Nanotherapeutic Approaches to Overcome Drug Resistance in Cancers

Subjects: Pharmacology & Pharmacy
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Keywords: cancer ; nanotherapeutics ; cancer stem cells ; drug resistance ; tumor microenvironment

1. Emerging and Innovative Nanotherapeutics-Based Strategies against Drug-Resistant Cancers

Nanotherapeutics serve not so much to overcome the chemotherapeutic treatment, but rather to overcome the chemoresistance of cancers, improve pharmacokinetics of the drugs, and decrease or eliminate their systemic toxicity and so on. The foremost objective of the nanotherapeutics-based approach is to target specific cancer cells and their microenvironment with minimal toxicity by delivering chemotherapeutic agents efficiently to the target site. Moreover, the development of nanotherapeutics in the past few years indicates its considerable potential in the cancer therapeutic domain. Aside from cancer therapies, nanotechnology-based medicines have significant potential implications in the diagnostic imaging of many drug-resistant cancers. Nanoscale delivery systems for cancer-specific targeting have demonstrated enormous potential in the past few years with the development of strategies for specifically targeting specific cells, particularly cancer stem cells (CSCs), the tumor microenvironment, and various tumor components, using a variety of emerging and innovative approaches. The innovative approaches include the nano-therapies based approach to target specific components of the tumor environment (cellular and non-cellular component), employment of RNA interference technique (siRNA and miRNA based specific delivery), self-assembly based prodrug-based approach, exosome-based delivery, stimuli responsive delivery, advanced delivery systems for targeting the CSCs and integrin, and others for specific cancer therapy [1][2][3]. Various emerging and innovative strategies currently ongoing for specific targeting of tumor cells and microenvironment were reviewed and discussed in detail along with their advantages and associated challenges.

1.1. Nanotherapeutis-Based Approaches for Targeting Tumor Microenvironment (TME)

Tumor microenvironment (TME) plays a vital role in imparting tumor heterogeneity and disease progression. The heterogeneity of TME and its components, such as cells, interstitial fluid, and extracellular matrix (ECM), act as physical barriers and do not allow drugs to permeate the tumor tissue. As a result, there are marked gradients of cell proliferation and drug concentrations which influence the tumor sensitivity towards drug treatment [4]. This condition induces anticancer drug resistance. MDR presents major unresolved challenges in cancer chemotherapeutics and about 50% of patients face tumor relapse problems due to MDR. TME and its components induce drug resistance through a variety of processes, including cell–cell and cell–ECM interactions, crosstalk between distinct cells, phenotypic changes, mechanosensing variation, and protective dormancy. Furthermore, additional factors—including the overexpression of efflux pumps such as ATP-binding cassette (ABC) transporters and P-glycoprotein (P-gp)—found on certain cancer cells contribute to drug efflux and resistance [5]. TME allows tumor cells to avoid the harm produced by traditional clinical cancer therapies such as chemotherapy, radiation, and surgery.

The local microenvironments of tumor cells and crosstalk between specific cancer cells plays a crucial role in tumor progression was elucidated more than a century ago according to Stephen Paget’s seed and soil hypothesis [6]. However, the role of non-neoplastic cells of TME in tumor development and metastasis was uncovered only in the last three

decades [7]. TME comprises both cellular and non-cellular components that play critical roles in the development of drug resistance. The cellular component of TME includes cancer associated fibroblasts, cancer associated vascular endothelial cells, cancer associated pericytes, cancer associated immune cells, lymphatic endothelial cells (LECs), and CSCs. The cellular components of TME by different nano-drugs systems. Non-cellular component characteristics of TME include hypoxia, an acidic environment, the extracellular matrix, cytokines, growth factors, and vascular networks [8]. TME's non-cellular components create a favorable and permissive environment for cancer cell proliferation. TME exhibit characteristics that separate them from normal tissue include their leaky vasculature, inadequate vascular perfusion, an acidic environment, changed pH dynamics, altered enzyme expression, altered metabolism, and hypoxic circumstances [9]. All these regions provide therapeutic opportunities which are exploited by nanocarrier-based drug delivery systems. In order to design chemotherapeutic and chemo preventive strategies to overcome drug-resistant cancers, in-depth knowledge of tumor biology is pertinent. Consequently, the targeting of both cancer cells and tumor microenvironment is necessary to achieve superior therapeutic efficacy. Therefore, in order to develop improved and efficient drug delivery systems, TME modification is a prerequisite through the better understanding of both TME stromal components functioning and its morphological features [10].

In the last two decades, various other cellular components of TME, such as CSCs, endothelial cells, and stromal cells, were identified and their role in tumor growth is established. All these cellular components vary greatly in terms of size, morphology, and expression of surface receptors, paving the way to target these cells individually in order to produce synergistic therapeutic effects [11]. The advancement of TME-enabled nanotherapy in the past few years demonstrated promising strategies and approaches for the modulation and targeting of TME in combating drug-resistant cancers by limiting disease progression [12]. Furthermore, a number of novel smart nanoparticles with transformational properties exhibited improved spatiotemporal control over particular tumor microenvironmental targeting. Because of their customizable size, surface coating, and capacity to include a vast number of therapeutic drugs, nanoparticles (NPs) have emerged as a viable platform for TME targeting. Emerging nanocarriers are being utilized for targeting TME and its components include nanoparticles (polymer- and lipid-based), liposomes, polymeric micelles, magnetic nanoparticles, polymer drug and nanoconjugates. A variety of nanocarriers are employed for targeting TME in order to overcome multi-drug resistance.

TME modulation and targeting using nanocarriers can be achieved either through passive targeting or active targeting. In the passive approach, tumor targeting is carried out by diffusion process and EPR effect is considered as crucial factor. In passive targeting, accumulation of nanocarriers is supported by abnormal leaky vasculature of tumor compartment. Nanocarriers are generally functionalized with specific ligands such as folic acid, transferrin, and aptamers in active targeting that could interact with overexpressed folate and transferrin specific receptors present on targeted cells. In active targeting, different ligands not only target cellular components of TME, but also non-cellular components such as hypoxic conditions and acidic environment. Physiological hypoxic conditions in tumor microenvironment contributes primarily to the tumor growth and cancer drug resistance.

In cancer nanotherapeutics targeting TME, monotherapy sometimes fail to produce the desired therapeutic effect. Thus, other strategies such as multifunctional nanomedicine and combination therapy were employed for enhancing the effectiveness of cancer therapy. Multifunctional nanomedicine utilizes encapsulation of various therapeutic cargos such as chemotherapeutic drugs, clustered regularly interspaced short palindromic repeats (CRISPR) nucleotides or RNA interference (RNAi) [13][14]. Thereafter, nanoparticles can migrate to target tumor sites to release therapeutic agents in a controlled manner through local or systemic administration.

