miRNA nanotherapeutics for cancer

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MicroRNAs (miRNAs) are noncoding RNA molecules that regulate gene expression through diverse mechanisms. Increasing evidence suggests that miRNA-based therapies, either restoring or repressing miRNA expression and activity, hold great promise. However, the efficient delivery of miRNAs to target tissues is a major challenge in the transition of miRNA therapy to the clinic. Cationic polymers or viral vectors are efficient delivery agents but their systemic toxicity and immunogenicity limit their clinical usage. Efficient targeting and sustained release of miRNAs/anti-miRNAs using nanoparticles (NPs) conjugated with antibodies and/or peptides could reduce the required therapeutic dosage while minimizing systemic and cellular toxicity. Given their importance in clinical oncology, here we focus on the development of miRNA nanoformulations to achieve enhanced cellular uptake, bioavailability, and accumulation at the tumor site.

Introduction

MicroRNAs (miRNAs) are 22 nucleotide-long, noncoding RNA molecules that act as regulators of gene expression and regulate a range of biological functions, including cell survival, proliferation, apoptosis, tumor growth, and metastasis [1]. miRNAs bind to a complimentary mRNA sequence and result in post-translation repression or degradation and silencing. miRNAs are formed by transcription of RNA polymerase II, which folds back to form a distinctive hairpin structure, whereas other small mRNAs are formed from longer hairpin structures [2]. Processing of miRNAs as primary (pri)-miRNA and pre-miRNA (in the nucleus); mature miRNA duplexes, RNA-induced silencing complex (RISC) strand-mediated complex, and complementary mRNA sequence formation cause translation repression and mRNA degradation (in the cytoplasm) [3,4].

miRNAs have significant roles in cancer, as evident from the more than 24,000 peer-reviewed reports (Fig. 1a) and clinical studies on this topic over the years. Although several miRNAs modulating carcinogenic processes have been identified, their clinical translation is limited because of their unsuccessful delivery at the tumor site and their broad functionality, which results in off-target effects. Lentiviral vectors have shown efficient cellular delivery, but their activation of oncogenes and/or excessive immunogenicity raise concerns over the safety of genomic integration. To overcome such limitations, nonviral miRNA delivery systems, such as polyethyleneimine (PEI)-based NPs, liposomes, polymeric micelles, dendrimers, magnetic NPs, and polymeric NPs (Fig. 1b), have been proposed. These delivery systems protect the degradation of miRNAs by nucleases and increase their half-life in the blood [5], can escape from endosomal and/or lysosomal degradation, and deliver miRNAs to the cytoplasm or nucleus (Fig. 1c). The first miRNA replacement therapy to enter clinical trials involved the restitution of a tumor suppressor miRNA (miR-34) in modified liposomes (MRX34, Mirna Therapeutics; http://www.mirnatherapeutics.com/pipeline/mirna-MRX34.html). MRX34 showed promising results in a Phase I clinical trial, where partial responses where observed in patients with renal cell

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1359-6446/
http://dx.doi.org/10.1016/j.drudis.2016.10.014

