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Multifunctional and multitargeted nanoparticles for drug delivery to overcome barriers of drug resistance in human cancers

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The recurrence and metastatic spread of cancer are major drawbacks in cancer treatment. Although chemotherapy is one of the most effective methods for the treatment of metastatic cancers, it is nonspecific and causes significant toxic damage. The development of drug resistance to chemotherapeutic agents through various mechanisms also limits their therapeutic potential. However, as we discuss here, the use of nanodelivery systems that are a combination of diagnostics and therapeutics (theranostics) is as relatively novel concept in the treatment of cancer. Such systems are likely to improve the therapeutic benefits of encapsulated drugs and can transit to the desired site, maintaining their pharmaceutical properties. The specific targeting of malignant cells using multifunctional nanoparticles exploits theranostics as an improved agent for delivering anticancer drugs and as a new solution for overriding drug resistance.

Cancer remains a major cause of mortality in humans and, over the past decade, immense effort has been invested in the development of new diagnostic tools and therapeutic strategies [1]. The success of cancer therapy depends on the ability of a therapeutic agent to destroy the tumor cells while minimally affecting normal nonmalignant cells. Intensive research is now focused on delivery systems to target therapeutic drugs specifically to the tumor site [2]. However, resistance to chemotherapeutic drugs is a major barrier to effective cancer treatment [3]. Chemotherapy fails because: (i) the tumor cells might be inherently resistant owing to genetic deformities; and/or (ii) they might acquire resistance following drug exposure [4].

Drug resistance is a cellular phenomenon that reflects an inability to demonstrate cytotoxicity at physiologically achievable drug concentrations in cancer cells. Multidrug resistance (MDR) occurs in over 50% of patients during cancer relapses, accounting in large part for the high mortality associated with cancer. Tumor resistance to chemotherapy results from the inefficient distribution of drugs and its failure to reduce tumor size after treatment [5]. The first incidence of MDR was observed in microorganisms, such as bacteria and viruses [6]. The phenomenon of MDR is complex and mainly involves the activation of energy-dependent drug efflux pumps [such as P-glycoprotein (P-gp)], altered expression of apoptotic proteins, such as B-cell lymphoma 2 (Bcl-2), survivin and caspase 3, and enhanced DNA repair, aiding in MDR. Regulation of P-gp and apoptotic proteins has become an area of study for researchers, and attempt are under way to target these MDR cells to make them susceptible to therapy [7].

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miRNA is an important molecule with a vital role in the development of MDR because it acts as both a tumor suppressor and a tumor promoter. MiRNAs are small, noncoding RNAs that regulate cell growth, differentiation and apoptosis [8]. One of the most crucial solutions to MDR that has been developed is aptamers. Aptamers are single-stranded structured oligonucleotides (both DNA and RNA) that bind to targets with high affinity and specificity. They are potential agents for effective therapy and diagnosis because of their small size, low immunogenicity and toxicity, long shelf life and strong binding affinity to their cognate receptors [9]. Multifunctional nanoparticles are a proverbial Swiss army knife of options in overcoming drug resistance in cancers. Nanoparticles have diameters in the range of 5–100 nm and have numerous functional moieties on their surface competent for multivalent conjugation for targeting, imaging and delivery of therapeutic agents. Nanoparticles protect the drug from quick metabolism and clearance and also assist in evading uptake by the reticuloendothelial system [10] and mononuclear phagocytes [11]. Multifunctional nanoparticles can direct the delivery of drugs for specific cytotoxicity and, consequently, overcome the problem of resistance by sensitizing cancer cells to chemotherapeutic agents. They can be conjugated with small molecule inhibitors, including nucleotides, such as miRNA [8], small interfering (si)RNA and aptamers for sensitizing tumor cells and overcoming MDR [12]. Nanoparticle-based multimodal imaging probes also have the potential for providing improved qualitative and quantitative imaging of cancers.

Nanoparticles as tools for targeted drug delivery

Nanotechnology provides an excellent platform for the rational drug delivery of chemotherapeutic agents to tumors upon systemic administration and circumvents the problem of MDR. Some of the best structures for drug delivery systems are nanotubes [13], fullerenes [14], dendrimers [15], nanoshells [16], micelles [16], liposomes [17], polymeric nanoparticles [18] and iron oxide nanoparticles. These engineered nanocarriers have many advantages, such as small particulate size, limited size-range distribution, target-specific binding, protective covering of drug molecules to improve stability, ability to deliver multiple therapeutic agents in a single formulation, and multimodal imaging and therapy to analyze the outcomes in real-time combination. Drug-resistant tumors exhibit decreased extracellular pH, absence of adequate lymphatic drainage, hypoxia and alteration in expression of oncogenes, tumor suppressors and apoptosis mediators [19]. Nanoparticles have the potential to override MDR by eliminating the minimal residual disease (MRD) population of cells accounting for the recurrence of cancer [20]. Hence, nanocarriers are taken up by the cells through endocytosis, enter the cells and then release the drug molecule inside the cells by particle disruption [11] (Fig. 1).

