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Review

Protein and Peptide-based Nanoparticles as an Emerging Strategy to Tackle Cancer Drug Resistance

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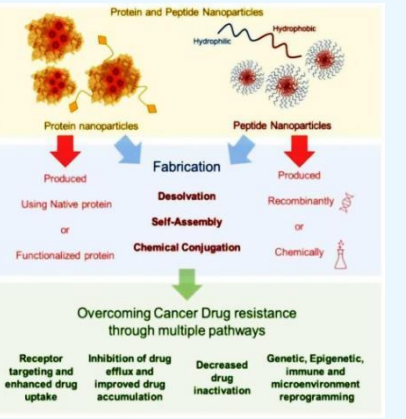
ABSTRACT: Cancer treatment has been confronted with serious challenges. These challenges stemmed from the heterogeneity of tumor microenvironment, as well as genetic and epigenetic factors related to cancer cells. The changes imposed by the tumor lead to the development of acquired resistance. Drug efflux, inhibition of apoptosis and immunosuppression are examples of resistance mechanisms imposed by cancer cells. Nanotechnology has sought to overcome these resistance mechanisms through enhanced cellular uptake, activation of alternative pathways by combined drug delivery, or targeting immune cells leading to their activation and proliferation. Several types of nanomedicine-based systems were implemented; however, protein and peptide nanoparticles remain one versatile tool in drug delivery, providing preferable physicochemical and biological implications. Notably, protein and peptide nanocarriers can also exhibit enhanced cellular uptake, the possibility of tuned drug release, and hence, better tumor targetability. Here, we present the methods of fabrication, physicochemical characteristics, biological mplications, and future perspectives, focusing on overcoming cancer drug resistance.



1. INTRODCTION

Cancer drug resistance represents a major hurdle, which may compromise the efficacy of administered chemotherapeutic agents [1]. The mechanisms of resistance to various therapies are encountered by an interplay between multiple factors [2]. Intrinsic factors, e.g., genetic and epigenetic aberrations, as well as extrinsic factors, such as variation in pH, hypoxia, and immuno-suppressive tumor microenvironment, are the main contributors to cancer drug resistance. Eventually, drug inactivation, drug efflux, resistance to apoptosis through shutting down apoptotic proteins, and suppression of immune cells are features of resistant tumors (**Figure 1**) [3, 4].

To address these limitations, nanomedicine-based delivery of therapeutics was investigated [5].

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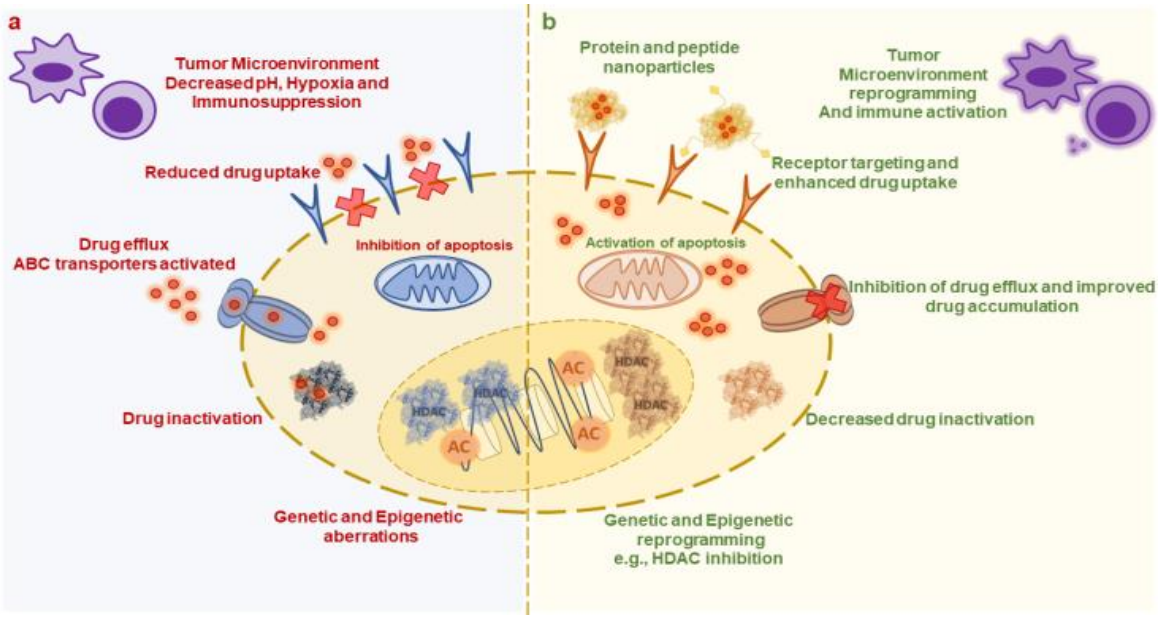

Figure 1. Mechanisms of cancer drug resistance (a), and implications of using protein nanoparticles in overcoming cancer drug resistance mechanisms exhibited by different tumor types (b).

The ultimate goal was to bypass theses resistance mechanisms through enhanced uptake and drug retention, combined drug delivery, and controlled drug release [6]. Several nanomaterials were investigated in order to overcome tumor resistance mechanisms, however, protein-based and peptide-based nanocarriers remain a safer, more biodegradable, and less immunogenic materials to be implemented [7]. The application of protein nanoparticles in the treatment in cancer treatment has been inspired by the FDA approval of Abraxane®, which is albumin-bound paclitaxel nanoparticles offering the advantages of overcoming the side effects of toxic adjuvants [8]. Furthermore, proteins and peptides are easily tuned and functionalized with other materials, which enhances active targeting potentials [9].

In this review, we presented the nanofabrication procedures of protein and peptide-based nanoparticles, with a special focus on the implications of these nano-systems in overcoming various pathways imposed by resistant tumors.

2. Albumin

Albumin is favored for the synthesis of nanocarriers, as it contains multiple drug binding sites, which allows the insertion of hydrophobic drugs. Furthermore, albumin nanoparticles can be produced through coacervation, controlled desolvation or emulsion formation. The two main types of albumin employed in nanoparticle preparation are human serum albumin (HSA) and bovine serum albumin (BSA) [10]. HSA is a globular plasma protein, consists of 585 amino-acids, and has a molecular weight of 66,500 Daltons (Da). On the other hand, BSA shows a molecular weight of 69,323 Da. BSA advantages include its lower cost compared to HSA and higher abundance together with its easier purification.

However, BSA may show relatively higher immunogenic reactions compared to HSA [3].

2.1. Preparation Methods of Albumin Nanoparticles

2.1.1. Emulsification

This technique has been widely implemented for synthesis of polymeric nanoparticles. There are two methods to stabilize albumin nanoparticles produced by emulsification method: (1) chemical or (2) thermal stabilization. Formation of albumin nanoparticles was conducted by homogenizing the oil phase. For example, cotton seed oil, which contained the albumin droplets, was heat stabilized at 175˚C to 180˚ C for 10 minutes [11]. Then the mixture was cooled and diluted with ethyl ether to reduce the oil viscosity for easier separation through centrifugation. Alternatively, chemical method was implemented by dissolving albumin in an aqueous solution, which was emulsified in cottonseed oil at 25˚C. Then, emulsified albumin was denatured after resuspension in ether containing 2,3-butadiene or formaldehyde as cross-linking agents [10].

2.1.2. Desolvation

In this process, albumin nanoparticles were prepared by virtue of phase separation through using ethanol or acetone. An aqueous solution of albumin was added in a dropwise manner to ethanol which allows the precipitation of albumin by phase separation due to its decreased water solubility. Then, a crosslinking agent, e.g., glutaraldehyde is added to ensure the stabilization of albumin nanoparticles through the interaction of albumin amino acids with the aldehyde groups of glutaraldehyde [10].

2.1.3. Thermal Induced Gelation

Thermal gelation is a process of using heat to induce aggregation by means of unfolding of the albumin through heat then protein-protein interactions occur such as hydrogen bonding, electrostatic, hydrophobic interactions, and disulfide-sulfhydryl interchange [10, 12]. Doxorubicin inclusion in albumin nanoparticles was obtained through Bovine Serum Albumin (BSA)-dextran and chitosan heating, which formed a gel like core of the nanoparticles that

includes trapped chitosan, chains, because of an electrostatic attraction force occurring between chitosan and Bovine Serum albumin (BSA). Finally, doxorubicin was included after adjustment of the solution pH to 7.4 [13].