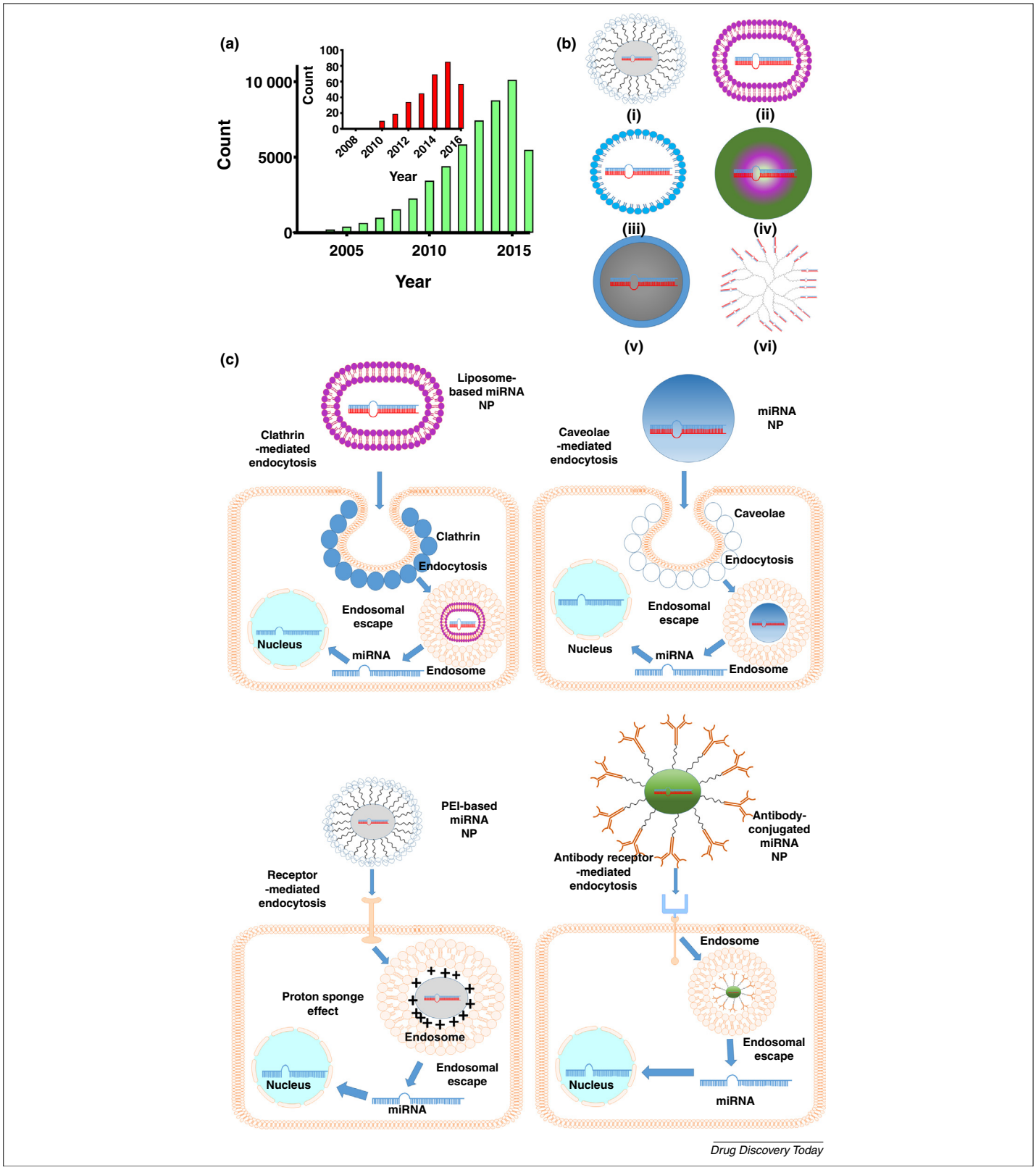

FIGURE 1

Scientific evidence and nano-based delivery of miRNAs for cancer therapeutics. **(a)** Publications reporting miRNA (green bars) and miRNA delivery (red bars in insert) using nanoparticle (NP) formulations from 2000 to July 2016. Data was collected from PubMed on July 26, 2016. **(b)** Structural differences in nanoparticle formulations used for miRNA delivery. **(i)** Polyethyleneimine (PEI) or cationic polymer-based nanoassemblies; **(ii)** liposomal formulations; **(iii)** polymer micelles; **(iv)** polymer NPs; **(v)** metal or magnetic NPs; and **(vi)** dendrimer-based formulations. **(c)** Possible routes of miRNA uptake mechanisms in cells: clathrin, caveolin, and receptor-mediated endocytosis. The proton sponge effect leads to the release of miRNAs from NPs.

carcinoma (RCC), acral melanoma, or hepatocellular carcinoma. Many patients with advanced-stage disease showed promising results while on treatment. Thus, the company plans to start Phase 2 trials with MRX34 for patients with RCC and melanoma by the end of 2016. Given the clinical impact of miRNAs in cancer, we review here the strategies implemented for the delivery of tumor suppressor miRNAs or anti-miRNAs using nanotechnology-based formulations for the treatment of various types of cancer.

Delivery of miRNAs: major obstacles and nanotechnology

Although accumulating scientific evidence proves significant roles of miRNAs in cancer, their translation into clinical application has multiple issues.

The main reasons include poor systemic stability, rapid clearance, and lack of efficient delivery. In general, oligonucleotides in the bloodstream have a half-life of a couple of minutes; however, suitable substitution can improve the half-life to several hours [6]. The kidney is one of the barriers that readily accumulates and clears oligonucleotides from the body via renal clearance. The liver is another organ that abundantly takes up these oligonucleotides for clearance from the body [6]. The other major barrier is the reticuloendothelial system (RES), in which Kupffer cells of liver and spleen macrophages eliminate these oligonucleotides from the circulation system. Phagocytosis of the oligonucleotide results in a phagosome, which is then integrated into the lysosome, where it is degraded by nucleases [6]. Nuclease activity in plasma and tissue degrade the oligonucleotide very rapidly. This phenomenon can be avoided by targeted delivery of NPs to cancer cells [7]. Nanocarrier-mediated oligonucleotide delivery is capable of crossing endothelial cells into the interstitial space of the tumor [6]. In addition, oligonucleotides can be delivered into the cytoplasm for translation via endocytosis using a nanocarrier that can escape endosomal degradation. There are many NPs or nanocarriers being used to deliver miRNAs, each uniquely formulated and with distinctive composition.