Targeted nanoparticles for effective therapy

The targeting of nanoparticles can be classified into passive and active targeting. For passive targeting, the nanoparticles extravasate at the site where the tumor vasculature is more permeable and leaky. Increased levels of these vascular mediators result in vasodilation and promotes the extravasation of bulky molecules and their maintenance in tumors [21]. This phenomenon, also known as the enhanced permeability and retention effect (EPR), enables increased accumulation and retention of macromolecular drugs in tumor tissue. This EPR effect is principally used for the passive delivery of drug-loaded nanoparticles. For example, a nanoformulation of iron-saturated bovine lactoferrin (Fe-bLf) for cancer therapy has been developed. Alginate-surrounded chitosan-coated Fe-bLf or paclitaxel (Taxol®) nanoparticles adsorbed onto nanocores of calcium phosphate nanocarriers (AECCP-FebLf NCs or AEC-CP-Taxol® nanocarriers) were shown to enhance the anticancer efficacy of drugs *in vitro* [18] (Fig. 2).

For active targeting, the overexpression of some receptors or epitopes can be used to target localized disease, such as cancer or inflammation. Ligands that specifically attach to surface epitopes that are highly expressed at these target sites have been joined to the surface of long circulating nanocarriers. Active targeting can improve the distribution of nanoparticles inside the tumor vasculature and to MDR cells. Active targeting ensures that increased amounts of drug inside the carrier can be delivered to the target cell through ligand–receptor interactions. In addition, a multitude of ligand moieties can be linked to the nanocarrier for enhanced binding to target cells, especially for those with lower binding affinities. Lastly, when ligand binds to the carrier, because of the small size of the conjugate, it can only be released at the diseased site but not normal vasculature, therefore preventing the ligand interacting with the target epitopes of normal tissues and the resulting adverse effects [22] (Tables 1 and 2).

The nonspecific nature of many current anticancer agents severely limits their effectiveness if the dosage is too high and the systemic toxic effects outweigh the beneficial anticancer effect. To reach pharmacologically active concentrations of the agent, there is often the risk of causing collateral damage to the body. Nanotechnology offers multiple ways of solving this issue by increasing the accumulation of an agent when administered systemically or intratumorally. The more prominent targeting molecules are specific surface macromolecules, such as folic acid receptors, aptamers and antibodies.

Folic acid receptors

Folic acid (FA) is a vitamin that is needed for reactions of carbon metabolism and nucleotide bases. It is taken up by the cells through folate receptors (FRs). There are two isoforms of FR: the alpha isoform (FR-α) is found on the apical surfaces of epithelial cells; and the beta form (FR-β) is found on activated macrophages. Both these forms are overexpressed in malignant cancer cells. Other forms, such as FR-γ and FR-δ, are rare but found on regulatory T cells. The expression of FR on cancer cells helps them to compete aggressively against other cells for folate intake. Overexpression of these FR on cancer cells is common owing to epigenetic changes. Given that FA helps in the production of nucleotide bases, actively dividing cancer cells require high amounts of FA for their DNA replication and, thus, show increased expression of FR on the cell surface. This can be exploited by the conjugation of folate to the nanoparticle, greatly increasing its affinity for tumor cells. In addition, the folate-treated nanoparticles depicted an increased affinity for the binding protein compared with free folate. This factor, in combination with the overexpression of the binding protein, promoted endocytosis of the treated nanoparticle. It was also shown that no uptake occurred with cancers that did not overexpress the FR. This

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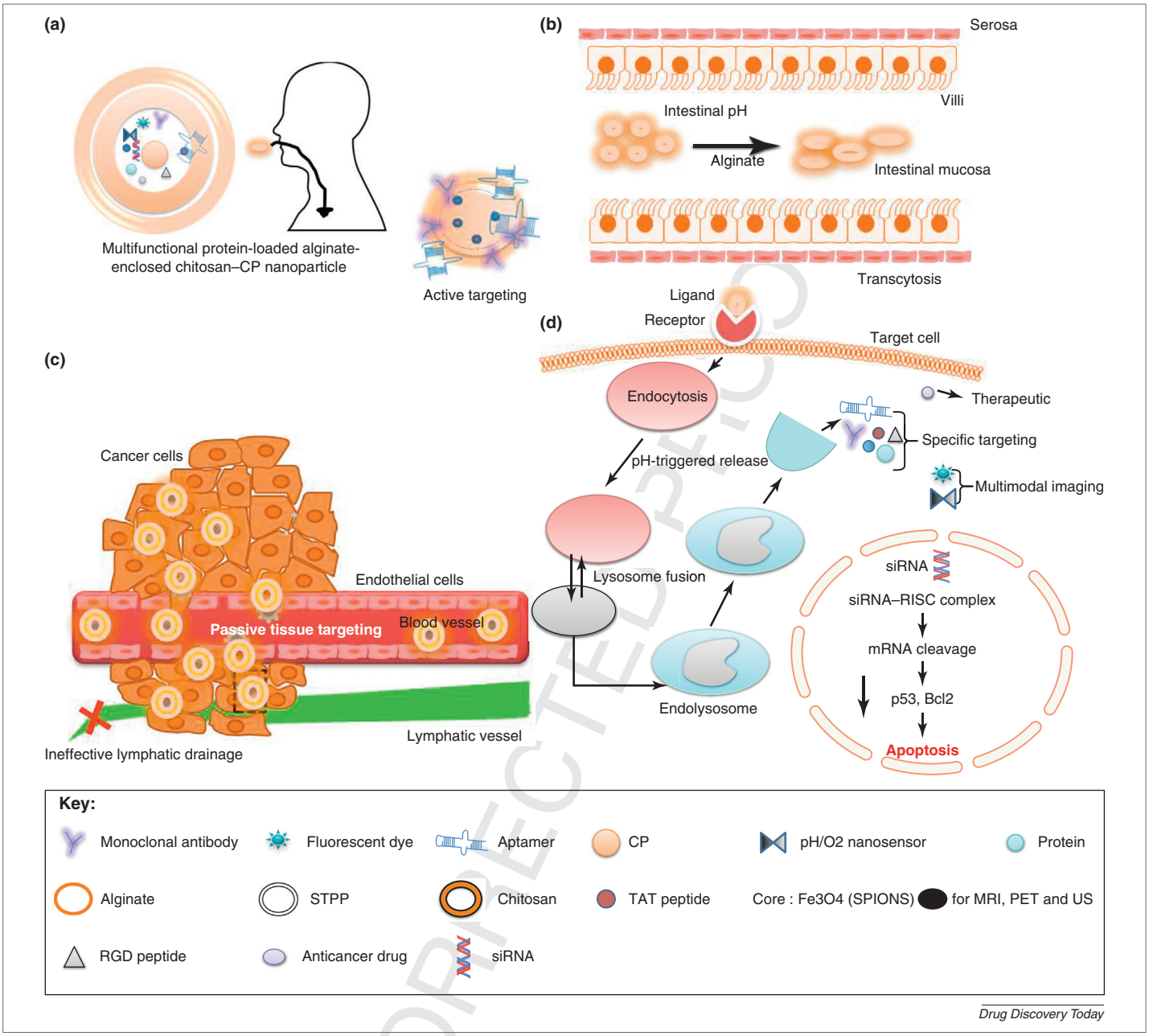

