EXPERT OPINION

informa healthcare

Nanocarrier systems for delivery of siRNA to ovarian cancer tissues

Eshrat Gharaei Fathabadi, Andrew N. Shelling & Raida Al-Kassas[†]
[†]*University of Auckland, School of Pharmacy, Faculty of Medical and Health Sciences, Auckland, New Zealand*

Introduction: Novel therapeutic strategies have been investigated for ovarian cancer to reduce toxicity and to improve outcomes for patients. Short interfering RNA (siRNA), which directs the sequence-specific degradation of target mRNA and provides specificity of gene knockdown, represents a unique class of potential therapeutics for ovarian cancer. However, siRNA molecules are rapidly degraded in plasma and are unable to passively diffuse through cellular membranes. Nanocarriers can efficiently protect siRNA from *in vivo* degradation and are able to deliver these active macromolecules to tumor cells even after intravenous administration.

Areas covered: Strategies of gene therapy and the role of siRNA in ovarian cancer treatment are introduced, followed by an overview of nanocarriers for siRNA delivery, the advantages of the systems and the types of targeting to tumor cells. Classes of nanocarriers for delivery of siRNA, their functionalities and modalities are discussed with emphasis on the promising vehicles.

Expert opinion: Gene silencing therapy based on siRNA represents a possible opportunity for treatment of ovarian cancer patients. However, this approach requires selection of suitable nanocarriers that can safely and effectively deliver siRNA to the target site to induce its effect. Very little work has been done in this field; therefore, it is a good direction for future development.

Keywords: nanoparticles, nonviral nanocarriers, ovarian cancer, siRNA delivery

Expert Opin. Drug Deliv. (2012) 9(7):743-754

1. Introduction

Over the last decade, the mapping of the human genome, along with improved understanding of signal transduction and the pathways responsible for tumor survival, has been transforming therapeutic oncology from more promiscuously targeted chemotherapeutic approaches toward more highly selective targeted therapeutics [1].

In ovarian cancer, the aggressive nature and poor prognosis associated with clinical presentation with advanced and recurrent ovarian carcinoma have led investigators to pursue alternative therapeutic strategies. Gene therapy is an alternative strategy in ovarian carcinoma that has shown promise in preclinical models and in Phase I trials [2]. Until this date, the majority of siRNA nanocarriers in clinical trials were designed for the treatment of various types of cancer; however, none of them is for targeting ovarian cancer [3].

2. Ovarian cancer

The various histological subtypes of ovarian cancer include serous, mucinous, clear cell, endometrioid, transitional cell/Brenner tumors and undifferentiated adenocarcinomas; these are different in their etiology and genetic abnormalities [4]. It is clear that epithelial ovarian cancer have highly aneuploid cells with multiple marker chromosomes and high heterogeneity both within and between individual cases [5].

10.1517/17425247.2012.683173 ISSN 1742-5247

Article highlights.

- Small interfering RNA (siRNA) is becoming as an innovative drug substance especially in the treatment of cancers.
- siRNA shows a promising approach for the treatment of ovarian cancer.
- Nanocarrier systems can be modified to be appropriate tools for the delivery of siRNA.
- In comparison with viral carriers that have been used for delivery of nucleic acids, nonviral carriers are much safer and more straightforward.
- Although great advances occurred in nonviral nanocarrier systems for delivery of siRNA, establishing an effective and efficient system is challenging.

This box summarizes key points contained in the article.

Ovarian cancer is a general term for a variety of distinct diseases that just happen to share a similar common location that is the ovary [6]. In addition, it is becoming clear that the majority of ovarian cancers originate from outside the ovary; for example, serous tumors appear to arise from the implantation of epithelium from the uterine tube [7]. Likewise, endometrioid and clear-cell tumors may have arisen from preexisting endometriosis. To improve the classification of ovarian tumors, it has been suggested that they should be grouped as type 1 and 2, which have different steps of development. Type 1 ovarian cancers include the less frequent low-grade and borderline serous cancers, endometrioid, mucinous and clear-cell cancers, which are characterized by *PTEN*, *PIK3CA*, *KRAS*, *BRAF, ERBB2* and *β-catenin* (*CTNNB1*) mutations but rarely involve mutations in *p53*, a commonly mutated tumor suppressor gene. Type 2 ovarian cancers include high-grade serous cancers, mixed malignant mesodermal tumors, carcinosarcomas and undifferentiated cancers [8], and mostly contain *TP53* mutations and also mutations in *NF1*, *BRCA1*, *BRCA2*, *RB1* and *CDK12* [9].