One of the most widely used groups of polymers for the delivery of nucleic acids to cells are cationic polymers, because, being positively charged, these can be conjugated to the negatively charged nucleic acids. They also present low toxicity and low immunogenic responses compared with other polymer-based systems for gene delivery [8]. Cationic polymers are subdivided into naturally derived and synthetically derived polymers. Naturally derived cationic polymers include chitosan (CS), dextran, gelatin, cellulose, and cyclodextrin polymer, whereas synthetically derived cationic polymers include PEI, poly(L-lysine), poly(amido amines), poly(amino-*co*-ester), poly-(2-*N*,*N*-dimethylaminoethylmethacrylate), and dendrimers, of which PEI and its conjugates have been widely exploited for gene delivery purposes [8]. Low-molecular-weight PEI polymers are considered to be efficient carriers for the delivery of small nucleic acids, miRNAs, and small interfering (si)RNAs because of their low toxicity compared with other transfection agents [9]. The main advantage of the PEI-based delivery system is the rapid uptake and release ('proton sponge effect') (Fig. 1c) of the nucleic acid inside the cell via an endocytic mechanism [9]. Schade *et al.* [10] showed that the combination of PEI with a magnetic NP formulation led to efficient delivery of the nucleic acid to target cells. Quantum dots (QDs) conjugated to PEI can enhance theranostic applications to provide imaging, gene delivery, and cellular labeling [11]. However, limitations associated with the PEI delivery system include poorer biodegradability inside the cell, leading to its accumulation and cytotoxicity [12]. Therefore, new research leading to improved PEI-based delivery systems is needed.

Liposomes are amphiphilic molecules that comprise phospholipids, are biocompatible and biodegradable, and, to a great extent, resemble the cell membrane of a human cell [13]. Given their resemblance to the cell membrane bilayer, liposomes have a tendency to pass through cell membranes and release their encapsulated payloads (i.e. miRNAs). The issues of low sensitivity or specificity and toxicity [13] can be overcome by surface modification, as detailed in Table 1. Polymeric micelles, which are highly soluble in water, have been largely identified as suitable carriers for, and distributors of, anticancer drug(s). These micelles have an outer and inner core that determine the different physicochemical properties of these nanocarriers [14]; for example, their surface composition, hydrophobicity, and crystallinity [14] determine the payload release. NPs comprising polymers, lipids, hybrids, and metal/metal oxides provide significant opportunities for targeted delivery [15]. NPs readily accumulate at tumor sites because of an 'enhanced permeability and retention' (EPR) effect [16].

Here, we discuss various novel strategies to circumvent antisense targeting and delivery to cancer cells through the use of nanotechnology.

Using nanotechnology formulations to deliver miRNAs to tumors

Breast cancer

miRNA nanoformulations targeting hyaluronic acid (HA) receptors, which are overexpressed in breast cancer, is a novel approach. A recent study showed that HA-CS NPs efficiently delivered tumor suppressor miR-34a and doxorubicin (Dox) to breast cancer cell-derived xenograft tumors in athymic nude mice, resulting in the increased inhibition of tumor growth and tumor volume compared with Dox-NPs or free Dox [17]. HA-CS-coated PEI-poly(D,L-lactide-*co*-glycolide) (PEI-PLGA) NPs conjugated with Dox and miR-542-3p both improved targeting and increased the uptake of NPs in triple-negative breast cancer cells [18]. Furthermore, delivery of PLGA-PEG NPs encapsulating antisense-miR-21 and orlistat or orlistat NP in combination with Dox significantly enhanced apoptotic effects in MDA-MB-231 and SKBR-3 triple-negative breast cancer cells [19]. In another study, PLGA-*b*-PEG NPs were successful in delivering anti-sense miR-21 and miR-10b in triple-negative breast cancer [20]. A nanoporous silicon microparticle modified by arginine-PEI in combination with miR-18a has been used to target breast cancer cells and resulted in a 90% knockdown of *ATMK* (an miR-18a target gene) and a significant reduction in tumor volume in a murine model of MDA-MB-231 cells [21]. Another CS-based nanoformulation incorporating negatively charged poly(γ-glutamic acid) (PGA) was conjugated with QD-miRNA let-7a-gold NP (QD-RNA-Au NP) for delivery to breast cancer cells where Dicer-mediated release of QD resulted in fluorescence, demonstrating its theranostic effectiveness [22]. Anti-miR-21 delivery with a PEI/poly(sodium 4-styrenesulfonates) (PSS)/grapheme oxide (GO) nanocomplex conjugated to

TABLE 1

Surface modification of NPs facilitates miRNA binding and successful drug delivery