FIGURE 1

The uptake of a multifunctional nanoparticle and its subsequent action. **(a)** Oral administration of an alginate-enclosed chitosan–calcium phosphate (CP) multifunctional nanoparticle loaded with protein and other moieties, such as antibody, aptamer, tat peptide, cell-penetrating peptide (CPP) for specific targeting of cancerous cells, an anticancer drug, siRNA, and a fluorescent dye and nanosensor for therapy and multimodal imaging, respectively. **(b)** In the intestine, owing to the alkaline pH, the alginate coating of the nanoparticles is degraded. These alginate coating-free nanoparticles then enter the circulation through endocytosis and/or transcytosis. **(c)** The nanoparticles are then released at the tumor site and are readily taken up by the tumor cells via the effects of enhanced permeability and retention. **(d)** Further uptake of the nanoparticles in the cancer cells occurs through receptor-mediated endocytosis, which is triggered by ligand–receptor interactions. The various agents are then released to carry out their respective functions. *Abbreviations*: PET, positron emission tomography scanning; RGD peptide, Arg-Gly-Asp peptide; STPP, sodium tripolyphosphate; US, ultrasound.

behavior could be used in a nanoconjugate to improve its specificity against certain cancer cell types [23]. However, this has its own issues because the FR is also strongly expressed by liver cells, which can result in the cytotoxic drugs affecting the liver [24,25].

Aptamers

Aptamers are single-stranded oligonucleotides that can attach to their target receptors with high affinity and selectivity [12]. They have an important role in diagnostics, therapeutics, in some cases, even exceeding the benefits of other molecular probes, such as antibodies. Compared with antibodies, aptamers have further benefits, such as biocompatibility and flexible modification, stability as dry powders or in solution for a prolonged period, ability to maintain reversible denaturation, absence of toxicity, immunogenicity and rapid tissue penetration [12]. Aptamers are a class of molecules that are characterized by their small size, low toxicity,

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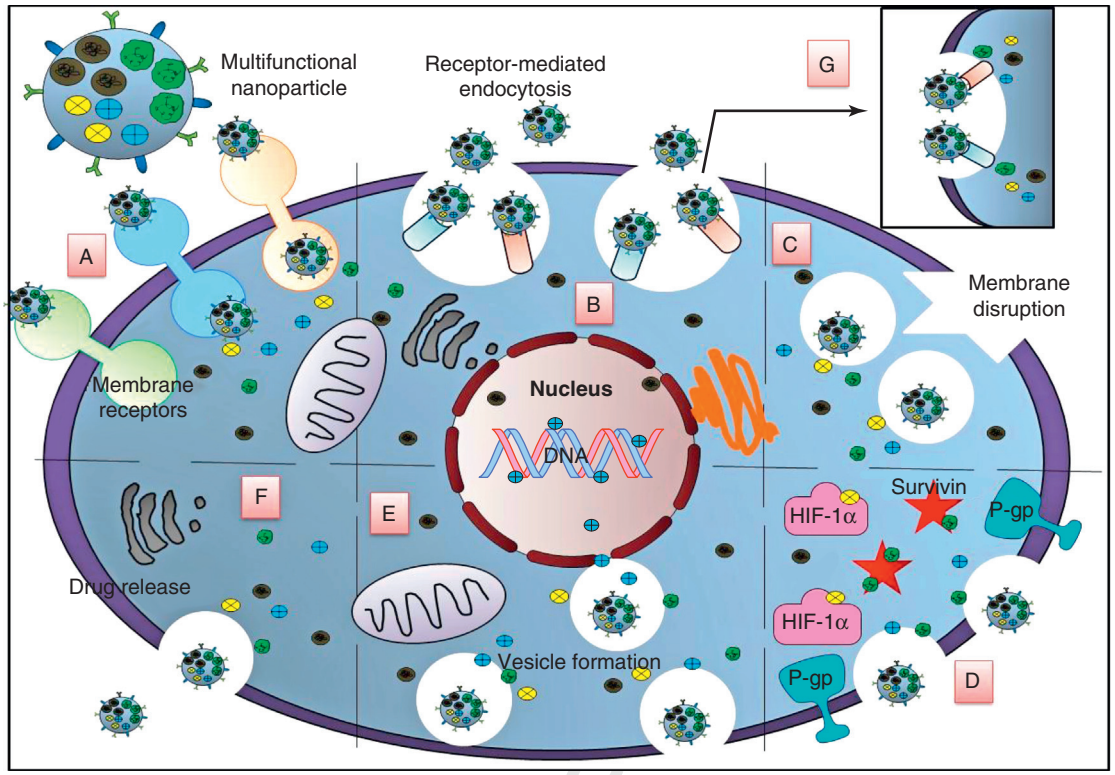