2.1.4. Self-assembly

Self-assembly requires the utilization of a hydrophilic part of albumin such as a primary amine group and conjugation of a hydrophobic material. This strategy will allow albumin to form polymeric micelles after increasing its hydrophobicity [14]. Xu et al prepared self-assembled albumin- drug conjugate where the inner core of albumin nanoparticles contained doxorubicin which was conjugated with albumin via disulfide bonds [14].

2.1.5. Nanoparticle Albumin-bound Technology (Nab-technology)

Nab- technology is based on emulsion evaporation cross-linking method. Aqueous solution of HSA was pre-saturated with 1% chloroform. Then, an oily phase was added dropwise on the aqueous phase which led to the formation of an emulsion, which was exposed to a low shear forces; mild homogenization. Later, a homogenizer was used on the crude emulsion at a high speed and pressure which led to the formation of nanosuspension of albumin nanoparticles, which was obtained by removal of the solvent. Furthermore, ultra-filtration was applied for purification, and to eliminate any contaminant. To improve the stability of nab-paclitaxel, lyophilization was employed to obtain solid powder [15].

2.2. Albumin Nanoparticles Against Cancer Drug Resistance

The following Table 1 summarizes examples of albumin nanoparticles which were used to overcome cancer drug resistance through increased drug uptake, using drug combinations, and overcoming high expression of ATP-binding cassette (ABC) transporters, namely multidrug resistance protein 1/P-gp/ABCB1 (MDR1), as well as multidrug resistance associated protein 1/ ABCC1 (MRP1), which allow the expulsion of anticancer drugs and block them from entering the tumor cells [16].

2.2.1. Breast Cancer

The most predominant cancer among women is breast cancer. Unfortunately, breast cancer has many ways to limit the effects of chemotherapeutic drugs through multidrug resistance against the treatment used for it. One of these major pathways is drug efflux where the primary mechanism was found to be the increasing production of the ATP-Binding Cassette (ABC) transporters which expels the drug from cancer cells. There are many MDR mechanisms such as P-glycoprotein and the ATP-Binding Cassette (ABC) transporters which are related to multidrug resistant cancer cells and their expression hinders the drug uptake inside tumor cells and lower the anticancer effect of most of anticancer drugs [17, 18].

Here, doxorubicin, which is a chemotherapeutic drug, is used in breast cancer treatment. Yang et al. developed a human serum albumin (HSA) nanoparticle which includes doxorubicin with a mean diameter of about 174 nm and it showed sustained release behavior. The uptake of doxorubicin (DOX) was increased significantly due to the targeted delivery with cetuximab. The nanoparticles led to decreased mRNA expression levels of the multidrug resistance protein 1 (MDR1) and P-glycoprotein (P-gp), in a DOX-resistant MCF-7 human breast cancer cell line (MCF-7/ADR) [17]. Additionally, the co-delivery of doxorubicin (DOX) with cyclopamine (CYC) in BSA nanoparticles was achieved with a diameter of about 150 nm. CYC showed a synergistic effect with doxorubicin by reversing its resistance in MDA-MB-231 breast cancer cell line, which led to increased intracellular accumulation of doxorubicin that resulted by down-regulation of P-glycoprotein (P-gp) expression [19].

2.2.2. Pancreatic Cancer

Recently, gemcitabine (GEM) was combined with albumin nanoparticles bound to paclitaxel (nab-PTX), which highly improved the efficacy in patients having metastatic PDAC vs. gemcitabine monotherapy. Nab-PTX treatment of GEM-resistant PDAC was enhanced by: (1) the albumin transporter protein, caveolin-1, can be upregulated by gemcitabine; (2) multistage nano-vectors (MSV) that increased retention of nab-PTX in the tumor [20]. Moreover, gemcitabine was included into albumin nanoparticles with a diameter of about 150 nm. The formulation was found to be effective in overcoming gemcitabine-resistance induced by multi drug resistance protein 1 (MDR1) and multidrug resistance associated protein 1/ ABCC1 (MRP1) overexpression, possibly due to consumption of the ATP needed by the efflux pumps by albumin component of the nanoparticles [16].

2.2.3. Colon Cancer

A facile and an easy way to overcome colorectal cancer is through combination therapy which overcomes multidrug resistance. Zhao et al. developed a combination of disulfiram / copper complex with regorafenib in albumin nanoparticles with a diameter of about less than 145 nm. The combination of drugs was highly efficient to hinder the proliferation of drug resistant tumor cells. The implications imposed by the combination therapy by albumin nanoparticles included: increased levels of reactive oxygen species (ROS), induction of autophagy and apoptosis, and more prominently, overcoming resistance of the tumor microenvironment through repolarization of the tumor-associated macrophages (TAMs) from type M2 to M1 [21]. Additionally, Chen and co-workers developed bovine serum albumin (BSA) nanoparticles loaded with a combination of doxorubicin and verapamil through self-assembly with a mean size of 50 nm. The combination was found out to have an enhanced intracellular permeation of doxorubicin mediated through the inhibition and blockade of efflux proteins by verapamil [22].

Table 1. Albumin nanoparticles implemented for overcoming cancer drug resistance

Drug	Particle Size (nm)	PDI	Method	Type of Cancer	Type of Cancer Drug Resistance Overcome	Outcome	Ref
Human Serum Albumin (HSA)							
Doxorubicin (DOX)	173.57 ± 1.30	0.027 ± 0.004	Desolvation-crosslinking method	Breast Cancer	Overexpression of P-glycoprotein (P-gp)	Increased uptake of Doxorubicin and death of the breast tumor cells	**[17]**
Docetaxel (DTX)	< 200	0.31	Micro emulsification technique	Breast Cancer	Overexpression of P-glycoprotein (P-gp)	Reduction of the tumor burden by 80%.	**[18]**
Docetaxel (DTX)	100-400	< 0.2	Crystallization with an antisolvent	Three cancer cell lines (In vitro)	Overexpression of P-glycoprotein (P-gp)	Albumin nanoparticles enhanced the targeting of docetaxel towards the multidrug-resistant NCI/ADR-RES tumor cells.	**[23]**
Doxorubicin (DOX)	496.4	0.213	Desolvation	Neuroblastoma	transporter-mediated drug efflux	Inhibition of the ABCB1-mediated drug efflux.	**24]**
Paclitaxel (PTX) and 2-methoxyestradiol (2-ME)	180± 12.31	N/A	Solvent evaporation	PTX-resistant esophageal cancer	transporter-mediated drug efflux	2-methoxyestradiol (2-ME) reversed the drug resistance towards Paclitaxel (PTX)	**[25]**
Doxorubicin (DOX)	340	N/A	Self-assembly method	Lung cancer	Extensive uptake by lung macrophages	Albumin nanoparticles enhanced the delivery of Doxorubicin to the pulmonary tumor cells	**[26]**
Gemcitabine (GEM)	150 ± 27	N/A	Nano-binding technology	Pancreatic cancer	Overexpression of ABC transporters as MDR1 and MRP1	GEM-HSA-NP overcame the overexpression of MDR1 and MRP1	**[16]**
Doxorubicin (DOX)	N/A	N/A	S-nitrosylation	Leukemia	Overexpression of P-glycoprotein (P-gp)	Poly-SNO-HAS enhanced the doxorubicin anticancer effects through reducing the expression of P-glycoprotein	**[27]**