Ovarian tumors can also be classified according to the kinds of cells from which the tumor started (cell of origin). The cell types of a normal ovarian tissue include surface epithelium, stromal cells, thecal cells and granulosa cells surrounding the oocytes; each of these may be involved in ovarian tumors [10]. However, it does appear that the vast majority of what have been thought to be primary epithelial ovarian and primary peritoneal carcinomas are secondary and the majority of epithelial ovarian carcinomas originate outside the ovary [7].

In the current genomic era, clinicians and laboratory investigators may move away from conventional histological typing and move toward gene expression and proteomic profiling [11]. Gene expression assays are now used in daily clinical practice in the care of many patients who are newly diagnosed with breast cancer. The assay includes genes related to cell proliferation, invasion and several reference genes; however, in ovarian cancer, gene expression profiles have so far been used to examine differential gene expression patterns between normal and tumor cells as a way to distinguish between histological subtypes [12].

This means that gene expression microarray and proteomic profiling not only enhance our understanding about the disease but also facilitate screening the response of thousands of mRNAs and proteins to anticancer agents [13].

Ovarian cancer treatment currently comprises a combination of surgery and chemotherapy with carboplatin and paclitaxel [14]. The overall survival rates in ovarian epithelial cancer remained high in the past 25 years, in spite of improvements surgical techniques and chemotherapeutic agents. Therefore, identification of carcinogenesis mechanisms in this group of patients may lead to the better targets for treatment. A lot of evidence suggests that correcting genetic defects in cancer cells may inhibit cell growth and reverse tumorigenicity [15].

3. Nucleic acid therapy in ovarian cancer

Nucleic acid therapy strategies have been used to modulate the activity of genes implicated in the pathogenesis of ovarian cancer [14]. Nucleic acid-based therapies for ovarian cancer could be based on the following strategies:

Tumor suppression genes encode cellular proteins that negatively regulate cell growth. Loss of function of these genes by mutation has been shown to play a role in many malignancies. In ovarian cancer, *p53* gene mutation leading to loss of function is common. Therefore, reintroducing a wild-type *p53* gene could be a viable therapy [15].

Oncogenes are another class of genes targeted for gene therapy. When these genes are amplified and/or overexpressed, they cause uncontrolled cellular proliferation. For example, in ovarian cancer, *HER-2/neu* oncogene amplification and overexpression are frequently observed, leading to altered activity of an important growth-regulatory pathway. One of the most promising strategies for oncogene silencing is using interference RNA (RNAi) technology as will be explained in more detail in the next section.

Another direct gene therapy approach involves the transfer of viral 'suicide genes' into the genome of tumor cells. The introduction of a foreign gene enabling the transduced cell to metabolize and thus toxify a prodrug has been proposed for several years. Examples are the herpes simplex virus-1 derived thymidine kinase (hsv1-tk) or the *E. coli*-derived cytosine deaminase [16]. Delivery of diphtheria toxin gene is another promising approach for killing ovarian cancer cells [17].

Molecular chemotherapy and alteration of drug sensitivity are other goals for gene therapy [2]. In this method, the gene encoding an enzyme is delivered to tumor cells, followed by administration of a prodrug, which is converted locally to an active drug by the enzyme. The producer cells as well as surrounding bystanders are subsequently killed [18,19]. Currently gene therapy strategies are limited in their clinical utility partly due to poor transfection efficiency of nucleic acid carriers.

Silencing of gene expression by siRNAs is rapidly becoming a powerful tool for genetic analysis and represents a potential strategy for therapeutic product development [20]. One area of great interest is the use of siRNA to treat cancers where genes are upregulated in tumors; therefore, targeting them for knockdown could lead to changes in tumor growth rates and survival [21].

siRNAs are short (usually 21 – 25 nucleotides long), double-stranded RNA molecules with two nucleotide overhangs at the 3′-ends. In the cytoplasm, siRNAs are loaded into a protein complex called the RNA-induced silencing complex (RISC). The loaded RISC complex then scans all intracellular mRNA for a target mRNA with a complementary sequence to the loaded siRNA. If a target mRNA is found by the loaded RISC, the target mRNA is cleaved and degraded, successfully inhibiting the translation of the target gene [22].

