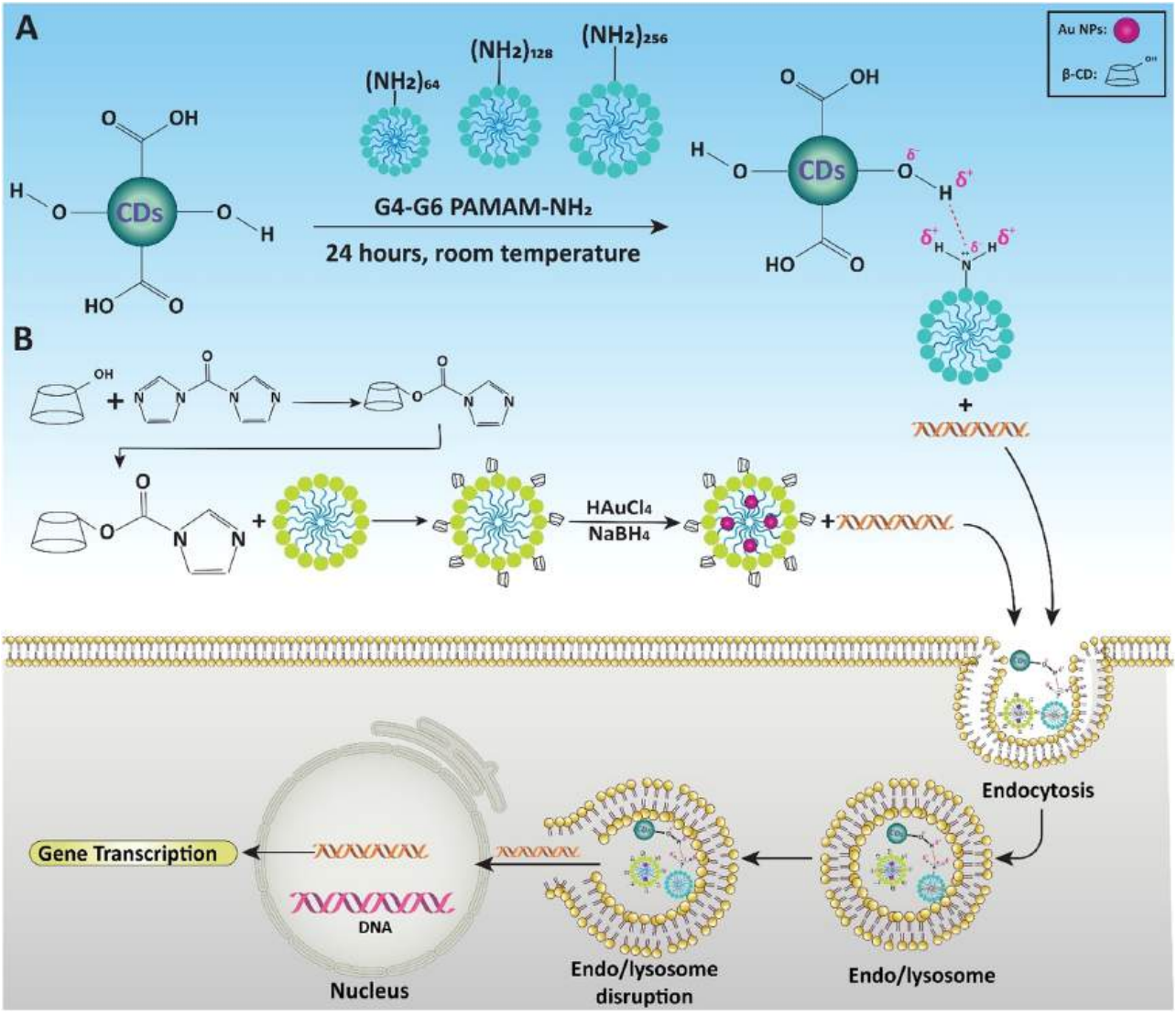

Fig. 8. Gene delivery behavior of anionic carbon dots (CDs)-dendrimers and gold nanoparticles (AuNPs)-dendrimers-β-cyclodextrin (A) Schematic illustration of the hydrothermal synthesis of anionic CDs and their electrostatic self-assembly with polyamidoamine dendrimers (Generations 4–6) to generate G4-G6 CDs@PAMAM Nanohybrids-. (b) (B) Schematic illustration of the synthesis of AuNPs-dendrimers-β-cyclodextrin
(a) Reproduced from [200] with permission from ACS. (b) Reproduced from [210] with permission from the Royal Society of Chemistry.