Drug	Particle Size (nm)	PDI	Method	Type of Cancer	Type of Cancer Drug Resistance Overcome	Outcome	Ref
Bovine Serum Albumin (BSA)							
Disulfiram/copper complex (DSF/Cu) and regorafenib (Rego)	< 145	N/A	Self-Assembly	Colon tumor cells	Non-uniform drug uptake and low concentrations of the anticancer drug at the tumor	Inhibition of cell growth of HCT8/ADR cells	**[21]**
Doxorubicin (DOX)	50	0.23	Desolvation	Invitro Cancer Cell lines	Overexpression of P-glycoprotein (P-gp)	MOLT-4 and MES-SA/DX5 cell lines proliferation inhibition	**[28]**
Paclitaxel (PTX) -Doxorubicin (DOX)	170–340	< 0.2	Ultrasonication and dialysis	Breast Cancer	Overexpression of P-glycoprotein (P-gp)	Inhibition of P-glycoprotein (P-gp) activity	**[29]**
Doxorubicin hydrochloride	50	N/A	Facile self-assembly method	HCT-15 cells	Overexpression of P-glycoprotein (P-gp)	Enhancement of the anticancer effect of DOX/VER/BSA nanoparticles.	**[22]**
Doxorubicin (DOX) and cyclopamine (CYC)	151	0.206	Self-assembly process of BSA under heating conditions	Breast cancer	Overexpression of ABC proteins as P-gp, BCRP and MRP1	Down-regulation of the expression of P-gp which led to enhancement of doxorubicin accumulation	**[19]**
Trichosanthin (TCS) protein and albendazole (ABZ)	98±1.65	0.18±0.01	Self-assembly	A549/T and HCT8/ADR cells	Caspase 9 phosphorylation and overexpression of P-glycoprotein (P-gp)	rTL/ABZ@BSA/Ag NPs led to inhibition of phosphorylation of caspase 9 and downregulation of P-gp	**[30]**
Paclitaxel (PTX)	< 180	N/A	Lipid film hydration method	Breast Cancer	Overexpression of P-glycoprotein (P-gp)	TLA/PTX@CS enhanced the paclitaxel drug concentration in the tumor cells	**[31]**
Paclitaxel (PTX)	20–50	N/A	(Albumin bound) nAb-PTX Particles injectable suspension was loaded into lyophilized MSV	pancreatic ductal adenocarcinoma	Low drug accumulation and reduced cav-1 expression	Enhanced accumulation of nAb-PTX in the cancer cells	**[20]**

Cet: Cetuximab; HSA: Human Serum Albumin; DOX: Doxorubicin; MDR1: multidrug resistance protein 1; siRNA: small interfering Ribo Nucleic Acid; P-gp: P-glycoprotein; SLNs: Solid Lipid Nanoparticles; DTX: Docetaxel; SA-SLN: Stearic acid based Solid Lipid Nanoparticles; NCs: Nano Crystals; DTX-F-alb: albumin-coated Docetaxel nanocrystals; NCI/ADR-RES: human multidrug-resistant ovarian cells; F127: Pluronic; ABCB1: ATP binding cassette subfamily B member 1; ATP: Adenosine Tri Phosphate; PTX: Paclitaxel; 2-ME: 2-methoxyestradiol; EC109: human esophageal cancer cell line; EC109/Taxol: Paclitaxel resistant cells; MDR: multidrug resistance; PLGA: Poly Lactic-co-Glycolic Acid; GEM: Gemcitabine; GEM-HSA-NP: gemcitabine-loaded human serum albumin nanoparticle; ABC: ATP Binding Cassette; MRP1: multidrug resistance associated protein 1/ ABCC1; Poly-SNO-HSA: poly-S-Nitrosated Human Serum Albumin; cGMP: cyclic Guanosine Monophosphate; DSF: Disulfiram; Cu: Copper; Rego: regorafenib; HCT8/ADR: drug-resistant human colon tumor cells; MOLT-4: human acute lymphoblastic leukemia cell line; MES-SA/DX5: human uterine sarcoma with P-glycoprotein; Dox-DBSA-NPs: Doxorubicin loaded Doughnut-shaped Bovine Serum Albumin Nanoparticles; HCT-15 Cells: human colorectal adenocarcinoma cells with inherent P-glycoprotein overexpression; BSA: Bovine Serum Albumin; VER: Verapamil; CYC: Cyclopamine; BCRP: Breast Cancer Resistance Proteins; MDA-MB-231 cell line: Breast cancer cells; TCS: Trichosanthin; ABZ: Albendazole; HCT8: Human Colon Cancer Cell Line; A549: Human Non-Small Cell Lung Carcinoma Cells; A549/T and HCT8/ADR: Drug Resistant Tumor Cells; ABZ@BSA/Ag NP: Albendazole encapsulated in the bovine serum albumin-coated silver nanoparticles; rTL: low-molecular-weight protamine; TPGS: α-tocopherol polyethylene 1000 glycol succinate; CS: chondroitin sulfate; TLA/PTX@CS: TPGS and CS dual-modified lipid-albumin nano system loaded with chemotherapeutic drug paclitaxel; MCF-7/MDR: multidrug-resistant MCF-7 human breast cancer cell line; CD44: cell surface adhesion receptor; PDAC: Pancreatic Ductal Adenocarcinoma; nAb-PTX: albumin-bound paclitaxel; MSV: multistage nanovectors; cav-1: caveolin-1

3. Lactoferrin

Lactoferrin (Lf) is a large reddish pink whey protein that consists of two globular lobes called N-lobe and C-lobe. Both lobes are composed of 700 amino acids, which are stabilized by disulfide bonds and connected with a flexible alpha helix [32-34].

3.1. Preparation Methods of Lactoferrin as a Carrier for Drug Delivery

3.1.1. Sol-in oil emulsion

Lactoferrin nanoparticles can be prepared by mixing the required drug with an aqueous solution of the lactoferrin protein along with oily phase. The drug becomes adsorbed onto the proteins forming aggregates, which can be dissociated by sonication. Finally, the mixture is set to cool in order to precipitate, resulting in the formation of solid protein-drug nanoparticles. Although this technique might have many side effects on the integrity of the protein structure, due to sonication and the harsh process of removal of the oil from the mixture, it gives us advantageous characteristics for the nanoparticles. They include small size of the nanoparticles of around 80 nm, a great drug loading capacity that reaches up to 50% drug loading, and the nanoparticles show a good pH-responsive abilities, as it has a higher rate of release at a pH of around 5.5, which enhances targeting of tumors [35-38].

3.1.2. Lactoferrin-drug Nanoconjugates

Lactoferrin chemical structure allows it to be able to form conjugates with hydrophobic moieties or molecules. Since lactoferrin has hydrophilic properties, it is used to enhance the solubility of hydrophobic drugs. Furthermore, conjugated molecules are driven by the self-assembly technique to form nano-conjugates or nano-micelles in aqueous solutions [39]. For instance, carbodiimide coupling was utilized for chemical conjugation of a chemotherapeutic drug, pemetrexed, with lactoferrin. Then, the coupled complex was connected to an aminated mesoporous silica nanoparticles that was loaded with a herbal drug, namely, ellagic acid [40].

3.1.3. Desolvation

Desolvation is one of the most favorable techniques for the preparation of lactoferrin nanoparticles, as it doesn't affect the stability and integrity of the protein structure since it does not require severe preparation conditions such as intense shearing or heat. Simply, within a specified pH, a miscible organic solvent with the required drug is added into an aqueous solution of lactoferrin. This leads to the presence of turbidity in the aqueous solution, which indicates the formation of nanoparticles. Then, glutaraldehyde was added as a cross-linking agent which allowed the nanoparticles to harden [41]. Optimization of the nanoparticles is mainly mediated through many factors, e.g., concentration of the protein, solvent ratio, temperature, sonication, flow rate, cross-linking agent, and pH. For example, as the temperature increases, this leads to unfolding of the protein particles, which exposes sulfhydryl groups, and forms cross-linking within the molecule itself (intra-crosslinking), and subsequently produces smaller nanoparticles [42].

3.1.4. Lactoferrin Shell-oily Core Nanocapsules

For the delivery of hydrophobic drugs, the polymeric oily core nanocapsule was used as it solubilizes these drugs and allows their controlled drug delivery. Mainly, poorly water-soluble polymers were used as Poly Lactic-co-Glycolic Acid (PLGA) and Polycaprolactone (PCL) for that shell formation in nanocapsules. In addition to the high cost of polymeric oily core nanocapsules, there are many concerns about their safety as there are many issues related to the formation of degradation acidic products and immune reactions [43]. Therefore, lactoferrin can be used as a shell-forming protein in the fabrication of oily core nanocarriers, by the virtue of electrostatic coating [39].