Therefore, when siRNAs are introduced into cells, they mediate posttranscriptional gene silencing of a specific target protein by disrupting messenger RNAs (mRNAs) containing complementary sequences [23].

siRNA must be delivered intracellularly into the target cells to exert their silencing effects on target genes. Therapeutic applications of siRNA have been hampered by their instability, poor cellular uptake and the lack of efficient delivery methods. Therefore, the development of siRNAs as therapeutic agents requires the development of carriers that stabilize siRNAs and facilitate their uptake by target cells [24].

4. Nanocarriers

Nanoscaled carriers (nanocarriers) are increasingly being applied for drug delivery, allowing therapeutic agents to be selectively targeted to an organ-, tissue- and cell-specific level and minimizing exposure of healthy tissue to drugs [25]. Therefore, the main aims of using nanocarriers are minimizing drug degradation upon administration, increasing bioavailability and preventing undesirable side effects of the drug. Nanocarriers are also expected to be easily prepared, biodegradable and have high loading capacity for drug [26].

Nanocarriers can be made using a variety of materials including polymers (polymeric nanoparticles, micelles or dendrimers), lipids (liposomes), viruses (viral nanoparticles) and even organometallic compounds such as nanotubes [27].

4.1 Nanocarriers for delivery of siRNA

The accessibility of the target organ highly determines the method of siRNA delivery [28]. In ovarian cancer, the main method of delivery can be systemic via intravenous injection. This means that siRNA formulations for systemic application face a series of hurdles before reaching the cytoplasm of the target cell. Even after reaching the cytoplasm, there are other barriers to be overcome before the siRNA can become effective (**Figure 1**). Therefore, the optimal *in vivo* systemic delivery systems for siRNA should be biocompatible, biodegradable, non-immunogenic, provide efficient delivery of siRNA into target cells or tissues (avoiding rapid hepatic or renal clearance) with protection of the active double-stranded siRNA products from attack by serum nucleases (protection against degradation) and specifically direct siRNA to target cells (target tissue-specific distribution). Furthermore, it should facilitate its intracellular uptake, escape from endosome/lysosome into cytosol and finally promote efficient gene silencing (allowing the interaction of siRNA with the endogenous RISC) [11,29].

Delivery systems for siRNA can be classified into two main groups: viral and nonviral delivery systems both having their advantages and disadvantages. Viral delivery systems are mostly used to deliver precursor molecules to induce RNAi. There are five viral carriers commonly used for RNAi including retrovirus, lentivirus, adenovirus, adeno-associated virus (AAV) and baculovirus. A recent study used a lentiviral system for siRNA delivery in ovarian cancer [30]. The advantage of these systems is the high transduction efficacy, due to the inherent ability of viruses to transport genetic material into cells. However, there is a potential for mutagenicity or oncogenesis, and the high cost of production limit their application. For these reasons, different kinds of nonviral siRNA delivery systems have been tested to avoid viral vectors [31,32].

Nonviral vectors seem to be promising tools for gene delivery, because they are relatively safe and can be modified through the incorporation of ligands for targeting specific cell types [33]. For instance, by incorporation of a water-soluble polymer such as polyethylene glycol (PEGylation) into a carrier, it could be possible to modify the polymer/siRNA complex, which is a major strategy to decrease nonspecific interactions, prolong the residence time and target the nanocarrier to a specific organ [34,35].

4.2 Targeting by nanocarriers

Nanocarriers for siRNA delivery for the treatment of cancer may be applied in two ways: active targeting and passive targeting [36].

In passive targeting, the drug is loaded into a nanocarrier and reaches to the target organ passively [37]. It has been reported that microvascular transport of macromolecules bigger than 5 nm in diameter is significantly inhibited in normal tissues. However, tumor endothelium has leaky and discontinuous vascular structures and allows the penetration of high-molecular-mass macromolecules, which is referred to as enhanced permeation and retention effect [28,38]. Therefore, passive targeting consists of the passage of nanocarriers through leaky tumor capillary endothelium into the tumor tissue and cells by passive diffusion. The passive targeting depends on the degree of tumor vascularization and angiogenesis. Furthermore, it is said that larger and long-circulating nanocarriers (up to 200 nm) are retained longer in the tumor, whereas smaller molecules easily diffuse [28,38,39]. However, there is a controversy regarding the best size of nanoparticles for targeting cancer, because nanoparticles are usually taken