absorbed by cells in culture, and fluorescence emission could be seen in all three places depending on the excitation wavelength used [200]. Furthermore, the G4-G6 carbon dots @ polyamidoamine nanohybrids were able to condense plasmid DNA completely, functioning as gene delivery vectors with much better transfection efficiencies than unmodified dendrimers. Nanohybrids based on G5 polyamidoamine dendrimers, on the other hand, were exhibited to form small, and homogeneous polyplexes along with mild cytotoxicity and high transfection efficiency as compared with other dendrimer generations (Fig. 8). Coupling of polypropyleneimine dendrimer with gold nanoparticles also showed promising efficiency in siRNA-mediated mRNA downregulation [107]. The gold nanoparticles help low generation polypropyleneimine dendrimers (G3) to condense siRNA at a high level while having little influence on the producing polyplexes. [201]. The polyplexes deliver siRNA to tumor cells and silence their target mRNAs with high efficiency. Low-generation dendrimers that have been augmented with gold nanoparticles are more effective in silencing mRNA than high-generation dendrimers. Furthermore, dendrimer-stabilized gold nanoparticles exhibit a considerable rise in gene transfection effectiveness and lower cytotoxicity in transfected cells on numerous cell lines [201,202]. Similarly, polyamidoamine dendrimers functionalized with gold nanorods could be used to deliver short hairpin RNA into MCF-7 breast tumor cells [203]. These nanocarriers besides owing to their interstice photothermal ability could be used to kill tumor cells by NIR light irradiation. The magnetic iron oxide (Fe_3O_4) nano-worm grafted onto polyamidoamine dendrimers can likewise enhance endosomal escape and decrease the expression of the EGFR protein, resulting in an increased siRNA transfection in glioblastoma *in vivo* [204]. Another research group found that polyamidoamine dendrimer modified with Fe_3O_4 can transfect NIH 3T3 cells, and the level of gene expression relies on the dendrimer generation, plasmid DNA concentration, and the N/P ratio [205]. They claimed that generation 6 polyamidoamine dendrimers at an N/P ratio of 10 were the best system to transfect high plasmid DNA without significant cytotoxicity. Mesoporous silica nanoparticles are another well-organized platform to be applied in clinical gene delivery application, because of their tunable pore diameter, particle size, and ease of surface functionalization [206]. The presence of a pore network provides a high surface area for high therapeutic loading capacity. It was indicated that mesoporous silica nanoparticles functionalized with Carbosilane H_3N dendrimer can transport single-stranded oligonucleotides into cells while also having the potential to deliver additional medications inserted into the silica mesopores [207].

Cyclodextrins, which are cyclic oligosaccharides with six, seven, or eight glucopyranose units and are categorized as α-, β-, and γ- cyclodextrins, are also excellent nanocarriers in gene delivery. Cyclodextrins interior cavities can encapsulate a wide range of hydrophobic therapeutic molecules [208]. Additionally, cyclodextrins can bind cholesterol, cholic acid, and other lipid molecules prevalent on cell membranes. As a result, conjugating cyclodextrins to a cationic dendrimer surface improves carrier solubility, stability, and biocompatibility as well as cell membrane affinity. [209]. For example, it was reported that modifying G2 polyamidoamine dendrimers with α-, β-, or γ- cyclodextrins significantly improve their transfection potential on NIH_3T_3 cells and RAW264.7 cells [210]. Besides, among various conjugates, G2-cyclodextrin is the most efficient vector for transfecting cell lines. Another study modified the G5 polyamidoamine dendrimers surface with β-cyclodextrin and AuNPs for delivery of plasmid DNA into 293 T cell lines [210]. The AuNPs-dendrimers-β-cyclodextrin could compact the plasmid DNA at an N/P ratio of 0.5:1 successfully and facilitated more efficient cellular gene delivery than AuNPs-dendrimers without β-cyclodextrin conjugation without substantial cytotoxicity. Overall, recent reports that have indicated the role of different dendrimers in the delivery of siRNA, miRNA, DNA, into tumor cells are summarized in Table 3.