Various nanoparticle-mediated approaches were reported to target TME in the past few years by creating nutrient deprived conditions for cancer cells together with exposure to various destructive mechanisms. Nanotherapeutics-based strategies are not only limited to improving chemotherapy, but also incorporate gene therapy and its applications for diagnostic and theragnostic domain. Consequently, nanoparticle-based approaches are reported to utilize either of the two mechanisms and expanded the nanotherapeutics in both directions. Currently, there are only a few clinically approved nano pharmaceuticals available in the market to treat cancer, namely, doxil®, Abraxane®, and Genexol®.

Nanotechnology-based products have shown promising outcomes in targeting TME and a few products are now clinically approved; however, their applications remain limited in treating certain types of cancers (although not all) [15]. TME provides conflicting attributes because, on the, it allows improved nanoparticles accumulation due to its leaky vasculature, and on the other hand, it also acts as a barrier for nanoparticles extravasation [16]. The TME barriers' contributions toward nanoparticles extravasation include high interstitial fluid pressure (HIFP), pericytes coverage, basement membrane, and composition of ECM. The interstitial fluid of the tumor environment is similar to blood plasma and comprises 50–60% of plasma proteins and electrolytes composition; however, the interstitial fluid pressure varies. The tumor interstitial fluid

pressure (IFP) is elevated (5–40 mm Hg) compared to normal IFP (range of −3 to 3 mm Hg). The pressure increases as the tumor growth progresses due to various factors such as rapid cell proliferation, presence of highly crosslinked collagen, modulated extracellular matrix, increased contractions of stromal cells, lack of pericyte coverage, high vascular permeability, lack of lymphatic drainage, and increased secretion of angiogenic factors and growth factors [10]. High tumor IFP exerts mechanical forces on cells and stimulates the proliferation tumor cell proliferation [17]. Pericytes coverage presents another barrier for nanoparticles-mediated drug delivery. It was indicated that pericytes dysfunction leads to loss of vascular coverage and plays an important role in disease progression [18]. Basement membrane represents another barrier of TME which performs the function of a sieve to modulate the nanoparticle extravasation from blood capillaries to the TME. Although the basement membrane does not induce the elevation of IFP, its structural complexity and thickness restricts the entry of nanoparticles or therapeutic agents' migration to TME [15]. Furthermore, ECM composition, and structural and componential complexity restrict the extravasation of nanoparticles. Apart from the ECM composition, nanoparticle distribution is influenced by the alignment and orientation of collagen fiber network. In addition to tumor cell growth, stromal cell density contributes to the solid stress by compressing the matrix into a disordered network and restricting nanoparticle penetration, both of which limit nanoparticle penetration. A general decrease in nanoparticles that extravasates from neighboring micro vessels compromises the ability of stromal cells to internalize therapeutic NP in cancer cells. Taken together, TME barriers such as the presence of stromal cells coverage, extensively cross-linked collagen networks, and interstitial fluid pressure, among others, restrict the entry of chemotherapeutic agents from reaching the target cell. This restriction ultimately reduces the therapeutic benefits in patients. Therefore, the remodeling of cellular and non-cellular components of TME is pertinent in order to improve drug delivery by facilitating the extravasation of nanoparticles to TME. The four main strategies employed for the enhancement of nanoparticles extravasation include the vascular normalization strategy, stress alleviation strategy, and stromal/tumor matrix normalization strategy [10][19]. The normalization of the vascular system, mediated, for example, by the metronomic dosage of some conventional chemotherapy (such as docetaxel), may enhance blood flow inside the tumor, but it also closes the pores in the capillary walls, which are typical of solid tumors and required for the EPR effect. As a result, vascular normalization may even limit the growth of nanosized systems within malignancies. All three strategies employ different nanoformulations such as nanoparticles and polymer micelles to enhance extravasation. The priming mechanisms of stromal normalization strategies include the degradation of ECM, modification of ECM, reduction of collagen content, and reduction of IFP. In the context of the vascular normalization strategy, blocking of VEGF receptors, vessel diameter reduction, inhibition of tubulin, and stromal cells present main priming mechanisms. Furthermore, the prime mechanisms of the stress alleviation strategy involve the inhibition of tubulin, depletion of stromal cells, and reduction of IFP. In the past few years, TME-responsive cancer nanotherapeutics showed fast development, with the design and development of various theranostic strategies for combating drug-resistant cancers. Although few nanoparticles formulations are currently in clinical trials, the multitude of pre-clinical testing being far more than for clinical testing presents an obvious barrier for translation into clinical settings. Although TME-enabled nanotherapy showed high performance outcomes for further clinical translation, still a number of challenges must be overcome to ensure the better feasibility of these targeted systems [20]. In order to enhance the clinical translatability of nanoparticles platforms, safety profile, formulation scalability, targeting efficiency, and selection of pre-clinical models act as major determining factors.

2. Nanotherapeutic Strategies for Targeting Cancer Stem Cells (CSCs)

Tumor heterogeneity represents major obstacle in cancer therapy as bulk of tumor harbors various cell types with differential sensitivity to chemotherapy [21]. One of the crucial factors responsible for tumor heterogeneity is considered as CSCs, which regulates the tumor microenvironment and exhibits self-renewal ability, invasiveness and high tumorigenicity [22][23]. CSCs are small group of cancerous cells responsible for tumor initiation, progression, relapse, and poor prognosis, highly influencing the available therapeutic processes; see [24]. CSCs are able to resist conventional therapies such as chemotherapy and radiotherapy owing to their intrinsic characteristics such as phenotypic plasticity capacity, maintenance of a slow dividing state, drug efflux transporters, overexpression of antiapoptotic proteins, highly efficient DNA repair system, detoxifying enzymes epithelial to mesenchymal transition, and sustained stemness features [25][26][27]. Additionally, CSCs' persistence in a hypoxic tumor microenvironment confers additional resistance to anticancer therapy [1]. Moreover, CSCs represents an important source responsible for resistance to traditional chemo and radiotherapy. Therefore, the development of efficient anticancer strategies which would specifically kill both tumor cells and CSCs would form the core of cancer therapeutics.