Nanoformulation	Modification	miRNA	Refs
PEI/poly(L-lysine)	HA	miR-542-3p	[18]
	E-selectin	miR-146a/miR-181b	[76]
	Polyarginine	miR-145	[30]
	Carboxymethyl-hexanoyl CS	miR-122	[77]
	Rabies virus glycoprotein	miR-124a	[12]
Liposomes	Chlorotoxin	miR-21	[78]
	Surfactant protein C	miR-486	[79]
	Ephrin-A1	Let-7a	[80]
	Cyclic RGD	miR-296	[81]
	Transferrin	miR-1	[82]
	Transferrin	miR-29b	[83]
	N-Lactobionyl-dioleoyl-phosphatidylethanolamine	miR-155	[84]
Gold NPs	Folic acid	miR-122	[85]
PEG-peptide-poly(ε-caprolactone) copolymer NPs	Gelatinase	miR-200c	[63]
Silica NPs	GD2	miR-34a	[86]
PLGA	Cyclic RGD	miR-132	[87]
	uPA	miR-10b and miR21	[20]
Magnetic NPs	PEI	miR-145/9/21	[88]
	Cy3-DNA probe	Let-7	[89]
	Lanthanide cations	miR-99a/486/21	[90]
	PEG	miR-16	[91]

adriamycin inhibited 40% of miR-21 and 45% of ABC transporter expression levels and resulted in a twofold increase in uptake of adriamycin [23]. In a recent study, intravenous injection of exosomes conjugated with epidermal growth factor (EGF) peptide targeting EGF receptor (EGFR)-expressing cells with encapsulated let-7a was shown to be effective in xenograft mouse models of breast cancer cells [24].

Prostate cancer

Various miRNAs, including miR-34a, -21, and -153, have been implicated in prostate tumorigenesis [25]. A recent study demonstrated that the delivery of CS-encapsulated miR-34a intrafemorally reduced bone tumor growth and volume by twofold [26]. Exosomes have been shown to effectively deliver anti-miR-21 oligonucleotides to prostate cancer cells, leading to a significant downregulation of miR-21 levels and decreased motility of prostate cancer cells [27]. miR-34a delivery has shown chemosensitization of paclitaxel treatment in prostate cancer cells by targeting the Bcl-2 protein [28]. Let-7c miRNA, conjugated with a NP-based system targeted for prostate cancer cells using anti-prostate specific membrane antigen (PSMA) antibody or aptamer conjugation, showed enhanced targeting and uptake. Gold NPs formulated for the delivery of miRNAs into cancer cells showed a payload that was approximately 10–20 times higher than that of lipofectamine, lower toxicity, efficient uptake, fast endosomal escape, and increased half-live [29]. Introduction of disulfide linkage in PEI (SSPEI) led to better biocompatibility and reduced the associated toxicity, whereas delivery of polyarginine peptide (R11)-labeled SSPEI NP showed specific uptake in prostate cancer cells [30]. This strategy not only reduced toxicity, but also enhanced the restitution of the tumor suppressor miR-145 to prostate cancer, resulting in decreased tumor burden in xenografted mice.

Pancreatic cancer

Deregulation of miRNAs has been shown in pancreatic cancer, leading to enhanced tumor growth and metastasis [31]. Various miRNAs, such as miR-221, -21, -375, -34a, and -145, have been implicated in pancreatic carcinogenesis. miR-221 has been known to function as an oncogene by promoting the growth of pancreatic ductal adenocarcinoma (PDAC) by regulating the key oncogenic PTEN-AKT pathway [32] and increased expression of matrix metalloproteases (MMP), such as MMP-2 and MMP-9 [33]. miR-145 functions as a tumor suppressor in pancreatic cancer and is known to target Mucin 13 (MUC13) to inhibit pancreatic cancer growth and invasion [34]. A magnetic NP formulation encapsulating miR-145 efficiently delivered miR-145 to the tumor site and downregulated the expression of oncogenic signaling, such as MUC13, HER2, and pAKT, to inhibit pancreatic cancer growth and invasion [35]. NP-encapsulated delivery of miRNA for pancreatic cancer treatment remains an unexplored field that has potential therapeutic value. In a previous report, tumor suppressor miR-34a restitution was achieved using an antibody-modified liposome/polycation delivery system in a Panc-1 xenograft mouse model [21]. Gold NPs with fluorophore-labeled hairpin DNA, so-called 'gold nanobeacons', were used to target and silence miR-21, an endogenous miRNA involved in cancer development and chemoresistance [36]. The miR-375 expression level in pancreatic cancer is associated with the carcinogenesis of pancreatic cancer cells. A solid lipid NP delivery system in conjugation with miR-375 efficiently reached pancreatic tumors and inhibited pancreatic cancer growth *in vitro* and in *in vivo* models. The delivery of miR-150-encapsulated NPs increased the expression of miR-150 in Colo-357 and HPAF cells by 28- and 26-fold, respectively, compared with transfection of miRNA via lipofectamine [37].