8. Co-delivery

Despite the fact that dendrimers containing a medication or a gene have a considerable influence on tumor cell resistance, drug and gene co-delivery via a single delivery mechanism might be more successful in cancer treatment. [211]. To overcome the limitations of systemic administration of combination therapy, such as cytotoxicity from chemotherapeutic agents, drug insolubility, and multi-drug resistance mechanisms, a co-delivery method is required. Furthermore, because of differences in drug and gene hydrophobicity, systemic stability, and molecular weight, an efficient drug and gene co-delivery method that can transport gene and drug to target tumor cells simultaneously without interfering with drug pharmacological behavior or release kinetics is required. [212]. The tree-like structure of dendrimers is suitable for encapsulating hydrophobic pharmaceuticals, and the primary amine group is used to electrostatically interact with the phosphorous groups of nucleic acids, allowing both drugs and genes to be delivered to tumor cells simultaneously [213]. Co-delivery of drugs and genes is typically employed for different purposes including promoting apoptosis, anti-angiogenic function, immunotherapy, etc. It is also well-established that suppressing drug efflux pumps with a siRNA-drug delivery system causes downregulation of drug efflux proteins as well as accumulation of anticancer agents in tumor cells, resulting in higher combination therapy efficacy than single molecular drug therapy [214]. For example, one study used functionalized polyethyleneimine dendrimer with 1,2-dioleoyl-sn-glycerol-3-phosphoethanolamine to deliver MDR-1 siRNA and doxorubicin to the NCI/ADR-RES cell line, which is resistant to doxorubicin [215]. MDR-1 siRNA caused intracellular doxorubicin accumulation and a significant increase in drug toxicity through downregulating P-glycoprotein (P-gp). Another study created an amine-terminated G5.0 polyamidoamine dendrimer-selenium conjugate as a co-delivery method to test the synergistic impact of simultaneous administration of MDR-1 siRNA with cisplatin as an efficient anticancer medication. [216]. They indicated that the expression of P-gp and MDR-associated proteins were dramatically reduced in A549 resistant tumor cells after treatment with this delivery method. Another combination therapy based on polyamidoamine dendrimer generation G6.0 encapsulating both paclitaxel chemical drug and siRNA was developed to block the phosphatidylinositol 3-kinase/Akt pathway in ovarian cancer [217]. Both *in vitro* and *in vivo* results demonstrated that the AKT siRNA-dendrimer/ paclitaxel entrapment complex displayed efficient gene silencing activity. Paclitaxel's therapeutic efficacy in ovarian carcinoma cells was also improved by the modified co-delivery method.

Doxorubicin and MMP-9-short hairpin RNA was encapsulated into a graphene oxide functionalized G3 polyamidoamine dendrimer to achieve effective breast cancer treatment. The fabricated complex provided a high drug loading of doxorubicin with pH-dependent release as well as a high transfection efficiency, which lead to suppressing MMP-9 protein expression in MCF-7 cells [164]. In a promising co-delivery system, PEG-G2 dendrimers were modified with PTP (plectin-1 targeted peptide, NH2-KTLLPTP-COOH) through disulfide linkages for targeted co-delivery of paclitaxel and nuclear receptor siRNA (siTR3) and *in vitro* and *in vivo* [218]. The targeted carrier specifically aggregates in pancreatic tumor cells through receptor-mediated cell endocytosis. The loaded paclitaxel and siRNA were then released from the carrier and applied their therapeutic efficacy due to the cleavage of disulfide bonds in the intracellular glutathione-rich reduction environment. *In vitro* studies indicated that the co-delivery system successfully facilitates cellular uptake and shows high gene transfection in pancreatic tumor cells (Panc-1 cell lines) [218]. Besides, the expression of anti-apoptotic proteins, including Bcl-2 and Survivin were decreased through siTR3 mediated knockdown of TR3. Interestingly, co-delivery of paclitaxel and siTR3 inhibited tumor cell proliferation in murine tumor models *in vivo*, which was also more effective than paclitaxel- and siTR3-based monotherapy. Overall, such co-delivery carriers have great potential applications in clinical cancer therapy.