3.1.5. Electrostatic Nanocomplexes

In an aqueous solution, electrostatic interactions with lactoferrin can occur, as it possesses a positive charge due to its high isoelectric point (pI= 8.5) compared to most proteins, which have an isoelectric point of 5. Negatively charged polysaccharides can form electrostatic nanocomplex at room temperature, which was followed up by heating up to 92˚C and aggregation of protein molecules was allowed [44].

Lactoferrin implications can be represented either as nanoparticles or as a targeting material. Table 2 summarizes some of the examples of the use of lactoferrin in overcoming the cancer drug resistance.

3.2. Lactoferrin Used to Overcome Many Types of Cancers and Their Resistance

3.2.1. Glioma

The major challenge of treatment of gliomas is the presence of the blood brain barrier which allows a limited number of molecules to pass through it. Therefore, nanomedicine techniques can be implemented to overcome this obstacle which allows ease of drug penetration through the blood brain barrier in order to treat gliomas [45-49]. Temozolomide-lactoferrin nanoparticles were used to treat gliomas. The nanoparticles were characterized by having a small diameter of 70 nm and a polydispersity index of 0.24. Lactoferrin allowed the increase of the uptake of temozolomide which led to the killing of cancer cells and reduction of the tumor size. Thereby, overcoming the resistance occurred [35]. Xu et al. developed polysaccharide nanoparticles containing curcuminoid for treatment and targeting of gliomas, where the nanoparticles were characterized by a diameter between 210-240 nm. Lactoferrin coating led to enhanced drug uptake through blood brain barrier and increased drug accumulation in the brain [50].

3.2.2. Prostate Cancer

Development of bovine lactoferrin nanoparticles, which includes doxorubicin against resistant prostate cancer, showed significant apoptosis induction and overcoming of the p-glycoprotein efflux system. It was postulated that bypassing, as well as suppressing P-gp are the main effects that led to enhanced cytotoxicity of DOX by 4-fold. Lf, as carrier, was regarded as the suppressor of P-gp, as well as improving drug accumulation through enhanced uptake through receptor-mediated endocytosis **(Figure 2)** [51].

3.2.3. Breast Cancer

Lactoferrin-coated−DOX−mesoporous maghemite nanoparticles with a diameter of 130 nm increased the uptake of doxorubicin into the tumor cells which led to tumor growth inhibition and reduction of the size of the tumor.

It was demonstrated that the enhanced uptake posed by Lf was responsible for overcoming drug resistance by enhanced cellular uptake. However, in vivo application was still compromised with off-target accumulation of nanoparticles, e.g., in liver and spleen [52].

4. Zein

Zein is an alcohol-soluble maize protein and is mainly soluble in 70–80% aqueous ethanol. Then, it is dispersed into water in order to produce zein nanoparticles as a precipitate, which can encapsulate hydrophobic drugs for drug delivery [53]. Zein has gained a wide interest in using it as a carrier for drug delivery because of its: (1) biocompatibility, (2) biodegradability, (3) enhanced bioavailability and (4) stability [54, 55]. Zein was implemented as a nanocarrier for drugs in an attempt to overcome cancer drug resistance as shown in Table 3.

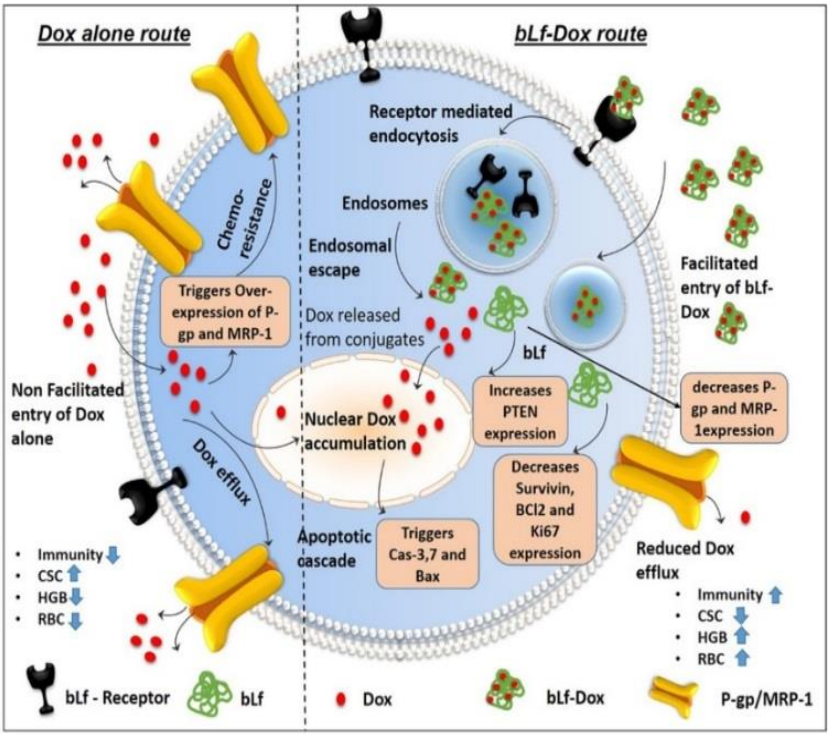

Figure 2. Implications of using DOX-loaded bovine lactoferrin nanoparticles in circumvention of cancer drug resistance through enhanced internalization, as well as decreased P-gp and MRP-1 expression, which improves the cytotoxicity of DOX. Moreover, bovine lactoferrin decreases the expression of P-gp, MRP-1, survivin and increases the expression of PTEN; hence, promoting apoptosis. The overall effect in vivo involves enhancing the anti-tumor immunity. Reprinted from Ref. [51].

Table 2. Lactoferrin-based nanocarriers to overcome cancer drug resistance

Drug	Particle Size (nm)	PDI	Method	Type of Cancer	Type of Cancer Drug Resistance Overcome	Outcome	Ref
Temozolomide (TMZ)	70 ± 10	0.24 ± 0.1	Sol-oil method	Glioma	DNA damage repair enzymes and poor drug uptake	Enhanced uptake of LfR and cytotoxicity on the GL261 cells	**[35]**
Fluorouracil (5-FU)	150 ± 20	0.332 ± 0.1	Sol-oil method	Malignant melanoma (B16F10) cell line.	Apoptosis resistance	5-FU-LfNPs showed an increase in melanoma cell cytotoxicity and enhanced retention in tumor cells	**[36]**
Etoposide (ETP)	< 200	N/A	Emulsification–diffusion method	Glioblastoma multiforme	Presence of the blood-brain barrier (BBB) which restricts the drug delivery	Nanoparticle enhanced the permeation and targeting of etoposide to the brain cancer cells.	**[56]**
Carboplatin (CPT) and Etoposide (ETP)	61.2 ± 3.94 for carboplatin-loaded NPs 45.15 ± 5.85 for etoposide-loaded NPs	N/A	Sol-oil method	Retinoblastoma (Rb) Y79 cells	Overexpression of P-glycoprotein (P-gp)	Sustained effect of the CPT and ETP-loaded Lf-Nps with enhancment of the intracellular uptake of the cytotoxic drugs.	**[57]**
Doxorubicin (DOX)	130 ± 1.48	N/A	Hydrothermal methods	Breast Cancer	Reduced drug uptake	Reduction of the tumor was a result of the production of ROS by LF-DOXO-MMNPs	**[52]**
Doxorubicin (DOX)	30	N/A	Sol-oil method	Glioblastoma (GBM)	Reduced drug accumulation at the tumor site	Enhanced permeation of the BBB (in vitro) and delivery of doxorubicin to brain tumors.	**[58]**
Curcuminoid (Cur)	210–240	0.095–0.101	Sol-oil method	Glioma cells	Reduced drug accumulation	Effective and improved targeting of gliomas through a combination of CSH,HA and Lf.	**[50]**

TMZ: Temozolomide; 5-FU: 5-Fluorouracil; LfR: Lf receptor; DNA: Deoxyribo Nucleic Acid; GL261: murine glioma cells; Sol: Solution; B16F10: malignant melanoma cell line; 5-FU-LfNPs: 5-FU loaded lactoferrin nanoparticles; BBB: Blood-Brain Barrier; ETP: Etoposide; CTP: Carboplatin; Rb: Retinoblastoma; P-gp: P-glycoprotein; Lf-Nps: Lactoferrin Nanoparticles; DOX: Doxorubicin; Lf-Doxo-MMNPs: lactoferrin−doxorubicin−mesoporous maghemite nanoparticles; DTX: Docetaxel; CST: Celastrol; DTX-LFCST: Docetaxel and Celastrol Lactoferrin dual drug nanoconjugate; MCF-7: human breast cancer cells ; : lactoferrin (Lf)-coupled mesoporous silica nanoparticles : Curcuminoid (Cur); curcuminoid; GBM :glioblastoma; CSH: Chitosan hydrochloride; HA: Hyaluronic acid; Lf: Lactoferrin.