In the past few decades, CSCs properties were focused on and emphasized finding different ways to specially targeting the CSCs population for improving conventional chemotherapeutic approaches [28][29][30]. In order to attempt this, nanotherapeutic-based approaches using nanoparticles were developed for specific targeting of CSCs to reduce the chances of cancer recurrence and provide better palliative care. The potential nanotherapeutic approaches utilized for

targeting CSCs in the past few years include crucial factors required for the survival of CSCs in the tumor microenvironment, such as specific surface biomarkers (CD44, CD133, EpCAM, aldehyde dehydrogenases), drug efflux pumps (ABC transporters) expression, different metabolic pathways, and signaling pathways (Wnt/β-catenin, Notch and Hedgehog) [27][30].

Recently, the efforts in understanding the properties and different mechanisms of targeting CSCs paved way for the development of innovative nanotherapeutics for targeting CSCs. One of the most important overexpressed markers on the surface of CSCs is the cluster of differentiation-44 (CD44). Rao et al. developed polymer nanoparticles with chitosan coating and loaded with chemotherapy agent for targeting CD44. The results demonstrated increased therapeutic efficiency in mammary tumor spheroids model as nanoparticles delivered to tumor microenvironment specifically targeted CD44 overexpressing CSCs due to the high affinity between CD44 receptors and chitosan [31]. Furthermore, CSCs targeted nanotherapeutics gained much attention and other important biomarker CD133 was also utilized for specifically targeting CSCs. However, a pertinent issue related with the utilization of CD44 and CD133 lies in selective removal of a subset of CSCs only and may promote phenotypic shift and differentiation in tumor unintentionally. This leads to the compensatory high proliferation of cells and ultimately promotes chemotherapeutic resistance [32]. Therefore, the utilization of a more ubiquitous marker that can better target the large population of CSCs would be a more feasible approach. Thereafter, researchers utilized riboflavin loaded intracellular vesicles with coating of ATP binding cassette subfamily G member 2 (ABCG2) for targeting CSCs specifically and they observed a higher accumulation of riboflavin within cytoplasm due to specific recognition properties [33]. In another, a pH responsive/hypoxia responsive riboflavin linked three-pronged nanoparticles were utilized for targeting both tumor cells and CSCs [34]. Herein, nanoparticles are loaded with three drugs, namely, irinotecan, cyclopamine, and erlotinib, which are able to kill undifferentiated CSCs, differentiated breast cancer specific MCF-7 cells and vascular niches in tumor microenvironment, respectively. Similarly, irinotecan conjugated riboflavin displayed exceptional anticancer efficacy with increased accumulation inside cancer cells. Wang et al. moved on to use salinomycin-loaded nanoparticles to selectively target and kill cervical CSCs [35]. In another one, chitosan poly (lactic-co-glycolic acid)-based nanoparticles loaded with curcumin and modified with sialic acid demonstrated blood–brain barrier permeability and inhibited proliferation of glioblastoma cells and brain CSCs through targeting the aldehyde dehydrogenase of CSCs [36]. In a recent nanotherapeutic strategy, nanoparticles co-loaded with chemotherapeutic drug, camptothecin, and differentiation-inducing agent, all-trans retinoic acid, demonstrated CSCs killing within tumors via dual strategy. The dual strategy involves first the promotion of CSCs differentiation in hypoxic conditions that lead to increase of reactive oxygen species; second, the promotion of the release of camptothecin and subsequent death due to increased levels of reactive oxygen species. This strategy reduces stemness0related drug resistance, enhancement of the chemotherapeutic and prevention of post-surgical tumor relapse response with controlled drug release in breast cancer models [37].

With the recent advancements in cancer nanotherapeutics, various emerging and innovative strategies have shown immense potential in targeting CSCs using photothermal therapy, magnetic hyperthermia, photodynamic therapy, and molecular targeting. The photo thermal therapy (PTT) field has shown promising results for the CSCs targeting nanotherapeutic approach as this method stimulates hyperthermic physiological responses with the conversion of light into heat using metal nanoparticles to eradicate CSCs [38]. Tian et al. utilized gold nanospheres functionalized as a surface biomarker for osteosarcoma stem cells, CD271 for targeted PTT, and reported the inhibition of cells and targeted death in osteosarcoma treatment [39]. Another promising strategy utilized a biocompatible polymeric micelles-based nanocarrier co-loaded with gold nanorods and Adriamycin for killing CSCs under laser ablation via targeting an important CSCs surface marker, EpCAM [40]. In another, a nanoparticle system based on electrospun polycaprolactone nanofibers encapsulating all-trans retinoic acid and hydroxylated multi-walled carbon nanotubes for targeting and killing glioma stem cells was presented. Herein, stem cells inhibition was displayed by increasing the local temperature under near-infrared illumination, which further suggests its increased sensitivity towards heat treatment [41]. In another strategy used to overcome the resistance of CSCs, Wu et al. employed nanoparticles coated with the membrane of melanoma cells for simultaneously targeting chemotherapy, photothermal therapy, and photoacoustic imaging. The results reported this strategy's enhanced targeting ability, along with excellent tumor ablation rate, and antitumor efficiency [42]. Another potential light-triggered minimal invasive cancer therapy for targeting CSCs includes photodynamic therapy (PDT) [43]. PDT produces reactive oxygen species (ROS) and free radicals with activation of a specific wavelength of excitation light and related to photosensitive agents in tumor tissues. PTT-based treatment promotes the autophagy, apoptosis, and necrosis of tumor cells, suggesting its role in reversing chemoresistance [44]. Crous et al. employed nanobioconjugate along with the photodynamic effects and indicated the significant destruction and eradication of lung CSCs [45]. In another, nanoparticles loaded with a bimodal metallacage and with PDT targeted CSCs by decreasing the cells mobility under laser irradiation [46]. In a similar approach, a combination chemotherapy wherein nanoparticle-based micelles were loaded with photosensitizer (mitoxantrone) and anti-EpCAM–CSCs biomarker reported better antitumor efficacy compared to

either near infrared irradiation or chemotherapy alone with simultaneous chemotherapy and PDT [47]. Cao et al. utilized MnO_2@Ce_6 nanoparticles and a PDT-based approach which revealed improvement in tumor microenvironment related therapy resistance by modulating tumor microenvironment by excess hydrogen protons and H_2O that resulted in subsequent eradiation of CSCs [48]. Furthermore, the nanotherapeutics approach combining both photo thermal therapy (PTT) and PDT was utilized and showed a beneficial role in minimizing the metastasis of different cancer types by specific CSCs targeting. Another nanotherapeutic approach for targeting CSCs includes magnetic hyperthermia using magnetic nanoparticles wherein increased cancerous tissue temperature serves as an operative therapy for cancer therapeutics [49]. Magnetic nanoparticles are used for cancer therapy in this technique because of their beneficial physiochemical qualities, such as size resemblance to biomolecules, magnetic properties, appropriate combination capabilities, and targeted drug delivery capacity [50]. Su et al. utilized superparamagnetic iron oxide nanoparticles modified with the anti-CD44 antibody and alternating magnetic field resulting in the significant inhibition of CSCs growth and subsequent death in the head and neck squamous cell carcinoma model via magnetic fluid hyperthermia [51]. In another one, a mesoporous silica nanoparticle under an alternating magnetic field demonstrated an efficient inhibition of tumor growth with the elimination of CSCs through the blockage of the hypoxia signaling pathway and hyperthermia [52].