Table 3
Summary of dendrimer-based gene delivery systems.

Type of dendrimer formulation	Gene agent	Cancer	Particle size and zeta potential	Encapsulation efficiency	Cell line/ Animal model	Remarks	Refs
G4-polyamidoamine (PAMAM)	siRNA-VEGFA	Head and neck squamous cell carcinoma	Not reported	Not reported	HNSCC	In an HN12 xenograft tumour model, localized administration of FR-targeted PAMAM dendrimer G4 complexed with siVEGFA caused significant tumour suppression.	[1]
G5-PAMAM	miRNA	Breast cancer	100–400 nm - 20 mV to + 20 mV	Not reported	MDA-MB-231 and / Female athymic nude mice (BALB/c)	The results of *in vivo* and *ex vivo* imaging in human breast cancer tumor-bearing mice demonstrated that TA-modification increased the concentration of NPs in the tumour.	[2]
Dendrigraft poly-L-lysine	Antisense oligodeoxynucleotide	Murine hepatocytes cancer cells (H22)	97–102 nm + 29 mV	166.7 mg/g	HepG2 and Hela cells/ Male Balb/ c mice	According to *in vivo* antitumor efficacy testing, this nanocomposite showed strong anticancer activity, with a tumour inhibitory rate of 77.99%.	[3]
G5- Polypropyleneimine (PPI)	siRNA	Ovarian cancer	100–200 nm + 1.10 ± 1.54 mV	Not reported	mice	When paclitaxel is used, tumour apoptosis is increased, and siRNA activity is increased.	[4]
G4-PAMAM	siRNA	Colon cancer	148 ± 5–227 ± 9 nm 18.4 ± 1.4 – 30.3 ± 1.0 mV	Not reported	KB cells, HeLa, A549 cells, Colon-26-luc cells/ BALB/c male mice	The proposed nanocarrier has a high blood circulation ability, a high tumour *in vivo*, selective siRNA transfer activity, and a good safety profile.	[5]
Dendrimer 1 C	siRNA	Colon and kidney cancer	12–17 nm + 18 to + 46 mV	Not reported	HepG2, Hep3B, HT-29, HEK 293 cells	The ionizable properties of dendrimer via tertiary amine functions is a viable and effective method for siRNA administration with a positive safety profile.	[6]
G5-PAMAM	siRNA	Kidney cancer	< 194 nm < 20 mV	94.8%	HepG2 cells	Increased dendriplexes accumulation in tumor	[7]
PAMAM	Bcl-2 siRNA curcumin	Cervical cancer	~180 nm	~82%	HeLa cell lines	AMAM-Cur/Bcl-2 siRNA NPs showed more effective cellular uptake, and higher inhibition of tumor cell proliferation compared to PAMAM-Cur nanoformulations	[8]
G3-PAMAM polyplexes gel	pDNA	Breast cancer	219.3 nm	Not reported	4T1 cell lines	NCs/pDNA polyplexes enable 2.3- and 2.1-times higher gene transfection to cancer cells than the counterpart materials of single G3 and G5 PAMAM dendrimers	[9]
G4-PAMAM Conjugated with PEG-DOPE	siRNA and doxorubicin	Colon and breast cancer	225 nm − 18 mV	Not reported	A2780 ADR, MDA-MB-231 and HCT 116 cell lines	HA-modified MDMs alleviated the toxicity from cationic charge, increase the cancer cell specificity and enhance the cancer cell killing effect in CD44 + cell line.	[10]
Hydroxy terminal PAMAM dendrimer	P53 and RG7388	Breast cancer	200 nm zeta potential in the range of − 20–20 mV	92.5%	P53-wild type MCF-7 cells (MCF-7/WT) and MDA-MB-435	*IV* administration of PAMPSF/p53/RG inhibited tumor growth of MDA-MB-435 and MCF-7/WT xenograft mice models and induced no substantial 3 wt loss.	[11]
		Lung cancer	< 10 nm + 14.5 mV				[12]

(*continued on next page*)

Table 3 (*continued*)