Ovarian cancer

The efficient delivery of anti-miR-21 to ovarian cancer cells has been observed to reduce the tumor burden [38]. A recent study showed a gold NP delivery system for anti-miR-21 to be an excellent platform to target and silence miR-21 in ovarian cancer cells, inhibiting the sphere-forming capacity of tumor-initiating cells. miR-155 is downregulated in ovarian tumor-associated dendritic cells (DCs) and is essential for optimal antigen presentation and activation of T cells by DCs [39]. PEI-based nanocomplexes were used to deliver miR-155 to tumor-associated DCs, which increased the expression of miR-155 *in vitro* and resulted in increased antitumor immunity, thus, increased survival of the mice (by 65%) [39]. miR-124 is downregulated in ovarian cancer and acts as a tumor suppressor by targeting proteins such as myc and increasing the expression of p27, subsequently leading to cell cycle arrest at G1 phase because of the loss of phospho-Rb and decreased expression of the myc protein [40]. Transfection of miR-124 in an ovarian cancer cell line reduced the invasive and migratory capability of ovarian cancer cells and increased their sensitivity to etoposide by twofold. While miR-124 is restored in ovarian cancer xenograft tumors using 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) NPs, it resulted in a significant decrease in tumor weight alone and in combination with etoposide [40].

Lung cancer

Recent studies demonstrated the feasibility of systemically delivering miRNA mimics and siRNAs simultaneously to lung adenocarcinoma cells using polymer-based NPs in a mouse model of lung cancer, eliciting a potent antitumor response [41]. It was shown that miR-34a acts as a tumor suppressor and is significantly downregulated in lung cancer [42]. miR-34a targets the p53 signaling pathway to regulate cell cycle progression and apoptosis induction in cancer cells [42]. Liposomes encapsulating miR-34a were effectively delivered to lung cancer cells to mediate the inhibition of cell cycle progression and activation of apoptosis in lung cancer cells, thereby causing a significant decrease in tumor growth and volume in an orthotopic mouse model of lung cancer [43]. miR-200c is a known negative regulator of ZEB1, which induces the epithelial–mesenchymal transition (EMT) in cancer cells [44]. Liposomal NP-encapsulated miR-200c delivered to lung cancer cells induced the activation of oxidative stress response genes and enhanced the radiosensitivity of lung cancer cells up to 1.5 times in an *in vivo* mice model [45]. Multifunctional aptamer conjugated to miRNA is another method of delivering tumor suppressor genes, such as *let7g*, to lung cancer cells, significantly reducing tumor volume compared with aptamer treatment alone [46]. miR-29b is downregulated in non-small cell lung cancer (NSCLC) cells and directly targets oncogene cyclin-dependent kinase 6 (CDK6) to regulate cell cycle progression in these cells [47,48]. Cationic lipoplex-based delivery of miR-29b to lung cancer cells effectively reduced CDK6 expression by almost 54% and reduced tumor volume by almost 50% *in vivo* [48].

Brain cancer

Poly(amido amine) (PAMAM) has been found to be an effective carrier for the delivery of miR-7 into glioma cells owing to its low toxicity, high solubilization, and delayed release [49]. Similarly, the successful delivery of tumor suppressor miRNA -conjugated NPs to brain cells is feasible. In another study, PLGA NPs encapsulating antisense miR-21 were found to be effective in the delivery and sustained silencing of miR-21 function in glioblastoma cells [50]. Mesoporous silica NPs containing polyarginine-peptide nucleic acid (R8-PNA) conjugates targeting miR221 were used to treat temozolomide (TMZ)-resistant glioma cells. These NPs, combined with TMZ treatment, led to a significant increase in apoptosis [51].

miRNA-mediated chemo-sensitization

Recently, the combination treatment of miRNA therapeutics with small-molecule anticancer drugs has received much attention because of its superior therapeutic benefit (Fig. 2). This approach has many advantages over conventional therapies, such as reverting the EMT, inhibiting drug resistance, promoting apoptosis and autophagy, suppressing tumor angiogenesis, and inhibiting the expression of efflux transporters, such as P-glycoprotein [52]. By actively targeting oncogenic miRNAs using an anti-miR system or restoring lost tumor suppressor miRNAs, it is possible to sensitize cancer cells to chemotherapeutic drugs. This treatment modality resulted in smaller tumor nodules *in vivo*, which are less likely to show tumor relapse. Co-delivery of miRNA/siRNA along with chemotherapeutic drugs is recommended because it has additive or synergistic effects. Chemotherapeutic drugs can inhibit cancer growth and proliferation but, over a period of time, cells acquire resistance against these drugs because of the increased expression of efflux transporters and antiapoptotic signaling. The miRNA/siRNA platform helps to overcome drug resistance by directly targeting efflux transporter expression and antiapoptotic signaling, thereby sensitizing cancer cells to chemotherapeutic drugs [53]. This co-delivery system is an exciting platform that holds promise as a better therapeutic modality for treating cancer cells and, thus, requires further investigation. Another study using a dual miRNA combination of miR-21 and miR-10b in triple-negative breast cancer showed promising outcomes for miRNA combination therapies with drugs or with other miRNAs [20]. A recent study demonstrated that lipid NP-loaded miR-34a with paclitaxel induced increased anticancer effects compared with paclitaxel or miRNA alone [54]. miR-205 is known to sensitize pancreatic cancer cells to gemcitabine by targeting chemoresistance markers (i.e. OCT3/4, CD44, and Tubulin β3) [55]. Gemcitabine-miR-205 conjugated micelles showed a highly significant reduction in tumor volume compared with gemcitabine alone in an *in vivo* pancreatic cancer model, thereby suggesting a synergistic or additive effect of the combination treatment on tumor cells [56]. miR-34a directly targets Notch-1 signaling in breast cancer, thereby inhibiting cell proliferation, invasion, and chemoresistance [57]. Dox conjugated to miR-34a HA-CS NPs not only targeted breast cancer cell migration by inhibiting Notch-1 signaling, but also sensitized cells to Dox by suppressing Dox-mediated activation of Bcl-2 expression at both the protein and transcriptome levels [57]. miR-21 is known to modulate sensitivity to chemotherapeutic drugs [58]. Co-delivery of an anti-miRNA system with chemotherapeutic drug also holds promise as an improved treatment strategy. miR-21 has been proven to be an oncogenic miRNA in various types of cancer, and suppresses PTEN expression, thus promoting Akt-mediated activation of Bcl-2 signaling and inducing chemoresistance in cancer cells [59]. Co-delivery of miR-21 inhibitor conjugated to