Molecular targeting is another nanotherapeutic technique for targeting specific CSCs by changing molecular and metabolic processes. MicroRNA21 is an oncogenic gene that, when overexpressed in triple-negative breast cancer, downregulates several tumor suppressors. As a result, downregulation would improve tumor suppression and reverse resistance. To attempt this, Yin et al. employed a three-way junction motif with the utilization of nanoparticles conjugated with the inhibitor of microRNA21, RNA aptamer and CD133 receptor for CSCs targeting. This approach specifically targeted both the triple-negative breast CSCs and cancer cells and indicated reduced cancer cell migration and upregulated tumor suppressors' expression in in vitro and in vivo [53]. Nanotherapeutics based on molecular targeting constitute a more effective way of targeting CSCs, resulting in tumor growth suppression and metastasis reduction via decreased CSC adhesion, migration, and number [54]. Taken together, nanotherapeutic techniques for targeting CSCs demonstrate enormous promise and may enable to overcome cancer treatment resistance. However, further understanding and novel target molecules and CSC characteristics will be necessary in the future to convert these techniques into clinical practice.

3. siRNA-Based Nanotherapeutic Strategies

Currently, targeting the suppression of the oncogenes' expressions along with targeted chemotherapy shows tremendous success and represents one of the foremost strategies in cancer treatment. Earlier, different gene therapy-based approaches were utilized for their knockdown of genes associated with cancer pathophysiology; however, none of them were able to provide the complete suppression of genes [55]. Thereafter, an alternative innovative genetic approach RNA interference (RNAi) was developed for the inhibition of specific messenger RNA (mRNA) expression by controlling uncontrolled cell growth and proliferation, especially in carcinoma cells [56]. The RNAi approach triggers a homology-dependent degradation of targeted mRNA and reversible specific gene silencing capability through the delivery of non-coding double stranded RNA (dsRNA) to cancer cells [57]. In RNAi, the non-coding short double stranded RNAs include short interfering RNAs (siRNAs) and micro RNAs (miRNAs), which show broad potential as therapeutics by silencing sequence-specific genes. In this section, it was discussed siRNA delivery-based strategies for cancer therapy; miRNA-based delivery is discussed in the subsequent section.

The basic strategy involved with siRNA delivery-based gene silencing involves the rational design of siRNA-based delivery systems and identification of targeted genes for the selective knockdown of susceptible oncogene expression. Free siRNA is anionic and hydrophilic dsRNA, with an average diameter of <10 nm, which prevents them from readily crossing cell membranes. The physicochemical and pharmacokinetics properties of siRNA such as short half-life, toxicity, reduced cellular uptake, and degradation vulnerability by serum nucleases, limit the in vivo systemic administration of naked siRNA. Nevertheless, naked siRNAs are rapidly cleared by cells through opsonization and phagocytosis processes by the mononuclear phagocytic system as a part of routine immune system-mediated clearance of foreign substances [58]. Furthermore, siRNA delivery into the targeted tissues is impeded by the presence of different biological barriers that ultimately hinder its effectiveness in vivo. Therefore, different delivery vehicles are required for transporting siRNA to the site of action in order to achieve the clinical potential.

With advancements in the domain of nanotechnology, nanoparticles with remarkable physicochemical features serve as the vehicle of choice for siRNA targeted delivery [59]. Nano-encapsulated siRNAs modifies its pharmacokinetic properties by improving the solubility, oral bioavailability, serum stability, and renal and hepatic elimination owing to their diminutive size. Moreover, encapsulating siRNA into nanoparticles improves cellular internalization and intracellular drug release while decreasing cancer cell resistance to siRNA employing stimuli-mediated nano-therapeutics [60]. Clinical application of

siRNA-based nanotherapies siRNA-based nanotherapeutics for cancer therapy offers several advantages over chemotherapeutic anticancer drugs, especially the undruggable targets in cancer treatment. The first and foremost advantage is the high degree of safety. Second, siRNA acts at the post-translational stage of gene expression; therefore, there is no interaction with DNA. As a result, risks of mutation and teratogenic risks that are more common with conventional gene therapy are negligible. Third, siRNA is highly efficacious and preferentially target any genes with minimal off-target effects and immunogenicity [61]. Fourth, siRNA-based delivery systems can be easily fabricated and modified [62]. Fifth, siRNA therapeutics exhibit a promising antiproliferative and tumor growth suppression effect through different signaling pathways [63]. Sixth, they can cause angiogenesis suppression by inhibiting VEGFs and VEGFR-1 receptors [64]. Seventh, the inhibition of tumor invasion and metastasis is conducted through the utilization of different chemokines CXCL8 and CXCL11 [65]. Eight, unrestricted choice of specificity and targets compared to other antibody-based drugs or small molecule drugs are advantageous.

To date, there are several are demonstrating its role in tumor treatment using nanoparticles-encapsulated siRNA-based delivery system. There are three main types of siRNA-based delivery systems in cancer chemotherapeutics, namely, lipid-based systems, polymers-based systems, and siRNA conjugates. In the lipid-based system, in order to form lipoplexes different cationic lipids, such as 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), N-trimethylammonium chloride (DOTMA), and N-[1-(2,3-dioleoyloxy) Propyl]-N, N, were utilized along with neutral lip‘ids, such as cholesterol (Chol), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DSPE), dioleoyl phosphatidylethanolamine (DOPE), and 1,2-dioleyl-sn-glycero-3-phosphocholine (DOPC). In lipoplexes, the incorporation of siRNAs into positively charged liposomes is carried out by electrostatic interactions [66]. In the polymer-based siRNA delivery system polyethyleneimine (PEI), poly-L-lysine (PLL) chitosan, cyclodextrin, hyaluronic acid, and poly ethylene glycol (PEG)-based nanocarriers were extensively utilized [67]. In the siRNA conjugate system, antibodies, aptamers, peptides, and dendrimers were utilized. Among these abovementioned siRNA delivery systems, lipid-based delivery system attracted much attention in cancer therapy, and a few are already in clinical trials.