Type of dendrimer formulation	Gene agent	Cancer	Particle size and zeta potential	Encapsulation efficiency	Cell line/ Animal model	Remarks	Refs
Folic acid conjugated PAMAM dendrimer	siRNA and *cis*-diamine platinum			Den-PEI 35.65 ± 5.65 Den-PEI-FA 40.52 ± 4.18%	H1299 cell lines	FRA-targeted NP exhibited improved cytotoxicity compared to non-targeted NP against lung cancer cells	
PAMAM incorporated PEG liposomes	Anti-VEGF siRNA	Breast cancer	130 nm + 4 mV	96%	SKBR-3 cell lines	Higher sequence-specific inhibition of VEGF expression and cell growth than the respective G2-Chol40%/ siRNA dendriplexes	[13]
Amphiphilic phospholipid peptide dendrimers	siRNA	Prostate cancer	~100 nm siRNA/DSPE-KK2: 22.3 mV; siRNA/ DSPE-KK2K4: 18.5 mV	Not reported	PC-3 cell lines	Improved intracellular uptake and endosomal release of siRNA complexes, resulting in more potent gene silencing and anticancer effects both *in vitro* and *in vivo*	[14]
10-bromodecanoic acid modified PAMAM dendrimer	AS1411 and shRNA plasmid	Lung cancers	128–230 nm 12.76–19.13 mV	Not reported	A549 cell lines	Down-regulation of the anti-apoptotic gene (bcl-xl) up to 25%, apoptosis induced (14% late apoptosis) in targeted cells with strong cell selectivity	[15]
G5-PAMAM decorated zwitterion carboxybetaine acrylamide (CBAA) and lysosome-targeting agent morpholine	AuNPs and pDNA	Cervical cancer	100–400 nm + 30 mV	Not reported	Hela cell lines	Hypermethylated in cancer 1 (HIC1) protein expression assay data reveal that the expression of HIC1 gene in cancer cells enables effective inhibition of cell migration	[16]
Ruthenium-based Carbosilane dendrimer	siRNA	Leukaemia	318.5 ± 54.5 nm − 40–45 mV	Not reported	HL-60 cell line	Carbosilane dendrimer can transfect cancer cells with anti-cancer siRNAs and can prevent them from degrading when they are exposed to nuclease enzymes	[17]
Histidine and Arginine modified PAMAM dendrimer	pDNA and polyanionic peptide repebody	Breast cancer, Liver cancer	185.5 nm 23.1 mV	Not reported	MDA-MB-231, HepG2 cell line	Resulted in high efficiency and low cytotoxicity	[18]
Diethylenetriamine and tetraethylenepentamine-modified G 4.5-PAMAM dendrimer	siRNA	Cervical Cancer	472–191 nm, 215–101 nm	Not reported	Hela cell line	Enhanced cellular uptake compared to naked cy3-siRNA and untreated control with good cellular internalization and protected from nuclease degradation	[19]
PEGylated generation 3-dia-minobutyric Polypropyleneimine dendrimer	pDNA and Camptothecin	Prostate cancer	109 ± 4 nm to 105 ± 1 nm	Not reported	PC3-Luc cell line	Showed approximately 70% release of Camptothecin and greater than 85% of DNA condensation	[20]
Histidine-lauric acid-based green surfactant and PAMAM dendrimers	Docetaxel and SIRT 1 shRNA	Breast cancer	262.33 ± 3.87 nm	70.56%	MDA-MB-231 cells	released docetaxel over time, enhanced uptake by MDA-MB-231 cells, and had higher transfection potential than free DTX or naked shRNA	[21]
D3K2 and D3G2 poly(lysine) dendrimer	DNA	Human cervix adenocarcinoma,	Not reported	Not reported	Hela cell, HMEC-1	Greater cytotoxicity towards cancer cells. Moreover, D3K2 dendrimer showed more transfection efficacy and efficiently delivered plasmid DNA to both cancer and normal cells.	[22]