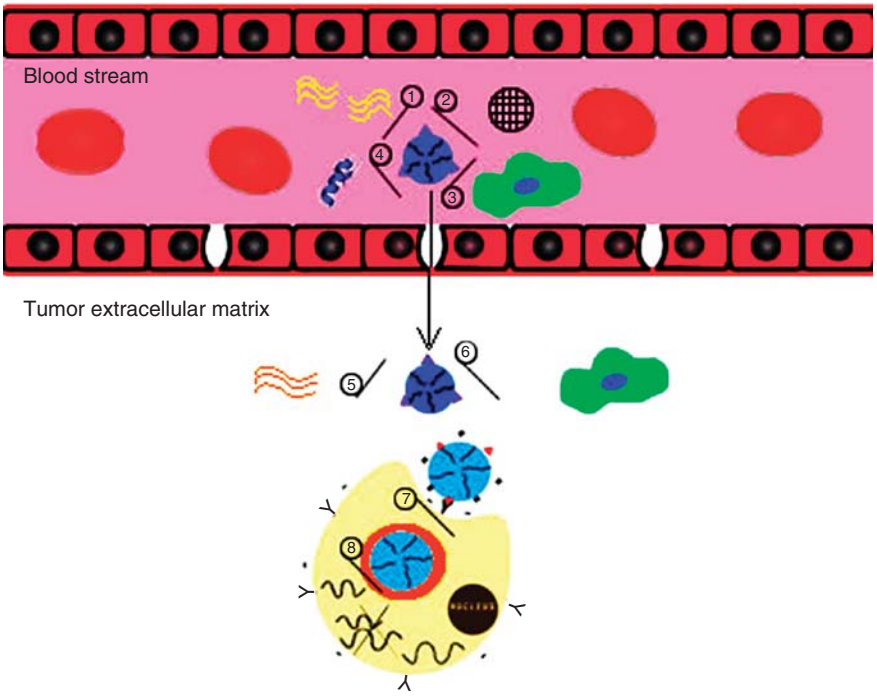

Figure 1. The main barriers encounter to the systemic delivery of small interfering RNA (siRNA) nanocarriers toward the tumor cells. 1. Aggregation by serum proteins. 2. Kidney filtration. 3. Uptake by phagocytes. 4. Enzymatic degradation by endogenous nuclease. 5. Diffusion through extracellular matrix. 6. Uptake by resident macrophages. 7. Uptake by the target cell. 8. Exit the endosome and then release from the carrier. The figure is original and has not previously been published.

up by the reticuloendothelial system (RES), depending on their size and surface characteristics. Hydrophilic nanoparticles that are below 35 nm show much lower uptake by the RES and, therefore, have longer blood circulation times and improved pharmacokinetic properties [40].

In active targeting, targeting ligands are attached at the surface of the nanocarrier for binding to appropriate receptors expressed at the target site [39,41]. The addition of a targeting moiety onto the surface of nanoparticles aims to increase cellular binding and internalization through receptor- mediated endocytosis [42]. Some of the ligands that can be conjugated to the surface of nanoparticles for active targeting to ovarian cancer cells were listed in **Table 1**.

4.3 siRNA loading in nanocarriers

In general, nanocarriers are loaded with siRNA effectors by ionic interactions between the negative charges of the nucleic acid phosphate groups and a polycation such as chitosan or a cationic phospholipid, resulting in the formation of a polyelectrolyte complex. Other loading strategies include covalent binding of nucleic acids onto the nanocarrier surface or with a hydrophilic polymer such as the polyethylene glycol (PEG) before the formation of polyelectrolyte complex with polycations [43]. Another way for delivery of siRNA to ovarian cancer cells have been examined by Tarapore *et al.*, based on using packaging RNA (pRNA) of bacteriophage phi29 DNA-packaging motor. In their study, siRNA was targeted to silence metallotionein IIA in ovarian cancer cells [44].

Various nanocarriers that have potential to deliver siRNA *in vivo* are described below.

4.3.1 *Lipid-based delivery systems*

Lipid-based gene delivery systems consist mainly of cationic lipids (non-cholesterol backbone), cholesterol-based cationic lipids, cationic liposomes and neutral liposomes. Conjugation of siRNA with lipophilic molecules such as cholesterol [45] could also be listed as a lipid-based delivery system.