4.1. Methods of Preparation of Zein Nanoparticles

4.1.1. Antisolvent Nanoprecipitation Technique

This method depends upon the precipitation and formation of the nanoparticles as a result of the induction of supersaturation. This is achieved by the addition of solute to the solution leading to the precipitation of the nanoparticles. Also, this method depends primarily on the protein solubility, pH of solvent, ionic strength and electrolytes present in the solution [59].

4.1.2. Liquid-liquid Dispersion Method

Liquid-liquid dispersion method depends on the differential solubility for zein in ethanol and water. Zein is soluble in alcohol, hence, after addition of water to alcohol, this leads to dilution of the alcohol concentration and induces a decrease in the solubility of zein and its precipitation in the form of nanoparticles [60].

4.1.3. Electrohydrodynamic Atomization Method

This method is also called electro-spraying, where it depends on the presence of an electric field to separate the liquid into charged molecules, where the solution moves through a metallic capillary or a needle. By adjusting the strength of the electric field, we can obtain many nanoparticles with multiple and different properties. This method is advantageous for producing nanoparticles with an increased drug encapsulation efficiency[61].

4.2. Examples for the Effect of Zein Nanoparticles on Different Types of Cancer Drug Resistance

4.2.1. Colorectal cancer

Frequent use of oxaliplatin, which is the main chemotherapy drug for colorectal cancer patients, is being limited due to the emerging resistance from cancer cells and harsh side effects such as peripheral neuropathy, hypersensitivity reactions and toxicity to bone marrow [62-65]. Liu et al. formulated zein nanoparticles with a diameter < 350 nm, loaded with a combination of curcumin and oxaliplatin. Effective synergism between the two drugs was achieved, where oxaliplatin induced CD44 expression, which increased the cellular uptake of the nanoparticles and enhanced the anticancer effects of curcumin. CD44-induced expression by oxaliplatin in HCT116 and HT29 cells was found to be related to the increased uptake of HZ-CUR nanoparticles. This mechanism was verified through the assay of the curcumin intracellular content after the inhibition of the expression of CD44, where the results showed significant reduction in the curcumin intracellular content in the HCT116 and HT29 cells. This showed that the endocytosis mechanism is important for the HZ-CUR nanoparticles uptake [66].

4.2.2. Hepatocellular Carcinoma

Lovastatin (LVS) incorporated into zein nanoparticles with a diameter of 67.2 ± 4.1 nm showed effective drug delivery towards the hepatocellular carcinoma cells and inhibited the proliferation of the tumor cells which was confirmed by morphological changes assessment of the cells. It showed superiority over lovastatin (LVS) alone in inducing apoptosis that was documented through caspase 3 assessment. The main mechanism of enhanced cytotoxicity was mediated by improved cellular uptake by zein nanoparticles. [67-70].

4.2.3. Prostate Cancer

Prostate cancer has been regarded as a metastatic and resistant type of cancer. Therefore, combination therapy was regarded to overcome hurdles that, compromise therapeutic outcomes in prostate cancer. In this regard, Histone deacetylase inhibitors could be implemented to induce apoptosis and autophagic cell death [71]. Therefore, vorinostat (Vor), a pan- histone deacetylase inhibitor [71], in combination with bortezomib (Bor) were coloaded in zein nanoparticles, prepared by phase separation method. These nanoparticles showed enhanced uptake, and more importantly, overcoming drug efflux mechanism through pH-controlled drug release in tumor cells [72].

5. Gelatin

Gelatin is a naturally occurring protein which can be collected through the collagen hydrolysis. Because of its biocompatibility, biodegradability due to presence of multiple functional groups and its low cost, gelatin became an attractive substance for nano drug delivery systems. It is composed of repeated triplets of alanine, proline and glycine amino-acids which are the main cause of the triple helical structure of gelatin. There are two types of gelatins (type A or type B) which mainly depends on the mechanism of hydrolysis of collagen, either through acidic or alkaline hydrolysis and each has a different mechanism of drug release for many nanoparticles [73-76].

5.1. Methods of Preparation of Gelatin Nanoparticles

5.1.1. Desolvation

This method depends on using a dehydrating agent such as alcohol or acetone to the gelatin aqueous solution which dehydrates the gelatin leading to changes in its conformational structure. Then, a cross-linking agent is used to harden the formed particles. Addition of another dehydrating (desolvating) agent might be needed to obtain uniformed sized and smaller nanoparticles. Also, another reported method is the one-step desolation process, where in this case, the pH of the gelatin is modified to reach the neutral values around 7.0 so that the gelatin molecules remain neutral and more susceptible to desolvation. The temperature for the process was set to 37°C, in order to ensure that the molecular weight of gelatin molecules is uniform [74, 77-80].

5.1.2. Emulsification-solvent Evaporation

The method depends on the preparation of a water-in-oil emulsion. The gelatin was mixed with a drug in the water phase of the emulsion. Then the aqueous phase was mixed with the oily phase which is composed of polymethylmethacrylate organic solution or paraffin oil. The process is followed by usage of a cross-linking agent [86-88].

Table 3. Selected examples for Zein as a nanocarrier for overcoming cancer drug resistance

Drug	Particle Size (nm)	PDI	Method	Type of Cancer	Type of Cancer Drug Resistance Overcome	Outcome	Ref
Paclitaxel (PTX)	189.1 ± 0.5	0.27 ± 0.01	Liquid–liquid phase separation method	Human breast adenocarcinoma (MCF-7 cells)	Reduced Drug Uptake	PTX-Loaded NPs showed increased uptake by the MCF-7 cells which led to their apoptosis.	**[81]**
Exmestane (EXM) and resveratrol (RVS)	141.4 ± 2.2	0.123 ± 0.01	Interfacial deposition technique	Human (MCF-7) and murine (4T1) breast cancer cell lines	Overexpression of P-glycoprotein (P-gp)	ZNCs showed marked reduction of the tumor burden compared to the free drug combination of EXM and RES.	**[82]**
Vorinostat (Vor) and bortezomib (Bor)	160	0.20	Phase separation method	Prostate cancer	Overexpression of P-glycoprotein (P-gp)	ZNP/VB showed prominent uptake by the prostate cancer cells which led to the induction of apoptosis.	**[83]**
Lovastatin	67.2 ± 4.1	N/A	Liquid–liquid phase separation	Hepatocellular carcinoma (HepG2 cells)	Reduced drug uptake by HepG2 cells	ZN nanoparticles led to the enhancement of the effects of lovastatin in inhibition of HepG2 cells proliferation.	**[70]**
Curcumin (Cur) and Oxaliplatin	< 350	< 0.35	Antisolvent coprecipitation method	Colorectal cancer cells(CRC)	Reduced drug uptake	HZ-CUR led to enhancment of the drug uptake and increased anticancer effects against CRC.	**[66]**
Doxorubicin (DOX)	200 to 250	0.15–0.20	Phase separation method	HeLa cells	Reduced drug uptake	DOX-Zein-NPs showed enhanced uptake and cytotoxicty against Hela Cells.	**[84]**
Honokiol (HNK)	210.4	N/A	Antisolvent precipitation and electrostatic deposition methods	Breast cancer cells	Reduced drug uptake	HA-Zein-HNK enhanced cellular uptake of HNK and cytotoxicty against the tumor cells.	**[85]**

HNK: Honokiol; PTX: Paclitaxel; EXM: Exmestane; RVS: Resveratrol; Cur: Curcumin; DOX: Doxorubicin; Bor: Bortezomib; Vor: Vorinostat; MCF-7 cells: Human breast adenocarcinoma; 4T1: breast cancer cell lines; HepG2 cells: Hepatocellular carcinoma; CRC: Colorectal cancer cells; PTX-Loaded NPs: Paclitaxel loaded nanoparticles; ZNCs: Zein Nano Capsules; ZNP/VB: Vorinostat and bortezomib-loaded zein nanoparticles; ZN: Zein; HZ-CUR: CUR-encapsulated hyaluronic acid–zein composite nanoparticles; HA: Hyaluronic Acid; HA-Zein-HNK: zein/hyaluronic acid core-shell nanoparticles loaded with honokiol; DOX-Zein-NPs: Doxorubicin loaded zein nanoparticles.