Since the last few decades, pharmaceutical industries focused on clinical ones using siRNA-based nanotherapies which were initiated in 2010 and several synthetic siRNA-based nanotherapeutics were explored in the past few years for treating recurrent and aggressive tumors. The first clinical trial of nanoparticles-mediated siRNA delivery CALAA-01 was published in 2010 by Calando Pharmaceuticals [68]. CALAA-01 comprises different components such as cyclodextrin-based polymer (CDP), external PEG chains to improve the stability of nanoparticles in biological fluids, a human transferrin protein (TF) to target TF receptors (TFR) on cancer cells surface, and a siRNA specific for M2 subunit target of the ribonucleotide reductase protein (RRM2). Moreover, intratumoral downregulation of RRM2 leads to the induction of apoptosis in cancer cells [69]. However, this was only preliminary as it utilized only small set of patients. Thereafter, in 2014, the phase I clinical trial of liposomal siRNA-based delivery system termed as Atu027 was published by Silence Therapeutics GmbH. The structure of Atu027 contains a neutral, fusogenic DPhyPE helperlipid, PEGylated lipid MPEG-2000-DSPE (molar ratio: 50/49/1), and a AtuFect01 for targeting protein kinase N3 [70]. The phase I clinical trial dose-escalation of Atu027 demonstrated disease stabilization for 41% of patients suffering from metastatic pancreatic cancer. The efficacy of Atu027 was tested together with gemcitabine in a clinical trial for the treatment of cancer. Another clinical one employed using the biodegradable polymer matrix loaded with KRASG12D-targeting siRNA for prolonged delivery regionally within the tumor tissue by Silenseed Ltd. A phase I/IIa clinical study was conducted using this delivery system together with gemtabicine in patients with non-operable locally advanced pancreatic cancer. The results of clinical trial demonstrated no evidence of tumor progression and disease stability [71]. Furthermore, a multinational randomized phase II clinical trial using this delivery system is currently in progress. Another clinical one using a lipid nanoparticles-based siRNA delivery system called DCR-PHXC-101 was developed by Dicerna pharmaceuticals for downregulating the expression of the transcription factor Myc. In this dose-escalation clinical one, safety, pharmacodynamics, pharmacokinetics, and clinical activity of DCR-MYC were explored in patients with lymphoma I, advanced solid tumors, and multiple myeloma. Among all patients receiving treatment, the majority of patients demonstrated shrinkage in tumor and sustained metabolic response [72]. The most recent anticancer siRNA-mediated nanotherapeutics clinical trial conducted was using EphA2-siRNA-DOPC. Herein, EphA2, tyrosine kinases receptors serve as the target protein. The upregulation of EphA2 was reported in several ones related to breast, prostate, lung, pancreas, and most importantly, ovarian cancer, and causes tumor invasion, metastasis and angiogenesis. Herein, EphA2-siRNA was encapsulated in liposomal nanoparticles 1,2-dioleoyl-sn-glycero-3-phospahtidylcholine (DOPC) and combinedly termed as EPHARNA (EphA2-siRNA-DOPC) for their specifically target of EphA2 expression in the tumor [73]. The simultaneous administration of EPHARNA and paclitaxel demonstrated an anti-angiogenic effect and drastic reduction in tumor growth in several in vitro and in vivo [74]. Other in vivo toxicological ones reported no observed adverse events and no major toxicities at a dose range of 75–225 mcg/kg after a single or double administration of DOPC nanoliposomes [75]. The phase I clinical trial

of EphA2-siRNA-DOPC started in 2015, with patients suffering from advanced metastatic solid cancer receiving two weekly intravenous doses over two hours of EPHARNA, and is still continuing [76].

Although the lipid nanoparticles-mediated delivery of siRNA using ApoE coated lipid nanoparticles indicated high internalization into liver cancer cells, the siRNA-based delivery systems for other cancers are still under exploration. Despite the promising results of the improved siRNA delivery system for cancer treatment and several clinical trials, still not a single anticancer siRNA drug has been FDA approved for commercial usage [77]. This might be due to the problems associated with delivery to target tissues. As siRNA presents a huge potential for cancer treatment, in addition to the identification and utilization of internalization pathways for specific target cells, attempting to overcome the delivery problems would pave a way to the design of innovative siRNA-based delivery systems for cancer therapeutics.

4. MicroRNA (miRNA)-Based Nanotherapeutic Strategies

In the past few years, RNA-based therapeutics have shown immense potential in cancer nanotherapeutics. RNA-based therapeutics can be mediated either as inhibitors of target protein expression using siRNA and miRNA or as upregulators using mRNA [78]. miRNA-based cancer therapeutics have shown tremendous implications in the pathophysiological processes of cancer as emerging gene regulators. miRNAs are tiny, endogenous, noncoding RNAs that control gene expression in a variety of physiological activities, including cell growth and proliferation, differentiation, cell cycle, apoptosis, and tissue development [79]. The deregulated miRNAs affect the multiple biological pathways and leads to cellular transformation, malignancy, and cancer progression [80]. The differential expression of miRNAs in different tissues related to cancer enables them to target a multitude of transcripts related to cancer signaling pathways. The upregulation and downregulation of miRNAs leads to the suppression of tumor suppressor genes and increased expression of oncomers, respectively, which indicate their functions as both oncogenes and a tumor suppressor. For example, miR-10b, miR-125b, and miR-145 are downregulated, while miR-21 and miR-155 are upregulated in cancer development, suggesting their dual roles as tumor suppressors and oncogenes, respectively [81][82]. Owing to miRNAs' functions as both tumor suppressor and oncogenic miRNAs, they can modulate multiple signaling cascades related to cancer and metastasis via the transcriptional effect. Therefore, miRNAs can be targeted in cancer therapeutics either as synthetic anti-miR sequences for an upregulated miRNAs or as miRNAs mimics for downregulated miRNAs [83]. In this context, miRNAs may be silenced to upregulate the tumor suppressor genes or degrade the anti-apoptotic genes. Taken together, the regulatory potential of miRNAs makes them a new, promising, individualized therapeutic strategy for cancer therapeutics.