9. Biosafety concerns and translational medicine

In general, the main goal of nanomedicine is to deliver therapeutics and imaging agents to a target site effectively and with minimal toxicity to the normal tissue/organ [219,220]. Recent studies demonstrate the efficacy of dendrimers to conjugate or encapsulate drugs for the treatment of various diseases, such as cancer [221], infections [222], or inflammatory diseases [88] via different routes of administration (e.g., intravenous, intraperitoneal, intranasal, ocular, transdermal, pulmonary, and oral) [20]. In addition, they can reduce the dosage of drugs [20], improve the pharmacokinetic and pharmacodynamic profile of drugs [20,211], and increase the delivery of drugs exactly to the desired site [223]. In addition, they can potentially be used as sensitive diagnostic tools to detect diseases with higher sensitivity and specificity [223,224]. However, although dendrimers are revolutionizing nanomedicine by developing therapeutic and more precise diagnostic tools, lack of knowledge about tissue- and organ-level toxicity, biocompatibility, biodegradability, lifetime of the nanostructures in the bloodstream, and development of multifunctional dendrimers are still challenges on the way to biosafety assessment [20,225]. For example, cationic dendrimers with positive surface groups such as amine exhibited much higher cytotoxicity than dendrimers with anionic and neutral terminals [226]. It was shown that lower generation dendrimers exhibiter lower toxicity than higher generation dendrimers when their cytotoxicity was investigated using MTT assays on different cell lines [227]. At the molecular level, dendrimers showed strong binding affinity to vitamins, amphiphilic lipids, bile acids, nucleic acids, and proteins, indicating that the administration of dendrimer-based therapeutics in physiological systems could affect the function of the biomacromolecules [228].

Currently, the construction of dendrimers with biocompatible surface functional groups is probably the best way to solve the problem of toxicity of dendrimers and allow nanoparticles to have a long circulation time [228–230]. To this end, dendrimers with PEG, acetyl, lysine, arginine carboxyl, cyclodextrin, mannose, and galactose end groups showed lower toxicity than polycationic dendrimers [228]. The long term toxicity of dendrimers after multiple administrations and their accumulation in different organs remain the major challenge in this research area and should be addressed in future studies. This is particularly true for dendrimers which are non-degradable.

A good criterion to evaluate the research and development of the pharmaceutical industry is the number of new drugs brought to the market [220]. The current studies confirm that the productivity of research and development has declined noticeably in recent decades, along with a decline in the return on investment [220]. Therefore, emerging technologies, including nanotechnology, have opened a new paradigm to solve the problems associated with the pharmaceutical industry [231]. These technologies combine chemistry, physics, biology, and engineering to have a major impact on the treatment of various human diseases such as cancer, central nervous system disorders, and cardiovascular diseases [232]. Moreover, a wide variety of nanoformulations with favorable pharmacokinetic, pharmacodynamics, and enhanced absorption, distribution, metabolism, and excretion properties have been developed using these technologies [233]. Among the numerous nanomedicine formulations, dendrimers as carriers have shown new possibilities in nanomedicine for the treatment of patients [220]. This is mainly due to the unique properties of the nanostructures, including biocompatibility, biodegradability, monodispersity, ease of surface functionalization, safety in the body, and non-immunogenicity [20,234]. Some of the tunable dendrimers are already commercially available, such as poly(propylene imine) and poly(amidoamine) as well [20]. Although dendrimers are making steady progress in the development and manufacture of biocompatible delivery systems, they are still in their infancy, and many translational requirements should be met before the initiation of entering clinical trials and commercialization [20]. For example, the interaction of therapeutic NPs with blood proteins and the quality of their production must be carefully and thoroughly tested [20]. They must meet the strict regulations of the United States Food and Drug Administration and the approval protocols of the European Medicines Agency. Such translational requirements are the basic prerequisite for the introduction of candidates into the clinic. Pharmacokinetic profiles, distribution in blood and tissues, metabolism, excretion, toxicity, and accumulation in target tissues are guidelines published by the Food and Drug Administration that should be considered before initiating Phase I clinical trials of nanoformulations [220, 235]. However, despite numerous publications, there are only two biologically active dendrimers for clinical applications [220]. Moreover, some dendrimers have passed through the valley of death between early research and clinical application [20].