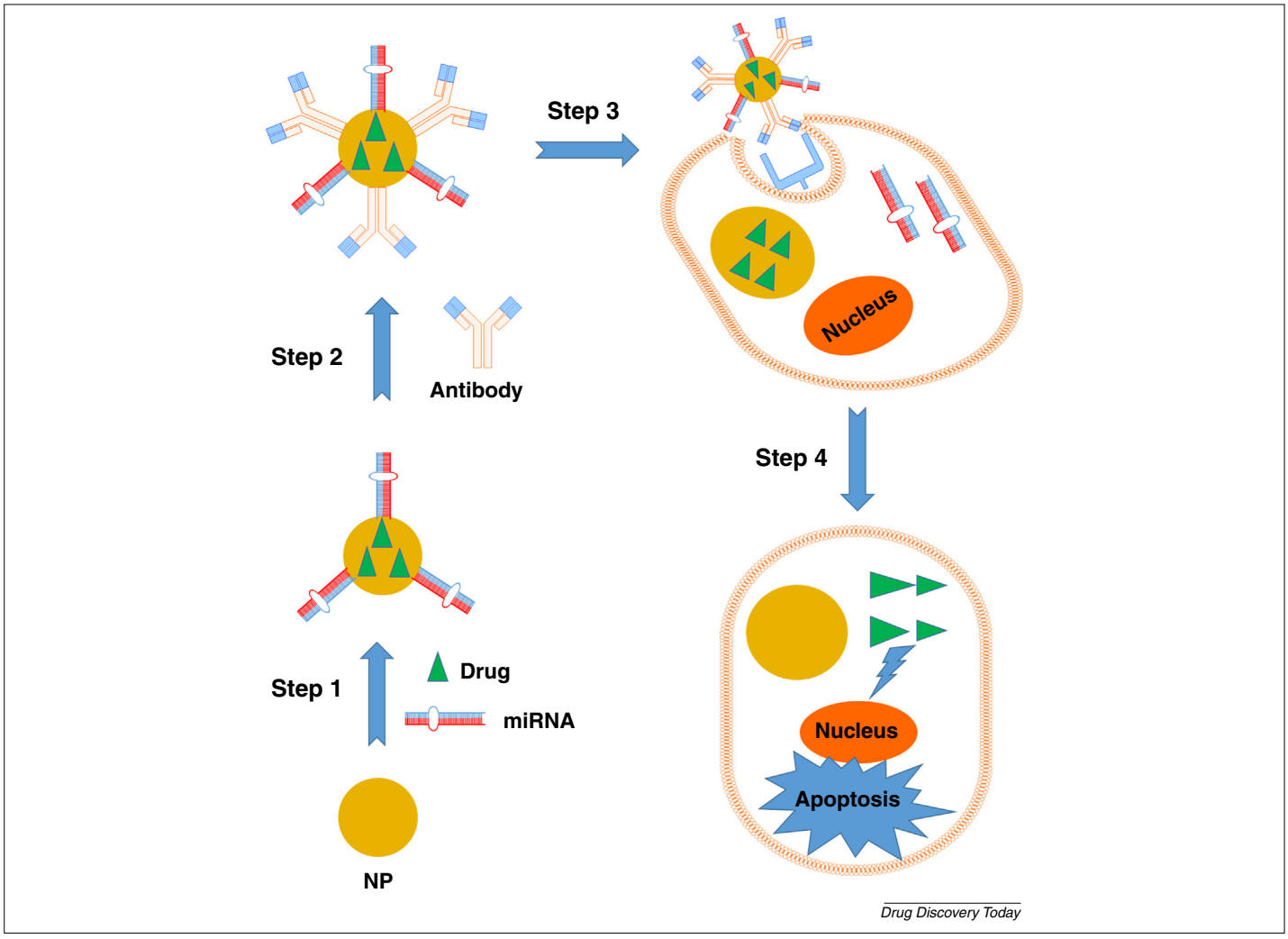

FIGURE 2

miRNA-mediated chemosensitization of cancer cells for improved therapeutics. Schematic representation: **(Step 1)** loading of nanoparticles (NPs) with miRNA and drug molecules. **(Step 2)** antibody conjugation reaction for targeted delivery; **(Step 3)** targeted binding and intracellular release of miRNA induces chemosensitization; and Step 4: drug release promotes apoptosis in cancer cells.