5.1.3. Nanoprecipitation

In this method, which is mainly described as a solvent displacement mechanism because of the solvent's miscibility, gelatin aqueous phase is mixed with ethanol which contains the drug, stabilizing agents, and crosslinking agents, where the nanoparticles are precipitated which shows how easy the method is to be performed. The method has many advantages such as being rapid, feasible to use, and the process is not complicated. Also, it does not need extensive shearing force, ultrasound vibrations (sonication) or even high temperature degrees. Moreover, the process does not have an oily-aqueous interface [89-92].

5.1.4. Self-assembly

There are two ways to form gelatin nanoparticles by means of self-assembly: (1) chemical modification; where gelatin is conjugated with multiple hydrophobic molecules in order to form a modified amphiphilic gelatin copolymer. This conjugate is capable of obtaining conformational changes when it dissolves in water leading to self-assembly as in the form of a micelle nanosphere, showing the aggregation of the hydrophobic moieties towards the core of the micelles, leaving the hydrophilic parts forming the outer layer of the micelles[93]. (2) Mixing; in this case, gelatin and the required drug solution are mixed directly, allowing the interaction between them via hydrophobic forces to take place, for instance, hydrogen bonding [94]. Table 4 demonstrates some of the examples of drugs formulated into gelatin nanoparticles to overcome cancer drug resistance.

5.2. Examples for the Use of Gelatin Nanoparticles to Overcome Various Types of Cancers

5.2.1. Breast Cancer

Amjadi et al. developed a co-drug delivery gelatin nanoparticle which delivered a combination of betanin (natural biological compound) and doxorubicin (a cytotoxic drug) towards the tumor cells. The nanoparticles had a diameter of around 160 nm. The potency of the nanoparticles was assessed through MTT assay which showed enhanced potency and cytotoxicity of doxorubicin as a result of the presence of betanin as a synergistic effect. Moreover, nanoformulation was superior to free drugs due to overcoming cancer drug resistance and elevated levels of cellular uptake and induction of apoptosis on the tumor cells [72].

5.2.2. Colon Cancer

Carboplatin gelatin-based nanoparticle of a diameter of 16 nm was found to be twice as effective as the free carboplatin on HCT 116 colon cancer cells. Also, the nanoparticles showed enhanced apoptotic activity and did not induce any drug resistance in colon cancer cells, as demonstrated by measuring MDR1 expression. The nanoformulation showed to be two-times more effective at a lower concentration than the free carboplatin which showed less side effects and massive therapeutic advantage [95].

6. Peptide- based Nanoparticles for Overcoming Cancer Drug Resistance

Peptides are a sequence of amino acids, which can be implemented as a versatile tool for anti-cancer drug delivery. They can be used as a monotherapy or functionalized with other materials. Furthermore, peptides can deliver various therapeutics either conjugated or loaded into a self-assembled peptide structure. Generally, there are two directions for designing a peptide. One method, a top-down technique, is obtaining the peptide sequence from a natural protein (structure-based design). Another method, a bottom-up technique, is employed by peptide library screening. Then, the peptide is constructed based on the expected interaction with desired target [96].

Synthesis of the peptide can be conducted through: (1) a biological method, using the recombinant DNA technology, and subsequently the production of the peptide from a prokaryotic or a eukaryotic host. (2) a chemical method, where the peptide is synthesized based on a reaction producing a peptide bond between amino acids. From all the chemical methods implemented, the most widely discussed are the ring- opening polymerization (ROP) of N- carboxyanhydride method, and the solid phase peptide synthesis (SPPS) [97]. The former method is usually sought for the synthesis of larger peptide sequences, however, the SPPS method can be implemented for synthesizing shorter peptide sequences [97]. The most reported methods of peptide nanoparticle synthesis are either through self-assembly of the peptide conjugates or through physical loading of the drug molecules within the amphiphilic structure of a peptide-amphiphilic block copolymer [97, 98]. Peptide- based nanoparticles have several implications in drug delivery as self-assembled nanocarriers, for imaging, and most importantly, to overcome cancer drug resistance as targeting or therapeutic peptides (Table 5).

6.1. Self-assembled Peptide Nanoparticles with Physically Loaded Cargos

To overcome cancer drug resistance on resistant breast cancer model, a pegylated self-assembling diblock copolymer comprised of a hydrophobic backbone as poly(phenylalanine) and a hydrophilic poly(histidine) was fabricated. This polypeptide was synthesized implementing the process of polymerization of the L-phenylalanine-N-carboxyanhydride (Phe-NCA) and His-N-carboxyanhydride (NCA) in a double step, where the pegylated histidine was first synthesized followed by the synthesis of the peptide block copolymer. The rationale for the polypeptide design followed the conception that histidine polypeptide is a pH-responsive peptide. This character would allow the histidine polypeptide to exhibit a protonation-deprotonation step, and therefore, create a pH- dependent buffering effect that would allow enhanced endosomal escape for intracellular drug delivery. This self-assembled triblock copolymer was loaded with doxorubicin (DOX) and quercetin (QRC) via dialysis method, where QRC was found to enhance the apoptotic potential of DOX. Free drugs combination was found to be less effective against the resistant type of breast cancer (MDA-MB-231), while the drug-loaded peptide nanoparticles showed an enhanced effect. Overcoming the resistant tumor was probably directed by more enhanced uptake and accumulation, bypassing the drug efflux pathways [103].

On another avenue, peptides can be synthesized in a branched manner, resembling dendrimers. These dendrimer- like peptides

can self-assemble and form supramolecular structures with hydrophobic cavities and generation- tuned hydrophilic dendrons. Therefore, hydrophobic cargos, e.g., chemotherapeutic agents, can be incorporated in the core of the assembled amphiphilic peptide dendrimer. In this regard, amphiphilic peptide dendrimers are synthesized comprised of hydrophobic C18 alkyl chains and hydrophilic polylysine peptides with different dendron generations. The self-assembled dendrimer was produced upon addition to an aqueous environment allowing the encapsulation of DOX, as a hydrophobic chemotherapeutic agent. The dendrimer showed enhanced cytotoxicity compared to free drug against DOX-resistant MCF-7 breast cancer model, due to enhanced cellular uptake and tumor accumulation [104].