Cationic liposomes are vesicles composed of a phospholipid bilayer with an aqueous core [33]. A cationic lipid molecule is typically composed of a hydrophobic part bound to a hydrophilic cationic part by a linker or other designed molecules. The hydrophobic moiety is generally constituted either by aliphatic chains or by a cholesteryl tail. In liposomes, the cationic lipid is usually associated with a co-lipid such as dioleoylphosphatidyl ethanolamine (DOPE) to form stable liposomal bilayers. Such liposomes instantaneously interact with DNA as well as siRNA and form complexes commonly known as lipoplexes [46,47].

Cationic liposomes such as DOTAP, DOTAP/DOPE, DOTAP/DOPC, MVL5/DOPC and DOPE/CDAN are used for gene delivery.

The formulation of cationic lipids has been widely applied to *in vitro* nucleic acid transfection, and more than

Table 1. List of ligands that have been used for active targeting nanoparticles to ovarian cancer cells.

Ligand	Target	Ref.
Folic acid	Folate receptor alpha (FRα)	[94,44]
Epithelial growth factor (EGF)	EGF receptor	[103]
Artificial ligands targeting HER2	HER2 receptor	[104]
Transferrin	Transferrin receptor	[105]
Arginine-glycine-aspartic acid (RGD) peptide	Integrins	[106]
YSA* peptide	EphA2 receptor	[107]
Gonadotropin releasing hormone (GnRH) peptide analog	GnRH receptor	[108]
Aptamers‡	MUC1	[109]

*YSA peptide binds to the erythropoietin-producing hepatocellular (Eph) A2 receptor that is crucial for tumor cell vascular development and angiogenesis. Its one-letter code sequence is YSAYPDSVPMMS [107].
‡Aptamers are single-stranded DNA or RNA oligonucleotides that fold into well-defined 3D structures, which are able to recognize molecules, such as proteins, phospholipids, sugars and nucleic acids, with high affinity and specificity. A large number of aptamers have been raised against cancer-associated antigens [47].

30 products are commercially available for this purpose, including Lipofectin® (a 1:1 mixture of DOTMA and DOPE), Transfectam®, Lipofeace™, Lipofectamine™ and Lipotaxi™ [48].

The role of the cationic lipid is to facilitate nucleic acid binding and condensation leading to cationic liposome/micelle–nucleic acid complex (lipoplex) nanoparticles. The cationic lipids also facilitate nanoparticle interactions with cell wall membranes to trigger internalization (typically by endocytosis), and if well-designed, the nanoparticle can escape endosome. The neutral co-lipid DOPE is a natural fusogenic lipid that helps endosomolysis and improves intracellular trafficking of nucleic acids post nanoparticle internalization. However, DOPE has potentially limited utility *in vivo* because it makes nanoparticles unstable [49].

The main disadvantages for cationic liposomes are that they lack self-assembly and size-controllable properties. Some lipid reagents for *in vitro* siRNA delivery such as oligofectamine, lipofectamine, metafectene and siportamine are not suitable for *in vivo* delivery because of their toxicity.

Solid–lipid-based systems are known as nano-structured siRNA carriers, which, unlike the liposomes, possess a relatively firm core of physiological lipids such as glycerides coated with nonionic surfactants such as polysorbates for dispersion and steric stabilization [50].

Neutral phospholipids (such as DOPC, 1, 2-dioleoyl-*sn*-glycero-3-phosphatidylcholine) can construct neutral liposomes, which can be filled with siRNA for delivery to tumor tissue; however, neutral liposomes confer low transfection efficiency due to their lack of surface charges [51].

Examples of lipid-based siRNA delivery systems that have been used in studies of ovarian cancer are listed in **Table 2**.

4.3.2 *Polysaccharide-based delivery systems*

Polysaccharides have a wide range of molecular weights and large number of reactive groups, varying chemical composition, which contribute to their diversity in structure and property. Charged polysaccharides can be further divided into positively charged polysaccharides such as chitosan and negatively charged polysaccharides such as alginate. Due to the presence of various derivable groups on molecular chains, polysaccharides can be easily modified chemically and biochemically, resulting in many kinds of polysaccharide derivatives. As natural biomaterials, polysaccharides are highly stable, safe, nontoxic, hydrophilic and biodegradable. Most of the natural polysaccharides have hydrophilic groups such as hydroxyl, carboxyl and amino groups, which could form non-covalent bonds with biological tissues. This property is called bioadhesion [52]. Polysaccharides can form several nanocarrier systems including polyplex, polymer micelles, nanoplex, nanocapsules and nanogels.