FIGURE 2

Steps involved in the mechanism by which multimodular functional nanoparticles act on drug-resistant tumors. **(a)** Receptor-mediated endocytosis of nanoformulations by which nanoparticles enter the tumor cell. **(b)** The formation of vesicles facilitating the internalization of nanoparticles. **(c)** Membrane disruption of nanoparticles owing to the formation of vesicles. **(d)** Overexpression of drug-resistant markers such as P-glycoprotein (P-gp), B-cell lymphoma 2 (Bcl-2) and hypoxia inducible factor-1α (HIF-1) being targeted by specific antagonists present in the multifunctional nanoparticle. **(e)** Release of anticancer drug molecules that causes DNA damage in tumor cells. **(f)** Release of bioavailable proteins, such as lactoferrin, in tumor cells. **(g)** Advantages of using super-paramagnetic iron oxide nanoparticles (SPIONS), Cy5-5 and other imaging molecules to image the route of entry of nanoparticles and trace their action on the cancer cell. The figure highlights the advantages of, and concept behind, the use of multimodular multifunctional nanoparticles as theranostic agents.

TABLE 1

Types of nanoparticle used as therapeutic options

Type of nanoparticle	Advantages	Primary use	Refs
Nanotubes	Soluble; biocompatible; reduced toxicity	Antiviral, antibacterial, antioxidants and anticancer agents	[13]
Dendrimers	Self-assembling; can attain specific response to stimuli	Delivery of drug-like camptothecins and other anticancer drugs	[15]
Nanoshells	Dielectric core; specific accumulation near tumors	Used in imaging and treatment of cancer; excellent contrast agents	[16]
Micelles	Water-soluble and pH-resistant delivery of drugs	Successful and specific delivery of anticancer drugs	[16]
Liposomes	Deliver both hydrophilic and hydrophobic drugs, with less intense adverse effects	Achieves enhanced therapeutic effect owing to prolonged circulation in bloodstream	[17]
Polymeric nanoparticles	Multifunctional, bioavailable and biocompatible	Enhances specific antitumor immune response by delivery of anticancer drugs.	[18]
Iron oxide nanoparticles	Thernostic agent: can be used for diagnostics, therapeutics and imaging of tumors	Overcome drug-resistant tumors detected by MRI and CT scans	[70]
Gold nanoparticles	Flexible size and shape	Tumor imaging and guided hyperthermia	[73]
Silica-gold nanoshells	Robust; prevent thermal ablation	Detection of tumors at early stages	[73]
Quantum dots	Can be used at various wavelengths; easily conjugated to antibodies; small size	Fluorescent probes for imaging	[73]
Magnetite nanoparticles	Stable and individual dispersal of particles on application of external magnetic field	MRI contrast agents	[73]
Biotin functionalized dual-agent nanoparticles	Overcome drug resistance resulting from transporter proteins	Delivery of Taxol® to MDR cells	[74]
Mesoporous silica nanoparticles	Knockdown of P-gp and effective delivery of doxorubicin and siRNA for P-gp	Deliver doxorubicin to drug-resistant KB-V1 cells	[75]
Silver nanoparticles	Natural nanoparticles; environmental friendly	Antibacterial activity	[76]