6.2. Self- assembled Peptide-drug Conjugates

Instead of loading drugs physically into amphiphilic peptide micelles, hydrophobic drugs can be conjugated to the peptide backbone, imparting amphiphilic characteristics to the peptide. This method allows the peptide-drug conjugate to self-assemble into nanoparticles with the drug forming the hydrophobic core. This peptide could be further functionalized with other materials. For instance, Shim et al implemented a pro-apoptotic peptide (second mitochondria-derived activator of caspase; SMAC) to induce cancer cell death and overcome cancer drug resistance, which was demonstrated to be mediated by inhibitors of apoptosis proteins (IAP) [105, 106]. IAPs can induce cancer drug resistance through shutting down caspases in the cascade of apoptosis, however, SMAC directly interacts with IAPs and rectifies their action [106]. On the other hand, utilizing SMAC could be hindered by unfavorable stability and poor cellular permeability. To overcome these limitations, The peptide (SMAC) was conjugated to DOX through a spacer peptide, which is cleavable in response to the tumor upregulated cathepsin B. The peptide- drug conjugate (PD- conjugate) showed an enhanced cytotoxicity compared to free DOX against DOX- resistant MCF-7 cells, which was explained by: (1) enhanced uptake and cleavage of cathepsin B peptide, thereby, releasing both drugs at their site of action; (2) combinatorial drug delivery strategy, through direct inhibition of IAPs, due to the presence of SMAC in combination with DOX [107]. In another example, DOX was conjugated to a poly (aspartic acid) moiety (Asp8), which was further coupled to a diblock copolymer composed of a pegylated HIV-derived cell-penetrating peptide (TAT). The self-assembled structure included the DOX- Asp8 in the core, while the hydrophilic pegylated-TAT occupied the outer surface of the nanoparticles. Nanoparticles showed the ability to overcome cancer drug efflux mechanisms, which was mediated by P-gp overexpression. Also, intranuclear retention of DOX was enhanced. This effect was confirmed by introducing verapamil as an inhibitor of P-gp, where no difference was found between free DOX in combination with verapamil and DOX-NPs. On the other hand, the uptake and retention of DOX was compromised when administered as free drug [108].

Another example is a nanofiber consisting of a self-assembling peptide (Nap-GFFpYK) conjugated to etoposide via ester bond. The nanofiber showed an enhanced cytotoxicity by 20-fold compared to free drug, in the MDR1- overexpressing tumor cells. Additionally, apoptosis induced by nanofibers were 5-fold higher than free drug. These effects were attributed to nanofibers, demonstrating the ability of nanofibers to overcome cancer drug resistance, imposed by the upregulation of efflux genes. The mechanism of overcoming drug resistance is thought to be mediated by: (1) enhanced cellular uptake of nanofibers and (2) carboxylesterase- responsive release of etoposide, which decreases the rate of drug efflux out of tumor cells [109].

6.3. Hybrid Peptide-based Nanoparticles as Nanocarriers to Overcome Cancer Drug Resistance

Hybrid peptide-based nanoparticles are considered as another strategy implemented to overcome cancer drug resistance to other drugs or nano-systems. To address this concept, a DOX prodrug was constructed via coupling DOX with d-α-tocopherol polyethylene glycol 1000 succinate (TPGS). TPGS can act as a P-gp inhibitor. Therefore, TPGS-DOX (TD) conjugate coated with DSPE-PEG showed enhanced cellular uptake compared to free DOX, secondary to P-gp inhibition. However, it was demonstrated by Bao et al. that in spite of the ability of TDs to overcome drug resistance, decoration of the micelles with cyclic targeting peptide (cRGD), to produce hybrid nanoparticles, can enhance tumor accumulation of DOX, accounted by improved tumor penetration, by 2.15-fold more than TD NPs [110].

Tuned drug release may also play a pivotal role in overcoming drug resistance in tumor models. One way was by the fabrication of a hybrid polymeric-peptide nanoparticles encapsulating DOX. The polymer-peptide hybrid was comprised of monomethoxy (polyethylene glycol)-b-P (d,l-lactic-co-glycolic acid)-b-P (l-glutamic acid) (mPEG-PLGA-PGlu). Through controlling the length of poly (l-glutamic acid), the pH-sensitivity is determined depending on the ionization status, representing an on-off switch. Moreover, the biodegradability of PLGA and PGlu would enhance the NPs degradation in response to tumor enzymes. Thereby, this dual responsive behavior would allow endosomal escape, and inducing cytotoxic effects 2- to 3-fold higher than free DOX, due to enhanced uptake and retention imposed by NPs against DOX-resistant MCF-7 cells (**Figure 3**) [111].

Table 4. Examples of Gelatin nanoparticles reported to overcome cancer drug resistance

Drug	Particle Size (nm)	PDI	Method	Type of Cancer	Type of Cancer Drug Resistance Overcome	Outcome	Ref
Cisplatin/Epigallocatechin Gallate	75	0.15	Self-assembled	human lung adenocarcinoma cells (A549)	Drug Inactivation, efflux proteins and enhanced DNA repair	GE-Pt NPs enhanced cellular uptake of Cisplatin and show improved cytotoxicity against A549 cells.	**[99]**
wt-p53 plasmid and Gemcitabine	120-230	N/A	Desolvation	Pancreatic adenocarcinoma	Anti-apoptotic transcription factors.	Thiolated gelatin loaded nanoparticles showed enhancement in apoptosis induction and reduction in tumor burden.	**[100]**
Doxorubicin (DOX) and Betanin (BET)	162	< 0.2	Two-step desolvation method	breast cancer cells (MCF-7)	Poor drug uptake and antiapoptotic mechanisms	DOX@BET-PGNPs enhance the uptake and cytotoxicity of DOX on MCF-7 cells.	**[101]**
Carboplatin (CP)	16	0.607	Two step desolvation method	Colon Cancer Cells (HCT116)	Poor uptake of carboplatin and P-gp overexpression	CP-NPs-50 showed enhanced and selective cytotoxicity towards the tumor cells in comparison with the normal cells.	**[95]**
Etoposide	150 ±0.241	0.257	Two step desolvation method	Breast Cancer (MCF-7 Cells)	Antiapoptotic mechanisms and low drug uptake	EGNPs showed enhanced drug uptake which led to the death of MCF-7 cells	**[102]**

GE-Pt NP: EGCG/cisplatin-loaded gelatin nanoparticle; EGCG: Epigallocatechin Gallate; A549: human lung adenocarcinoma cells; wt-p53 gene: wildtype p53; DOX; Doxorubicin; BET: Betanin; PGNPs: methoxy poly (ethylene glycol)-poly ((2-dimethylamino) ethyl methacrylate-co-itaconic acid decorated Gelatin Nano Particles; MCF-7: Breast Cancer Cells; CP-NPs-50: CP-loaded gelatin-based NPs synthesized at 50°C; EGNPs: Etoposide loaded gelatin nanoparticles.

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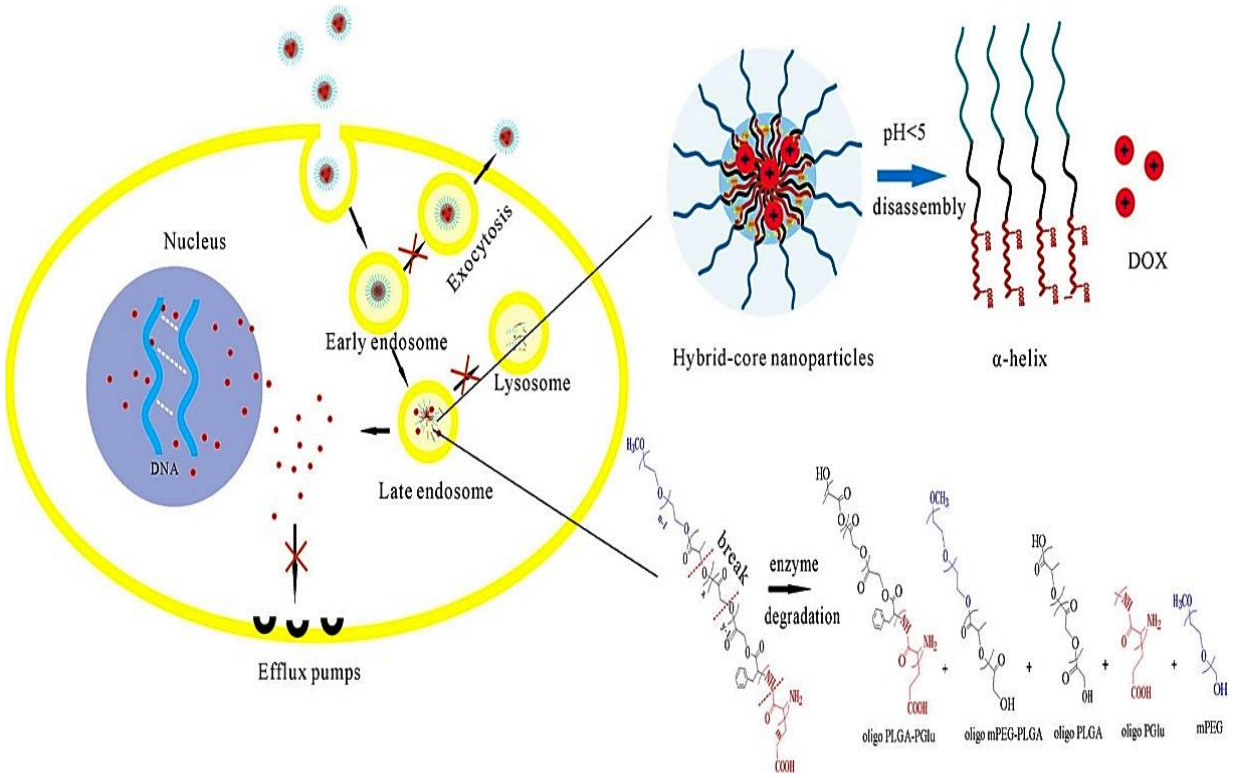