In the past few decades, several miRNAs-based delivery systems were focused; however, their clinical translation was limited due to their short half-life, degradation by nucleases, very low endosomal and/or lysosomal degradation, broad functionality, and off-target effects. In order to overcome these problems, nanotechnology-integrated miRNA delivery systems were developed for the cell-specific delivery of therapeutic miRNAs/anti-miRNAs using targeted miRNA mimics. Several nanoparticles-based platforms, such as lipid-based nanostructures, polymer-based nanomaterials, inorganic nanomaterials, dendrimers, polymeric micelles, and bioinspired nano vehicles, were employed for miRNA delivery in the past few years for targeted delivery [78]. Earlier it was utilized inorganic silica-based nanoparticles as a vehicle for miRNA delivery and demonstrated the delivery of miR-34a to neuroblastoma cells and induced apoptosis in tumor cells [84][85]. However, these inorganic nanoparticles-based delivery systems for miRNAs reported some challenges, such as lower loading efficacy, lower endosomal escape, and lack of cargo protection. Thereafter, polymer-based, and lipid-based nanoparticles-based platforms were utilized for miRNA delivery. In this cationic short polyurethane and branched polyethylenimine (PU-PEI)-based nanospheres containing miR-145 demonstrated significant downregulation of tumor growth in lung adenocarcinoma cells by inhibiting epithelial-mesenchymal trans differentiation [86].

The combination of PU-PEI-miR-145, radiotherapy, and cisplatin reduced the growth of metastatic tumors, indicating its promising role in miRNA-based cancer nanotherapeutics. Later, it was reported that the high molecular weight polyethylenimine (PEI), a high degree of branching, led to non-specific toxicity. Thereafter, researchers utilized low molecular weight PEI with a smaller degree of branching for miRNA delivery and demonstrated its efficient function. In an in vivo one, miR-145 and miR-33a mimics elevated programmed cell death and reduced tumor growth in colon cancer using low molecular weight polyethylenimine and suppressed the cancer cells proliferation [87]. The smaller degree of branching in low molecular weight polyethylenimine demonstrated reduced toxicities-associated issues which were otherwise observed with high molecular weight polyethylenimine.

The first miRNA-based cancer nanotherapeutics that entered clinical trials—Mirna Therapeutics—involve liposomes' modified tumor suppressor miRNA (miR-34), termed as MRX34. MRX34 demonstrated promising results in phase 1 and phase 2 clinical trials in patients with hepatocellular carcinoma, renal cell carcinoma (RCC), and acral melanoma.

Currently, five more miRNA-based cancer nanotherapeutics are currently in clinical trials either in phase 1 or phase II stage [79]. In the past few years, a combination approach employing the codelivery of miRNA, along with small molecule anticancer drugs, have indicated a superior therapeutic benefit in cancer nanotherapeutics. This combination approach provided several advantages over conventional chemotherapeutics in inhibiting drug resistance, reversing epithelial to mesenchymal transition (EMT), inducing apoptosis and autophagy, suppressing tumor angiogenesis, and inhibiting overexpression of efflux transporters (P-glycoprotein) [88]. The targeted delivery of miRNAs combined with chemotherapeutic drugs sensitizes the cancer cells to chemotherapeutic drugs using an anti-miR system-based replacement or restoration of tumor genes [89]. Thus, the synergistic effect of the combinational therapy helps to overcome drug resistance by directly targeting antiapoptotic signaling pathways and overexpressed efflux transporters. Shi et al. reported enhanced anticancer effects using lipid nanoparticles' loaded miR-34 and paclitaxel drug compared to miRNA or paclitaxel alone [90]. Another used polymer micelles coupled with miR-205 and gemcitabine to target markers such as OCT3/4, CD44, and Tubulin 3, showing a substantial reduction in tumor volume, implying that pancreatic cancer cells' sensitivity to gemcitabine was restored [91]. Targeted co-delivery of miR-34a with anticancer drug in breast cancer displayed inhibition in chemoresistance, cell proliferation, and tumor invasion by modulating Notch-1 signaling pathway [92]. Recently, the transfection of miR-126 mimic demonstrated an enhanced sensitivity of fourteen chemotherapy drugs (for example, trimetinib and alpelisib) through the inhibition of CDK4/6 and PIK3CA, which arrests cell cycle progression [93]. In another, miR-1291 delivery along with gemcitabine and nab-paclitaxel to pancreatic cancer reported induced DNA damage, mitotic block, induced apoptosis, and significant inhibition of tumor cells growth by upregulating the AT-rich interactive domain-containing protein 3B (ARID3B) gene [94]. In another one, poly lactic acid and poly dimethylaminoethyl methacrylate conjugated with miR-21 inhibitor and doxorubicin (Dox) exhibited excellent anticancer efficacy in glioblastoma cancer cells [95]. Furthermore, it was utilized the codelivery of miR-149 and miR-137 along with Dox to target neuroblastoma and pancreatic cancer cells and indicated restrained cell proliferation, promotion of apoptosis and sensitivity towards anticancer drug [96][97]. In a recent one, the injection of lipid nanoparticles conjugated with miR-634 and drug displayed induced apoptosis and reduced tumor growth in pancreatic cancer cells [98]. Although nanoparticles-mediated miRNA delivery has shown immense potential in the past few years, still, specific uptakes by cancer cells remain challenging due to the broad specificity of miRNAs. To overcome this challenge, nanoparticles are coated with either specific antibodies or ligands which are specifically expressed in cancer cells for targeted delivery. Polymeric micelles were conjugated with I-131-labeled prostate-specific membrane antigen (PSMA) antibody and demonstrated the co-delivery of miRNA and chemotherapeutic drugs to prostate cancer cells without any adverse effects [99]. Furthermore, nanoparticles conjugated with aptamers also showed promising results in the co-delivery of miRNA and drugs with enhanced cytotoxic activity against cancer cells [100][101]. Taken together, the combinational strategy by co-delivering anti-tumor miRNAs with chemo drugs synergistically enhanced the therapeutic efficacy with the reduction of cancer drug resistance. It signifies that this approach would provide a research direction and various hopeful avenues for cancer therapies.