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TABLE 2

Drug-resistance markers and nanoparticles to overcome them

Drug-resistant marker	Role	Nanoformulations used	Mechanism	Refs
Transporter proteins	Efflux of drug from cancer cells	PLA and PEG nanoparticles	Transport anticancer drugs without use of P-gp	[42]
HIF-1α	Causes hypoxia and decreasing drug influx	Nanotubes	siRNA against HIF-1α downregulating its action on cells	[62]
Survivin	Increases proliferation of cancer cells	Dendrimers plus magnetic nanoparticles	Antisense survivin to downregulate its action	[61]
Bcl-2	Antiapoptotic gene	Mesoporous silica nanoparticles	siRNA for Bcl-2 suppresses its action along with an anticancer drug	[58]
p53 mutations	Tumor suppressor gene, loses its role after mutations	Poly(D,L-lactide-co-glycolide)	Delivery of wild-type p53 DNA, antiproliferative activity against tumor cells	[25]
Transferrin receptor	Overexpressed in case of MDR	Liposomes	Attached to transferrin, direct uptake by cancer cells	[4]
Intracellular pH	Decreased pH causing ineffective drug activity	Poly(beta-amino ester)	Enhanced delivery of drugs even in acidic pH	[4]
Ceramide levels	Expression decreased in MDR cells	PEO-modified poly(ε-caprolactone)	Stimulates higher levels of ceramide in the cells	[43]
EGFR	Increased expression in most cancers	Functionalized quantum dots plus magnetic iron oxide nanoparticles	Systemic delivery of EGFR antibody; tumor targeting and imaging	[77]
Folate receptors	Overexpressed in breast, kidney, ovary, lung, brain and myeloid cancers	PAMAM dendrimers	Internalizes using excessive folate receptors on cells and enhances delivery of drugs	[78]
HER2/neu receptors	Overexpression causes drug resistance in breast cancer	Herceptin–dextran iron oxide nanoparticles	High level of accumulation in tumors reduces tumor volume	[79]
PSMA	Causes prostate cancer when upregulated	PLGA–PEG nanoparticles	Endocytosed into PSMA1 cells, causing death of cells	[80]
Lipoprotein receptor-related protein	Overexpressed in gliomas and brain cancers	PEG–poly(ε-caprolactone) nanoparticles	Can pass through blood–brain barrier and penetrate tumor tissue	[81]

immunogenicity and long shelf life. They are also manufactured synthetically and can be developed against nearly any target protein through repetitive cycles of *in vitro* screening of a combinatorial oligonucleotide library for target binding. Aptamers can also be made to target molecules or toxins that do not elicit immune responses. Aptamers by themselves can also have a therapeutic effect, as seen with AS1411 against human tumor xenografts *in vivo* [26]. These molecules can also modulate the activities of proteins implicated in pathological conditions, thereby becoming a functional pharmaceutical agent. For instance, Macugen® (pegaptanib), which is manufactured by Pfizer, is an aptamer-based anti-vascular endothelial growth factor (VEGF) treatment for age-related macular degeneration (ARMD). AS1411 is an aptamer that specifically targets nucleolin, which is a *bcl-2* mRNA-binding protein involved in cell proliferation and is present on the surface of many cancer cells [27].

Aptamers are exploited as targeting moieties specifically when developed by cell-based systemic evolution of ligands by exponential enrichment (SELEX) and can distinguish diseased cells from healthy cells, thus enabling the selective transport of therapeutic compounds to target cells. Given their small size, aptamers can target tumor cells and deliver small molecules, such as proteins, drugs or siRNA, through the microvasculature or tumor interstitium. Aptamers can be conjugated with nanoparticles for targeted drug delivery. By functionalizing the exterior of gold (Au) nanoparticles with an RNA aptamer that binds to prostrate-specific membrane antigen (PSMA), nanoparticle–aptamer complexes were utilized for targeted molecular computed tomography imaging and treatment of prostate cancer. Hydrogels are polymeric chain networks that are water insoluble and superabsorbent. Hydrogels that crosslink DNA aptamers with linear polyacrylamide chains have been developed. Competitive binding of the target to the aptamer results in reducing the crosslinking density and dissolution of hydrogel. An *in situ* injectable hydrogel linked with nucleic acid aptamers has been shown to direct the production of proteins for human disease treatment [27]. Aptamer micelle nanostructure comprising hydrophilic aptamers bound to hydrophobic lipids by polyethylene glycol (PEG) has been shown to target Ramos cells (from the human Burkitt's lymphoma cell line) [28]. Multitargeted locked nucleic acid (LNA)–aptamer complexes with anticancer-loaded Fe-bLf dopamine surface-modified Fe_3O_4 nanoparticles for specific tumor killing have also been developed. It was observed that 100% Fe-saturated Fe-bLf acts as a potent natural adjuvant for enhancing cancer chemotherapy [29]. Superparamagnetic iron oxide (Fe_3O_4) nanoparticles (SPIONs) are biodegradable, nontoxic and candidate platforms for the formation of theranostics nanoformulations [30]. These nanocarriers are ideal for encapsulation of SPIONs for both imaging or as therapy and drug molecules. LNA–nucleolin DNA aptamers and LNA–epithelial cell adhesion molecule (EpCAM) RNA aptamers, which are overexpressed on cancer cell surfaces and loaded individually or in combination on these Fe-bLf-SPIONS, have also been developed. These LNA–aptamer complexes of Fe-bLf nanocarriers caused cell death in breast and colon cancer cells *in vitro* [29]. This suggests that SPION-loaded LNA–aptamers can kill cancer cells without harming normal cells.

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The success with aptamers has become evident because several aptamers have now reached clinical trials, including EYE001 targeting the VEGF receptor; E100300 targeting platelet-derived growth factor (PDGF) [31]; REG1, which targets factor IXa [32]; ARC 1779 used for inhibiting acute coronary syndrome; AS1411 for cancer; and ARC1905 for ARMD. Aptamer-stimulated cell-type siRNA delivery has also been fabricated. For example, cell type-specific release of anti-HIV siRNAs via fusion to an anti-gp120 aptamer has been established [33].