10. Conclusion and future perspectives

Chemotherapy and combinatorial techniques are the current cancer treatment options, but their dose is typically limited in clinical practice due to a lack of selection, detection, drug release, and adverse effects. Thus, cancer therapy using dendrimers has been developed with certain unique properties and can be a suitable option for targeted, cell-specific, and controlled release applications. Various dendrimers are available for drugs and gene delivery in both *in vitro* and *in vivo* conditions, and these dendrimers show great promise for targeted cancer treatment. Furthermore, it has been found that co-delivery of drugs is a more efficient method in cancer treatment than a single drug or gene delivery. Though, since the safety concerns are connected to the positive charges of the dendrimer, long-term administration of dendrimers may have negative effects or be harmful to body organs. According to the current research, tumors have an acidic pH of 5–6 whereas the body's systemic circulation has an alkaline pH of 7.4. This difference in pH can be used as a significant element in the development of next-generation pH-sensitive-dendrimers. Scientific and medical scientists are analyzing the scope of dendrimers not only in cancer but also in other chronic diseases. Unlike other polymers, dendrimers have a branched structure that can be decorated with a wide variety of molecules for reflexive trapping and release of drug molecules to the specific region. Approaches to target various inflammatory pathways using these nanoscale dendrimers have not been yet explored. So, there is an urgent need, where these dendrimers can be used for molecular targeting that is not discovered yet. It has been observed that just one anti-cancer formulation progresses from the preclinical phase to Phase-I clinical studies, which can be interpreted as a hurdle during the transition from bench to bedside. DEP® docetaxel has demonstrated significant growth, promising outcomes, and success in clinical trials, all these advancements could motivate more ventures to take these dendrimers to the next phases.

Surface modification of dendrimers is an exciting strategy for improving their selectivity towards tumor cells. Hyaluronic acid, folic acid, aptamers, and other bioactive molecules have been utilized in this case. This strategy is based on the identification of receptors on the surface of cells. CD44 and folate receptors, for example, are upregulated on cancer cells and may be preferentially targeted by hyaluronic acid and folic acid-modified dendrimers. Furthermore, such alterations impair dendrimer uptake in cancer cells. Furthermore, such alterations affect cancer cell uptake of dendrimers. The non-modified-dendrimers do not induce endocytic pathway, while surface modified-dendrimers use endocytosis, mainly caveolae- and clathrin-mediated endocytosis for penetration into tumor cells. Furthermore, the surface charge of dendrimers affects endocytosis and anionic dendrimers enter tumor cells by binding to positive proteins on the surface of the cell membrane. The limitation of current works is that there is no experiment evaluating the association between particle size and dendrimer endocytosis that can be the focus of future experiments. It is assumed that discovering more opportunities to synthesize various dendrimer-based hybrid nanomedicine to target various inflammatory pathways has not been investigated much. Such studies can provide better insight about molecular

events in good manufacturing practices conditions and considering trials to examine in high-level animal models will no uncertainty help to speed up the field of cancer nanomedicine and ultimately accelerate efficient clinical translation.

CRediT authorship contribution statement

Asmita Deka Dey: Writing – original draft preparation. **Ashkan Bigham:** Writing – original draft preparation. **Yasaman Esmaeili:** Writing – original draft preparation. **Milad Ashrafizadeh:** Writing – original draft preparation. **Farnaz Dabbagh Moghaddam:** Writing – original draft preparation, Scheme illustration. **Shing Cheng Tan:** Writing – original draft preparation. **Satar Yousefiasl:** Writing – original draft preparation, Scheme illustration; **Saurav Sharma:** Writing-Original draft preparation. **Aziz Maleki:** Writing – original draft preparation. **Navid Rabiee:** Writing – original draft preparation. **Alan Prem Kumar:** Writing – original draft preparation. **Vijay Kumar Thakur:** Writing – review & editing. **Gorka Orive:** Writing – review & editing. **Esmaeel Sharifi:** Writing – review & editing, Commenting and Editing. **Arun Kumar:** Conceptualization, Writing – original draft preparation, Writing – review & editing. **Pooyan Makvandi**: Conceptualization, Figure preparation, Writing – review & editing, Commenting and Editing.

Conflict of interest

The authors declare no conflict of interest

Acknowledgment

A.P.K. was supported by a grant from the Ministry of Education - Singapore Tier 2 (MOE-T2EP30120-0016).

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