5. Self-Assembly Prodrug (SAP)-Based Nanotherapeutic Strategies

Conventional chemotherapy using anticancer medicines has several limitations, including low solubility, bioavailability, and, most crucially, MDR. To address the limits of free pharmaceuticals, a strong and effective nanotherapeutic technique, the self-assembling prodrugs-based approach, has emerged as a promising treatment option for cancer. This approach offers a strong and successful nanotherapeutic technique that received much attention in the past few years for the targeted delivery of poorly soluble anti-cancer medicines. SAP nanotherapeutics (SAPNS) are a very well-designed method, with various inherent benefits over free drugs that were previously clinically unmet by traditional approaches. SAPNs have better physicochemical qualities in terms of solubility, drug loading, chemical stability, and blood circulation. Second, they have better pharmacodynamic characteristics that favorably alter PK, drug release, and tumor uptake, while minimizing adverse effects. Third, this approach reduces systemic non-specific toxicities and serves as an effective carrier for the targeted administration of poorly soluble drugs in vivo. Fourth, the greater medication accumulation at the tumor site of the targeted delivery based on the enhanced permeability and retention (EPR) effect is a factor to consider [102][103]. Additionally, this SAPNs-based strategy utilizes a nanoparticle-mediated endocytosis cellular absorption mechanism, which aids in bypassing MDR-related issues. This endocytosis-mediated cellular absorption process circumvents drug efflux transporters, which are known to pump out free drugs.

After the last two decades, the self-assembling prodrugs (SAP) method has attracted considerable attention as a strong therapeutic platform for the enhancement of targeted tumor treatment [103][104][105][106]. SAPNs are classified into three types: lipid-drugs, polymer-drugs, and drug-drug conjugates [107]. Early, it was largely used hydrophilic polyethylene glycol (PEG) for combination with lipophilic medicines due to its ease of formulation, high hydrophilicity, and biocompatibility, which allowed for the avoidance of solubility and bioavailability difficulties associated with free drugs [108][109]. PEG-based

prodrugs do not only self-assemble to different nanoformulations, such as polymeric micelles, but also provide synergistic anti-cancer activity by co-delivering the water-insoluble chemotherapeutics incorporated in their hydrophobic core [110][111]. Thereafter, another robust strategy using lipid-based modification emerged for the formulation of hard-to-formulate drugs by facilitating their self-assembly into nanoparticles of different shapes [112][113]. In this, doxorubicin (DOX)-derivatized α-d-tocopherol succinate prodrug (N-DOX-TOS) and were able to form nano-assembly in aqueous solution after stabilization with TOS and demonstrated improved anticancer efficacy compared to unmodified DOX [114]. In another one, self-assembling doxorubicin prodrug PEG_{2K}-DOX demonstrated their effective reversal of doxorubicin related drug resistance with enhanced plasma pharmacokinetics and in vivo therapeutic efficiency against MDR xenograft tumors when compared to doxorubicin alone [115]. Yang et al. reported an improvement in the sensitivity of cisplatin to triple-negative breast cancer using platinum Pt (IV) prodrugs based on cisplatin and chemosensitizer adjudin (ADD), which havw ability to self-assemble into nanosheets. This Pt (IV)-ADD-based self-assembled prodrug nanotherapeutics indicated an improved in vivo tumor growth inhibition with 266-fold lower IC_{50} value [116]. Recently, a synergistic Pt (IV) prodrug, Npx-pp-Pt (IV) demonstrated dual responsive behaviors for deactivating the dual drug resistance-related pathways to reverse cisplatin resistance. Herein, the in situ supramolecular self-assembly of prodrug into nanofiber structure revealed the enhanced cellular uptake of cisplatin and significant damage of the cisplatin-resistance cancer cells through cyclooxygenase-2 and nuclear factor kappa B-mediated apoptosis pathways, with a 80% tumor inhibition rate [106]. Furthermore, by exploiting the unique physicochemical properties of different drugs, amphiphilic drugs (hydrophilic drug conjugated with hydrophobic drug) can self-assemble into various nanoparticles shapes with improved pharmacokinetics, bioavailability, and antitumor efficacy [117]. In the co-delivery-based combination cancer therapy, different drugs are physically loaded in different nanocarriers. However, no physical drug loading is required with the drug–drug conjugate approach, as it already contains two distinctly pharmaceutically active agents [102][118]. Moreover, the self-assembled prodrug nanotherapeutics approach utilizes a drugs cocktail that alleviates the nonuniform biodistribution of anticancer agents and also ensures well-controlled targeted dual-drug delivery to reverse multi-drug resistance in cancer therapeutics.

6. Exosomes-Based Nanotherapeutics Strategies

Exosomes represent a subclass of heterogeneous extracellular vesicles (EVs) of endosomal origin with a diameter of 40–150 nm, which are secreted from a variety of cells present in tumor microenvironment such as cancer cells, tumor associated fibroblasts, CSCs, and tumor associated immune cells [119]. In the tumor microenvironment, exosomes-mediated constant crosstalk between tumor cells and stromal forms a large part of the communication. Exosomes are involved in various cellular and pathological conditions and, through intercellular communication, deliver their cargo to the immediate surroundings, as well as at distant organs. The cargo of exosomes comprises proteins, lipids, nucleic acids, and metabolites that modulate stromal reactions, regulates immune response, promotes angiogenesis, and modify signaling pathways related to cancer in tumor microenvironment. Numerous in vitro and preclinical in vivo demonstrate that exosomes play a critical role in conferring drug resistance on cancer cells via intercellular interactions in a variety of cancer types, including pancreatic cancer, breast cancer, lung cancer, prostate cancer, colorectal cancer, glioblastoma, kidney cancer, neuroblastoma, ovarian cancer, gastric cancer, melanoma, and osteosarcoma [120][121]. Exosomes' cargo mediates chemoresistance through the regulation of drug efflux and metabolism, epithelial–mesenchymal transition, alteration of prosurvival signaling pathways, remodeling of tumor microenvironment, and increase concentration of plastic CSCs [122]. Along with their crucial involvement in establishing drug resistance in cancer, exosomes also transmit drug resistance phenotypes to other cancer cells and serve as biomarkers for monitoring drug resistance in cancer. Exosomes, by virtue of their function in chemoresistance, might also be used as a therapeutic target for overcoming drug resistance in cancer cells.