Figure 3. Tuned drug release through the fabrication of hybrid polymer-peptide nanoparticles. pH-sensitive release of DOX was controlled through manipulating the length of poly (glutamic acid) chain, which imparts an on-off switch characteristic. The hybrid nanoparticles represent a promising strategy to overcome cancer drug resistance secondary to enhanced drug accumulation and retention. Reprinted from Ref. [111]

6.4. Cell-penetrating Peptide-based Nanoparticles as Nanocarriers to Overcome Cancer Drug Resistance

Cell-penetrating peptides (CPP) are regarded as sequences of amino-acids, comprised of less than 30 amino-acids, which are either cationic or amphipathic. The charged nature of the CPP facilitates the internalization of their cargo into the cells without disrupting the cell membrane [112]. CPP showed several features that allow their tumor targetability. Specific tumor selectivity, as well as pH- responsiveness could be significantly attributed to the ability of CPP to enhance the cellular uptake of their cargo, overcoming cancer drug resistance. In this regard, Zhang et al demonstrated that a co-polymer comprised of polyethylene glycol and polyethyleneimine coupled through a disulfide bond (PEG-SS-PEI), and modified with CPP, can enhance the delivery of siRNA. In this work, PEG-SS-PEI NPs were modified with the pH-sensitive peptide made of repeated units of glutamic acid-alanine-leucine-alanine "GALA" and the triple-negative breast cancer (TNBC) selective peptide; cysteine-arginine-glutamic acid-lysine-alanine (CREKA). These NPs were complexed with siRNAs against epidermal growth factor receptor (EGFR) and growth-promoting bromodomain-containing protein 4 (BRD4). The functionalization procedure was conducted based on the reaction of a maleimide terminal of PEG with the mercapto group on the corresponding peptide. Following, the different ratios of the modified peptide-polymers were mixed with the siRNAs to induce the formation of the nanocomplex. Ultimately, NPs with combined CPPs showed enhanced cellular uptake (improved transfection efficiency) compared to either CPP alone, or free siRNA in MDA-MB-231 cell line. These results were translated in enhanced cytotoxicity, as well as the most effective gene silencing for EGFR and BDR4, which showed synergistic inhibitory action against MDA-MB-231 cell line [113]. In another attempt to enhance the selectivity of CPPs composed of multimers of leucine (L) of lysine (K) residues, which possess the capability to internalize in the cell at a much lower concentrations compared to the conventional CPPs, the addition of histidine residues enhanced – pH-responsive targeting of TNBC. LH2 showed improved cellular uptake at acidic conditions compared to LK2 peptide at very low concentrations reaching 10 nM. More importantly, simple mixing procedures forming peptide-drug complexes can induce synergistic antitumor activity in vitro, in addition to chemical conjugation strategy. These findings were demonstrated upon the formation of PTX-LH2 nano-rod complexes, which were effective in MDA-MB-231 mouse xenograft model at a 10-fold lower PTX dose [114].

Table 5. Selected examples of peptide-based nanoparticles in overcoming cancer drug resistance

Peptide	Drug(s)	Particle Size (nm)	PDI	Method	Type of Cancer	Mechanism of Cancer Drug Resistance Overcome	Outcome	Ref
Poly(phenylalanine)-b-poly(Lhistidine)-b-poly(ethylene glycol) (pPhe-pHis-PEG)	DOX and QRC	82	N/A	Solvent Exchange method	Breast Cancer (MDA-MB-231)	P-gp efflux	NPs-treated mice showed 3-fold smaller tumor volume than that of mice treated with free DOX/QRC.	**[103]**
Polylysine	DOX	AmPD KK2/DOX : 73 AmPD KK2K4/DOX: 80	N/A	Film Dispersion method	DOX resistant MCF-7R cells	Poor uptake and tumor accumulation	Enhanced cytotoxicity of the nanoformulatios against tumor spheroids compared to free DOX.	**[104]**
SMAC-FRRG-DOX	DOX	221.8 ± 15.9	0.274	Chemical Conjugation	DOX resistant MCF-7R cells	Poor tumor uptake and retention	Compared to free DOX, NPs led to enhanced DOX delivery by 6.8-fold in MCF-7 tumor bearing mice.	**[107]**
TAT-PEG-Asp_8	DOX	150	0.102	Chemical Conjugation	Drug-resistant human colon cancer HCT8/ADR cells	Poor drug uptake and drug efflux	4.3-fold higher uptake by nanoparticles compared to free DOX.	**[108]**
Nap-GFFpYK-etoposide	Etoposide	10	N/A	Chemical Conjugation	MDR1-overexpressing LLC/4T1 cells	P-gp efflux	Nanoparticles showed 20-fold enhancement in the cytotoxicity of etoposide.	**[109]**

DOX: Doxorubicin; QRC: Quercetin; NPs: Nanoparticles; SMAC: second mitochondria-derived activator of caspase; MDR1: multi-drug resistance protein 1.

7. Perspectives and Future Challenges

Proteins are attractive nanocarriers owing to their biocompatible characteristics. However, immunogenicity may be induced by some proteins derived from other non-human sources, for instance, bovine serum albumin [12]. Other expression systems that can be implemented to synthesize peptides through expression hosts can be immunogenic as well [115]. Moreover, employing organic solvents in the synthesis of protein or peptide nanoparticles may present a challenge in the toxicological profile of nanoparticles [116]. However, compared to other materials, proteins and peptides present a better choice for drug delivery applications. This is provided through the inherent targeting ability of proteins and peptides. Furthermore, the ability to functionalize these nanoparticles is considered a major advantage [10].

The therapeutic potential provided by peptides can offer advantages as monotherapy. Peptide nanoparticles, in particular, can overcome cancer resistance to radiotherapy. In this regard, a transformable peptide was synthesized linking an aggregation-induced emission moiety (AIE), a β-sheet-forming peptide domain, and a HER2 targeting peptide. The sequence was synthesized so that it can self-assemble in an aqueous solution, where the AIE and the β-sheet-forming peptide domain constitute the hydrophobic core and the HER2 targeting peptide forms the shell structure of the nanoparticles. Upon IV administration of the nanoparticles into tumor-bearing mice, the NPs were found to accumulate in the tumor. More importantly, the NPs can transform into a fibrillar structure. This structure can lead to the inhibition of HER2 receptor dimerization, and hence, its downstream signaling. Interestingly, the expression of

HER2 in breast cancer confers resistance to radiotherapy [117]. Thereby, the NPs showed an enhanced green fluorescence around the HER2+ MCF-7/C6 cell line, which displays five-fold more HER2 expression compared to the parent MCF-7 cells, conferring resistance to radiotherapy [85].

Genetically engineered peptides were also exploited for drug delivery and targeting applications. Elastin-like polypeptides in conjunction with poly (aspartic acid) moieties were produced secondary to transfection in E.Coli. The fusion protein also expressed a targeting peptide; iRGD. This fusion protein possessed the ability to self-assemble into micellar-like structure, accommodating PTX as a hydrophobic drug. Notably, integrins are overexpressed on resistant tumors and promote cancer progression[118]. The enhanced uptake exhibited by these nanoparticles can pave the way for such nanomedicine-based strategies to overcome cancer drug resistance by specifically targeting overexpressed moieties, e.g., integrins [119].

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Authors' contributions

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