Antibodies

Antibodies bound to nanoparticles have been used to deliver drugs to the site of disease via targeting the cell surface biomarkers to stimulate receptor-mediated endocytosis of the nanoparticle-encapsulated drug. For example, anti-HER-2-coated paclitaxel-loaded immune nanoparticles have been tested as a therapeutic tool for ovarian cancer [34]. Another example is the stimulation of apoptosis in colorectal HCT116 cancer cells using poly(lactic-co-glycolic acid) (PGLA) nanoparticles coated with conatumumab (AMG655) death receptor 5-specific antibodies (DR5-NP). Also, binding of radioisotopes with targeted antibodies has been exploited for both imaging and radioimmunotherapy. For instance, anti-CD20 ibritumomabtiuxetan (Zevalin Spectrum Pharmaceuticals, CA, USA) uses 90Y metal isotope for clinical therapy. Antibody nanoparticle conjugates stimulates the possible deactivation of the drug and its necessary discharge once internalized into endosomal and/or lysosomal vesicles via pH-labile or reducible linkers. Elevated drug:antibody ratios are probable with antibody nanoparticle complexes, thereby increasing the concentration of drug that is to be directed to the diseased region [35].

Drug resistance in human cancers

Drug resistance or MDR is now one of the major challenges facing the development of chemotherapeutic regimens. The mechanisms for MDR can be classified into at least five categories: (i) amplified drug efflux via ATP-driven efflux pumps; (ii) decreased influx; (iii) enhanced DNA repair; (iv) increased levels of detoxification enzymes, such as cytochrome p450 that metabolize rapidly and inactivate internalized drugs; and (v) inactivation of apoptotic pathways with simultaneous activation of antiapoptotic cellular defense modalities, including the upregulation of apoptotic inhibitors, such as survivin and antiapoptotic Bcl-2 family members [36,37]. Survivin has a vital role in stimulating MDR by directly suppressing caspase and procaspase signaling mechanisms, resulting in upregulation of MDR pathways, such as P-gp, multidrug resistance protein-1 (MRP-1) and protein-2 (MRP-2) signaling in cancer cells. Survivin is highly expressed in tumor cells, has a vital role in cell division and is a suppressor of apoptosis [38]. It also has a positive association with other molecules, such as hypoxia inducible factor-1α (HIF-1α), which is overexpressed in the case of hypoxia. It has been shown that there was a transcriptional increase in the survivin mRNA levels in conditions of hypoxia relating these two factors [39]. The *Bcl-2* family of proteins contains both antiapoptotic proteins, such as Bcl-2, Bcl-X_L and Mcl-1, and proapoptotic molecules, such as Bax, Bak and BH3 domain-only molecules. An abnormal increase in the anti- to proapoptotic Bcl-2 protein ratio has been observed and correlated to apoptosis resistance and resultant survival of cancer cells [40].

Transporter proteins

One of the chief mechanisms of MDR in cultured cancer cells is the expression of P-gp or the multidrug transporter, which is an energy-dependent drug efflux pump. This was one of the first members of ATP-dependent transporters known from the ATP-binding cassette (ABC) family [41]. These proteins have a role in the efflux of drugs and also in moving nutrients and other biologically essential molecules inside, outside, and across the plasma membranes and intracellular membranes of cells. P-gp expression occurs in many human cancers, including cancers of the gastrointestinal (GI) tract (small and large intestine, liver and pancreatic cancer), cancers of the hematopoietic system (myeloma, lymphoma and leukemia), cancers of the genitourinary system (kidney, ovary and testicle), and childhood cancers (neuroblastoma and fibrosarcoma) [42].

P-gp can identify and attach to a range of hydrophobic drugs as well as antiarrhythmics and antihistamines, cholesterol-lowering statins [43] and HIV protease inhibitors [44]. When these drugs bind to the receptors, they activate one of the ATP-binding domains of P-gp, resulting in ATP hydrolysis. This causes a conformational change in the structure of P-gp and, as a consequence, the drug is released into the extracellular space [45]. After this, hydrolysis of a second molecule of ATP occurs, which brings P-gp back into its original state so that it can go repeat the cycle of drug binding and release [46].

Numerous members of the ABC transporter protein family have a vital role in cancer cell resistance [47]. It has been shown that the expression of ABCB1, ABCC1 and other members of the ABC family has a direct effect on tumor drug resistance. For example, gliomas and tumor-derived endothelial cells that express ABCB1 and ABCC1 subfamily members have a poor survival rate [48]. Copper transporter-1 (CTR-1) has been shown to have a considerable role in cisplatin accumulation and its loss would confer a strong resistance to the drug [49]. In cisplatin-resistant cells, it is usually the decreased uptake relative to an increased expulsion that predominates. Higher concentrations of glutathione result in the expelling more readily of conjugations of cisplatin with glutathione from cells by the ATP-dependent glutathione S-conjugate export (GS-X) pump [50]. In addition, glutathione acts as an antioxidant and, thus, protects the cancer cells against the toxic effect of cisplatin [51].