Dox encapsulated in a star-branched copolymer comprising poly(-lactic acid) and poly(dimethylaminoethyl methacrylate) showed excellent anticancer efficacy. Tumor volume in glioma cells decreased by ninefold compared with control, suggesting a promising treatment approach combining gene delivery and chemotherapeutic drugs [60]. miR-200c targets class III beta-tubulin and CD44, improves the sensitivity of cancer cells to chemotherapeutic drugs, and reverts EMT by increasing E-cadherin expression [61,62]. Gelatinase-stimuli NPs for co-delivery of miR-200c and Dox in cancer stem cells (CSCs) resulted in a 75% decrease in tumor volume compared with control, again suggesting a synergistic effect of combination treatment [63]. Development of multidrug resistance in breast cancer cells occurs when the miR-21-mediated signaling pathway enhances expression of efflux transporters, such as P-glycoprotein [64,65]. Graphene NPs have excellent physical and mechanical properties. It was shown that adriamycin and miR-21 inhibitor encapsulation in graphene NPs had more pronounced antiproliferative effects on adriamycin-resistant MCF7 breast cancer cells [23]. Similarly, co-delivery of miR-21 inhibitor with 5-fluorouracil (5-FU) using a poly(amido amine) dendrimer showed an enhanced cytotoxic effect compared with 5-FU alone in glioblastoma cells [66]. Delivery of anti-miR-21 with poly(L-lysine)-modified PEI NPs to breast cancer cells increased cell cycle arrest in G1 phase, enhanced PDCD4 expression (a direct target of miR-21), and led to apoptosis by increased expression of caspase-3. Furthermore, it decreased the IC_{50} of Dox from 0.585 to 0.415 mg/ml and that of cisplatin from 1.051 to 0.940 mg/ml, showing the synergistic effect of anti-miR21 and chemotherapeutic drug treatment [67].

Targeted drug delivery system

A possible solution to the problem of cell-specific delivery of therapeutic miRNAs/anti-miRNAs is the utilization of targeted miRNA mimics. The problem with NP-mediated miRNA and/or drugs is that uptake by cancer cells is not specific; however, this can be circumvented utilizing targeted approaches by coating the surface of NPs with specific antibodies or ligands against proteins that are specifically expressed in cancer cells. The targeted delivery of curcumin (a natural product) was shown in C4-2 prostate cancer cell-derived xenograft tumors using PLGA NPs conjugated with

I-131-labeled PSMA antibody [68,69]. These results showed reduced prostate tumor burden, and the efficient delivery of curcumin to targeted prostate tumors with reduced or no uptake by other organs. This novel targeted PSMA delivery system can be used to co-deliver miRNAs and chemotherapeutic drugs to prostate cancer cells in a specific manner without any adverse effects. PSMA ligand-conjugated polymeric micelles have also been used to target prostate cancer cells. Transferrin-coated NPs have shown an excellent ability to pass through the blood–brain barrier and showed increased cytotoxic effects with zoledronic acid compared with free zoledronic acid in glioblastoma cells [70]. Folate receptors are highly expressed in breast cancer cells and NPs conjugated to the folate receptor for delivery of siRNA led to the specific uptake of NPs by breast cancer cells and the efficient delivery of siRNA to the cancer cells [71]. Aptamer-mediated delivery of short RNA therapeutics in cancer cells [72] has been studied, with promising results, and this system can be further enhanced by aptamer-conjugated drug NPs encapsulating short RNA therapeutics and chemotherapeutic drugs for enhanced cytotoxic activity.