In order to enhance the effect of chemotherapy, exosome-mediated chemoresistance inhibition is prerequisite. In this context, two possible strategies are available that include exosome biogenesis and trafficking suppression, depletion of exosome uptake by cancer cells, modulation of harmful exosomal cargo, and inhibition of exosome dissemination, removal of exosomes. Exosomes depletion and removal may restore drug sensitivity to chemotherapy to some extent. However, limited knowledge regarding the specific ways how exosomes are internalized by cancer cells and deliver their cargo pave the way for alternative strategies to overcome drug resistance. Therefore, the application of exosomes as drug and gene delivery vehicles for targeted cancer nanotherapeutics is an appealing platform owing to its natural composition, low toxicity, and low immunogenicity. In cancer nanotherapeutics, different synthetic nanoparticles such as liposomes, self-assembling peptides and nanosponges were extensively utilized for targeted cancer therapy [123][124]. Nonetheless, various challenges such as different biological barriers due to the tumor heterogeneity still remain, with the exogenous nanomaterials being utilized for targeted drug delivery to cancer cells [125]. To overcome the limitations of synthetic nanoparticles, one emerging approach is to develop and utilize natural nanocarriers for targeted delivery. Several intrinsic features of exosomes, such as the ability to pass through lipid bilayer of cell membrane, high delivery efficiency, good

stability in biological fluids, and high biocompatibility with low immunogenicity, support their potential as attractive nanocarriers for targeted drug or gene delivery [126][127]. Their specificity may further be improved upon by engineering exosomes with tumor-specific peptides, proteins, or antibodies for precise targeted drug delivery. The critical steps involved in utilizing exosomes as nanocarriers are the development of an efficient cargo loading method and choice of exosome-producing cells as these steps greatly impact the function, biodistribution, and immunogenicity of the exosomes. The exosomes-loading approaches include passive diffusion; electroporation, and loading the cargo to parental cells by incubation, overexpression, or transfection; and isolation of secreted exosomes through extrusion, freeze and thaw cycles, and sonication [128]. Regarding cell types, cells should be selected which are scalable and can produce large quantities of exosomes such as mesenchymal stem cells (MSCs) and bovine milk [129][130].

Exosomes loading with small molecule chemotherapeutic drugs attracted much attention in the past few decades. Researchers obtained paclitaxel-loaded exosomes from the centrifuged supernatant of chemo-resistant cells treated with paclitaxel. The supernatant contained drug loaded exosomes as the chemo-resistant cells natural tendency to flush out the drugs due to overexpression of drug efflux transporters [131]. Nevertheless, drug loading in exosomes demonstrated improved bioavailability, stability in biological fluids, and reduced off target effects. In this line, paclitaxel loaded exosomes increased the toxicity by 50-fold in drug-resistant cells by ensuring co-localization of exosomes carrier with cancer cells [132]. Despite encouraging results using exosomes as drug delivery vehicles, still a few challenges remain such as purification, large scale production, and efficient drug loading and storage. Exosomes subgroups' heterogeneity further slowdown the quality control processes and translation into clinical settings [121]. Therefore, the development of artificial exosomes through te advancements in nanobiotechnology opens several avenues for advanced drug delivery.

The nano bioengineered artificial exosomes or exosomes mimics carrying anticancer drugs as drug delivery vehicles present the current pro-active approach in cancer nanotherapeutics. Jang et al. developed exosome mimics by mixing the doxorubicin drug with whole monocyte or macrophage cells followed by passage through filters of different pore sizes. The developed exosome mimics were compared with natural exosomes loaded with doxorubicin and indicated similar properties, but a 100-fold higher production yield [133]. Several preclinical one utilized exosomes-based delivery approach for the targeted delivery of paclitaxel and doxorubicin to different cancer types, such as prostate, pancreatic, and lung cancer [130][134]. The results reported superior delivery of drugs through exosomes as compared to liposomes and free drugs. Kim et al. demonstrated exosomes-based successful delivery of paclitaxel to MDR cancer cells with overexpression of efflux transporters P-glycoprotein (P-gp). Paclitaxel loaded exosomes indicated the reversal of drug resistance by providing enhanced sensitivity towards MDR cancer cells by escaping P-gp-mediated drug efflux and inhibiting metastasis in a lung cancer xenograft model [132]. In a similar approach, gold nanoparticles' conjugated doxorubicin was loaded into exosomes and displayed an improved antitumor effect against lung cancer cells [135]. Furthermore, exploration of exosomes content escalated a vital role in the reversal of chemoresistance as they have a direct role in the development of chemoresistance [136]. Wu et al. (2020) utilized exosomes derived from bone marrow mesenchymal stem cells loaded with miR-193a for targeting leucine rich repeat and revealed reduced cisplatin resistance in non-small cell lung cancer [137]. In another one, engineered exosomes were employed for co-delivery of miR-21 inhibitor 5-fluoro-2,4(1H,3H)-pyrimidinedione(5-FU) for the reversal of drug resistance in colon cancer via targeted chemotherapy [138]. Shtam et al. showed a reduced level of DNA damage-repair protein and induction of apoptosis levels using exosome loaded with RAD51 siRNA in fibrosarcoma and cervical adenocarcinoma cell lines [139]. In a similar approach, exosomes derived from fibroblasts loaded with kras-siRNA indicated superior delivery and blunted tumor growth in pancreatic cancer [140]. In another, exosomes isolated from HEK-293 cells were transfected with HGF siRNA demonstrated reduced vascularization with reduction in levels of HGF and VEGF proteins in gastric cancer cells tumors compared to free siRNA [141]. Apart from siRNA, miRNA was also loaded within exomes for targeted delivery and inhibition of tumor growth. Several were reported improved nanotherapeutics using exosomes-loaded miRNA delivery (miR-143, miR146b, and miR-122) to human osteosarcoma cells, glioma cells, and hepatocellular carcinoma (HCC) cells, respectively [127][142][143]. Adipose tissue-derived MSCs (AMSCs) released exosomes transfected with miR-122 induced sorafenib chemosensitivity when co-cultured with hepatocyte carcinoma cells [142]. In a similar approach, co-culture exosomes derived from AMSCs carrying miR-199a induced chemosensitivity towards doxorubicin by downregulating mammalian target of rapamycin (mTOR) pathway and [143]. Furthermore, Kim et al. reported inducted apoptosis and cisplatin chemosensitivity using exosomes loaded with CRISPR/Cas9 and si-c-Met in ovarian cancer cells and human gastric adenocarcinoma cells, respectively [144][145]. Oxiplatin-resistant cancer-resistant cells demonstrated chemosensitivity and decreased motility with normal intestinal FHC cell-derived exosomes loaded miR-128-3p [146]. Recently, induced chemosensitivity towards trastuzumab and docetaxel were reported in breast cancer cells and tongue squamous cell carcinoma through exosomes-mediated delivery of miR-567 and miR-200c, respectively [147][148]. Moreover, exosomes-mediated targeted delivery holds promising strategy for reversing chemoresistance by delivering conventional drugs and various genetic materials. Overall, exosomes-based targeted delivery of drugs and genes are a new and emerging approach which holds much promise for

drug resistance reversal. However, further exploration of the different sources of exosomes, side effects, and safety would be pertinent for cancer nanotherapeutics in order to attain higher delivery efficacy for anticancer molecules at lower doses without any side effects.

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