Hypoxia

Hypoxic cells have a characteristic of decreased drug influx. Tumors are known to have an hypoxic and/or necrotic core and a proliferating outer shell of cells. Hypoxic cells accumulate lower amounts of therapeutically applicable doses of drug through the blood supply owing to the decreased oxygen and slowed cell cycle production. Drugs that achieve their end product via reactive oxygen species production, drugs that are cell cycle dependent and therapies that target rapidly proliferating cells are not useful in such cells. In addition, hypoxic cells also have active mechanisms to induce MDR, thereby increasing their survival probability [52]. HIF-1 formed under conditions of hypoxia binds to hypoxia-response elements (HREs), which upregulate the expression of the genes encoding angiogenic factors, such as VEGF, and glycolysis-related proteins; for example, glucose transporters 1 and 3 (Glut-1 and 3) and glycolytic enzymes. HIF-1 is a αβ heterodimer whose β subunit is not significantly changed by hypoxia and is constitutively expressed, whereas the levels of the α subunit

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increase under hypoxic conditions and degrade under normal conditions [53]. Thus, hypoxia in tumor cells results in the enhanced expression of HIF-1 and corresponding *HIF1A*, which promotes the formation of new blood vessels and tumor growth and proliferation by enhanced expression of angiogenic factors and glycolytic enzymes, leading to tumor resistance.

Xenobiotics

Xenobiotics often modify high-density apolipoprotein, which leads to enhanced hepatic drug elimination, decreasing its effective plasma concentration. Increased expression of drug-metabolizing enzymes (such as glutathione) in response to the presence of drugs results in deactivation of the drug by forming conjugates that are eliminated. For example, resistance to cisplatin cells results from the overexpression of dihydrodiol dehydrogenase [54]. Cytochrome p450 (CYP450) is a cluster of enzymes that change the chemical structure of drugs for their removal. Genetic mutations in CYP450 structure and expression lead to functional differences in drug absorption and elimination, which ultimately lead to the deactivation of internalized drugs [55].

Tumor suppressor genes

p53 is a cell cycle regulator that prevents the production of defective cells. The drugs that enhance DNA damage lead to p53-mediated cell death. Loss of p53 function enables the cells with damaged DNA to continue replicating and become resistant to DNA-damaging drugs. In a variety of tumors, p53 deletion has been reported to cause MDR [4]. Other genes, such as those encoding HRAS and bcl-2/Bax, have been shown to be present in increased amounts in MDR. In MDR cancer cell lines, the levels of the enzyme glucosylceramide synthase (GCS), which metabolizes ceramide to a glycosylated derivative, glycosylceramide, is high. These enzymes exert their effect by diminishing the proapoptotic function of ceramide; therefore, decreasing their intracellular levels might aid in diverting MDR cancer cells to apoptosis [56].

Multifunctional nanoparticles for overcoming drug resistance

The aim of multifunctional nanoparticles is to attain multiple outcomes using one delivery system. Multifunctional nanoparticle delivery system deals with three factors: (i) enable specific targeting and aid in uptake through surface modification; (ii) bypass rapid RES clearance, thereby enabling prolonged circulation and higher uptake; and (iii) loading of higher concentrations of multiple therapeutic agents that can override MDR and result in a therapeutic effect [57]. p53 mutations were one of the first markers of drug resistance in cancer cells. The original role of p53 was the suppression of tumor growth, but over a period of time, cancer cells became able to cause p53 mutations, leading to resistance of therapeutic options. Nanoparticles in the form of PLGA have been used to deliver wild-type p53 DNA into cancer cells. This delivery resulted in sustained antiproliferative activity, proving its potential role in cancer treatment [58].

Inhibition of drug resistance proteins through use of nanoparticles

Liposomes coupled to transferrin directed to the cancer cells that express increased levels of the transferrin receptor, have been utilized for the co-encapsulation of doxorubicin (Dox) and verapamil (Tf-LDox/VER) and this formulation resulted in increased cytotoxicity in drug-resistant human erythroleukemia K562 cells [59]. In another study, nanoparticles comprising anionic surfactant sodium bis(2-ethylexyl) sulfosuccinate, also known as aerosol OT (AOT), and an anionic polysaccharide alginate were exploited as carriers for the concurrent delivery of Dox and the P-gp inhibitor methylene blue, resulting in increased cytotoxicity in drug-resistant NCI/ADR-RES [60].

Polymeric nanoparticles escaping drug efflux pumps

A new polymeric micelle comprising two block copolymers of poly(L-lactic acid)(PLLA)-b-poly(ethylene glycol), (PEG)-b-poly(L-histidine)-TAT and poly(L-histidine)-b-PEG laden with Dox has been considered to transport anticancer drugs more efficiently. Experimentation with this nanovehicle on tumor xenografts of various drug-resistant tumor cells, including ovarian, breast, non-small cell lung, as well as nasopharyngeal carcinoma, resulted in significant regression in the size of all tumors following treatment [61]. An acidic pH-activated mechanism that disrupts the endosomal membrane upon receptor-mediated endocytosis liberating nanoparticles carrying drug has also been described. For example, a mixed polymeric micelle system comprising poly histidine (His)-co-phenylalanine (Phe)-b-PEG and PLLA-b-PEG-folate, carrying Dox and conjugated with FA, have been efficiently targeted to FA-overexpressing carcinomas. These micelles resulted in successful destruction of both sensitive and drug-resistant human ovarian cancer cells (A2780/DoxR) [62].