These examples show that the targeted delivery of short RNA therapeutics combined with chemotherapeutic drugs not only increases uptake by cancer cells specifically, but also inhibits chemoresistance in cancer cells and enhances their cytotoxic effects, suggesting a synergistic effect of combinational therapy. Interleukin (IL)-10 is an important immunoregulatory cytokine and has an important role in T regulatory cell function [73]. Let-7b directly suppresses IL-10 expression in T cells and expression of let-7b regulates tumor-associated macrophages (TAMs) and tumor infiltrating DCs (TIDCs), leading to increased immune responses against the cancer cells [74]. Conjugation of let-7b miRNA to mannose moieties (TAMs and TIDCs express high levels of mannose receptors) and pH-responsive PEG-histamine-modified alginate, which disintegrates in acidic microenvironments, led to the targeted delivery of let-7b. This nanocomplex formulation showed improved survivability in tumor-bearing mice, with nearly 50% of let-7b-treated mice still alive 50 days after the start of treatment. In addition, this formulation resulted in decreased tumor weight and volume along with decreased levels of M2 macrophage markers, with a subsequent increase in M1-specific gene (*iNOS*) and decreased expression of TIDCs markers, such as CD40 and CD80 [74]. A miRNA nanobeacon constructed with HA and miR-34a efficiently targeted cancer cells and endocytosis through the CD44 receptor, which was observed both *in vitro* and *in vivo* [75] (Fig. 3). Such specific targeting would improve therapeutic outcomes. A future goal would be to construct a unique nanoplatform that has inbuilt therapeutic components in addition to miRNAs, and a specific targeted moiety to specifically kill or eradicate cancer cells.

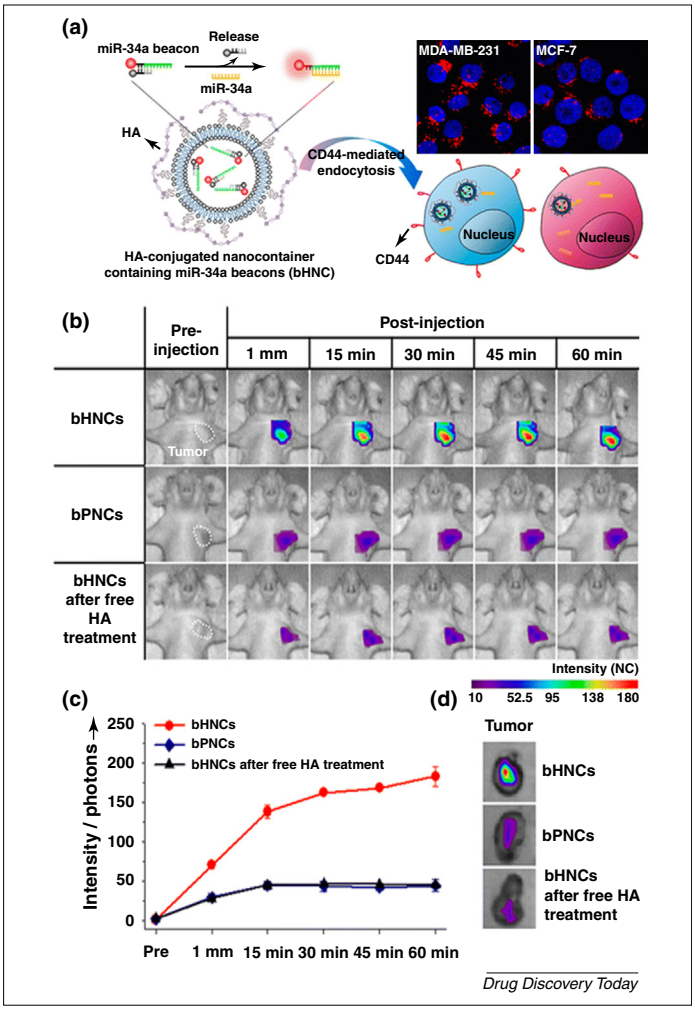

FIGURE 3

Hyaluronic acid (HA) conjugation offers superior targeted delivery of miRNAs in breast cancer cells. **(a)** Schematic representation of a miR-34a-targeted nanoformulation preparation and *in vitro* specific targeting of triple-negative breast cancer cells. **(b)** *In vivo* whole-mice imaging of miR-34a nanoparticles (NPs) in an orthotopic breast cancer mouse model at different time points after intravenous injection. **(c)** Photon intensity of tumor region after injection of NPs. **(d)** *Ex vivo* excised tumor tissue exhibiting superior targeting potential of HA-conjugated NPs. Reproduced, with permission, from [75].

Concluding remarks

The successful delivery of miRNAs to cancer cells is a major hurdle in cancer therapeutics. In this review, we have focused on various advanced nanoformulations and new methodologies for the successful delivery of miRNAs to tumor cells. With these complexation, encapsulation, and conjugation nanotechnology strategies, miRNAs can be delivered to the tumor in passive, active, and stimuli-responsive ways. Furthermore, because of the high fibrosis and heterogeneity of tumor tissues, a single theranostic nanoformulation with simultaneous therapeutic and imaging capabilities is of current interest. In addition, nanosystems have shown reduced systemic toxicity, which has been an important concern with earlier conventional transfection systems.

Acknowledgements

This work was supported by NIH U01CA162106, R01CA210192, R01 CA206069, R01CA142736, R01CA204552, K22 CA174841; Department of Defense ARMY GRANT W81XWH-14-1-0154; and College of Pharmacy/University of Tennessee Health Science Center Seed Grant.

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