Iron oxide nanoparticles

Metallic nanoparticles that dock antitumor agents have been examined as a new approach to escape drug efflux pumps. These include magnetic Fe_3O_4 nanoparticles (MNPs) [63] and Au nanoparticles. Fe_3O_4 superparamagnetic nanoparticles are biocompatible and could be exploited for the tissue-specific discharge of therapeutic agents and magnetic field-assisted radionuclide therapy [64]. Hence, metallic nanoparticle systems were considered as possible modalities to conquer Dox resistance in drug-sensitive K562 cells, whereas the Au nanoparticles proved effective against drug-resistant lung cancer cells [65]. The inclusion of a chemosensitizer inside the drug-loaded metallic nanoparticles had an improved therapeutic effect. A natural compound, tetrandrine (Tet), which is a bisbenzylisoquinoline alkaloid extracted from a Chinese herb commonly called as *Stephania* root, has proved to be a potential chemosensitizer of tumors that overexpress P-gp [66]. Fe_3O_4 MNPs laden with Dox and Tet resulted in reversal of resistance in leukemia cells.

Specific silencing of Bcl2, HIF1α and survivin by nanoparticles

Mesoporous silica nanoparticles (MSNs) have been used for the concurrent release of Dox and Bcl2 siRNA [67]. Dox-rich MSNs tailored with amine-terminated polyamidoamine (PAMAM) dendrimers attached to Bcl2 siRNA successfully overpowered the MDR phenotype (A2780/AD cells), probably by an inhibitory action of these PAMAM dendrimer-based nanoparticles on P-gp-mediated drug efflux. Multihydroxylated metallofullerene nanoparticles, represented as $[Gd@C_{82}(OH)_{22}]_n$ were filled with the DNA-alkylating

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agent cisplatin [2], which attaches and stimulates crosslinking of DNA, thereby activating cancer cell death through apoptosis. Introduction of cisplatin-resistant human prostate cancer cells (CP-r PC-3-luc) to cisplatin in the presence of $[Gd@C_{82}(OH)_{22}]_n$ nanoparticles displayed reduction in the growth of cisplatin-resistant tumors *in vivo* and diminished the continued existence of drug-resistant cells as depicted by both optical imaging and magnetic resonance imaging (MRI) [64].

Survivin is a negative regulator of apoptosis and is normally expressed in increased amounts in MDR cancer cells; therefore, multiple treatments concentrate on downregulating the expression of survivin [38]. One such system used PAMAM dendrimer-modified magnetic nanoparticles to transport antisense survivin oligodeoxynucleotides (asODN) to a diverse number of cancer cells. These asODN PAMAM magnetic nanoparticles suppressed survivin mRNA and protein expression in the three cancer cell lines: two breast cancer cells lines (MCF-7 and MDA-MB-435) and liver cancer cells (HepG2). Delivery of siRNA against HIF-1α with pristine single-walled carbon nanotubes (SWCNTs) was achieved in pancreatic cell lines. Intratumoral administration of these nanoparticles significantly inhibited the activity of HIF-1α in the tumor cells [67].

Intracellular pH modification by nanoparticles

MDR cells are often associated with decreased pH. Various strategies focus on either altering the intracellular pH or on the use of pH-sensitive constructs to administer the release of therapeutic agents. Novel pH-responsive polymers, such as poly(β-amino ester) (PbAE, soluble below pH 6.5), administered into nanoparticles to localize the efflux of agents in the acidic cellular environment of tumors and acidic subcellular endosomal and/or lysosomal compartments have been developed [68]. For example, a nanoparticle formulation of poly(ethylene oxide) (PEO)-PbAE nanoparticles encapsulating paclitaxel has been studied in *in vitro* and *in vivo* models and is shown to elevate the intracellular (ovarian cancer cell; SKOV3) and intratumor levels of paclitaxel, resulting in increased cytotoxicity of paclitaxel as depicted by enhanced cell death *in vitro* and reduced tumor volume *in vivo* [69]. These PEO-PbAE nanoparticle systems and analogous pH-sensitive nanoparticulate drug transporters could drastically condense the adverse effects correlated with chemotherapy.pH-sensitive polymeric micelles formulated from the two block copolymers poly(L-histidine)-β-PEG-folate and PLLA-β-PEG-folate laden with drug have also been exploited to target MDR breast cancer cells at pH 6.8 [70].

Concluding remarks and prospects

In this review, we have highlighted the various factors that are involved in drug resistance, and the challenges that researchers face in designing therapeutic measures for cancer. One of the difficulties of using chemotherapy is the development of MDR tumor cells. The use of nanoparticles and related multifaceted drug delivery techniques is a possible way of overcoming drug resistance in tumors. Multifunctional nanoparticles have increased the expectations for cancer treatment, leading to early diagnosis and precise detection. This might enable researchers to not only improve the prognosis of patients with cancer, but also to keep track of the effectiveness of the therapy such patients. The imaging that can result from using magnetic nanoparticles can also help in their detection using MRI and CT scans. Thus, research based on nanoparticles to overcome drug resistance provides a clear opportunity to reverse the phenomenon of drug resistance to drug sensitivity.

Uncited references

Q4 [71,72,82].

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Q5

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