Polysaccharide derivatives, being hydrophilic and water-soluble, can be modified with hydrophobic molecules or oligomers that these biopolymers in turn can self-assemble in water to form nanoparticles or nanogels useful for drug delivery applications [53].

Chitosan, alginate and dextran are polysaccharides that have been used for delivery of siRNA [54,55]. Examples of polysaccharide-based nanocarrier systems used for delivery of siRNA in ovarian cancer are summarized in **Table 2**.

4.3.3 *Protein- and peptide-based delivery systems*

Nanoparticle technology for protein-based gene delivery, so-called proticles for the delivery of siRNA, has also been explored. This technology uses an initial complex between human serum albumin and protamine and by incorporation of siRNA, the nanoparticles self-assemble. Albumin, collagen and gelatin are promising proteins for delivery of siRNA. The endogenous protein albumin is a suitable material for nanoparticle fabrication since it is readily available in large quantities, and it metabolizes *in vivo* to produce innocuous degradation products. It can be conveniently fabricated into nanoparticles via coacervation techniques. The size of the nanoparticles can be effectively controlled at the coacervation process [56].

Cationic peptides, for example poly-L-lysine (PLL) can be complexed with oligonucleotides, protect them against nuclease digestion and enhance the cellular uptake via nonspecific endocytosis. To get more specificity in the targeting of cells, various ligands such as folic acid, steroids, transferrin, mannose, growth factors can be conjugated to PLL to improve the uptake of the oligonucleotide–peptide complexes or conjugates via receptor-mediated endocytosis. Another functionality of these peptides is their endosomolytic potential. Peptide transduction domains (PTDs) have been found that they can cross the cellular membrane by themselves despite their high molecule size. PTDs have been used for the delivery of a wide range of molecules including peptides, proteins and antisense oligonucleotides [57].

Table 2. Nanocarrier systems for delivery of small interfering RNA (siRNA) in ovarian cancer.

Nanocarrier	Targeted siRNA	*In vivo*/*in vitro*	Ref.
Lipid based			
Cationic liposome (Lipofectamine 2000)	RhoC	*In vitro*	[112]
Neutral liposomes (DOPC)	Anti IL-8 siRNA	*In vivo*	[113]
Neutral liposomes (DOPC)	EphA2	*In vivo*	[110,114-116]
Neutral liposomes (DOPC)	Focal adhesion kinase (Fak)	*In vivo*	[117]
Cationic liposome (Lipofectamine 2000)	Notch 1	*In vitro*	[118]
Lipid-like materials known as lipidoids	Poly(ADP-ribose) polymerase 1 (PARP1)	*In vivo*	[119]
Neutral liposomes (DOPC)	EphB4	*In vivo*	[120]
Neutral liposomes (DOPC)	ALDH1A1	*In vivo*	[121]
Cationic liposome (Lipofectamine 2000)	KLF6-SV1	*In vivo*	[122]
Cationic liposome (Lipofectamine™ 2000)	Plexin-B1	*In vitro*	[123]
Polysaccharide based			
Chitosan	Multiple growth promoting genes including periostin (POSTN)	*In vivo*	[124]
Chitosan	Polycomb repressor, EZH2	*In vivo*	[125]
Protein and peptide based			
Arginine peptide	HER-2-specific siRNA	*In vivo*	[126]
A triblock poly(amido amine)-poly(ethylene glycol)-poly-L-lysine (PAMAM-PEG-PLL)	BCL-2	*In vitro*	[127]
Synthetic polymer and copolymer based			
Linear low-molecular-weight JetPEI	HER-2	*In vivo*	[93]
Poly(*N*-isopropyl-methacrylamide)	EGFR	*In vitro*	[128]
Inorganic material based			
Mesoporous silicon	Bcl-2	*In vivo*	[129]

Arginine- and histidine-rich peptides mediate an endosomal escape of the oligonucleotide into the cytosol. Furthermore, special amino acid sequence motifs have the ability to transfer oligonucleotides to the cell nucleus, if they include a nuclear localization sequence (NLS-peptides) [58]. Natural arginine-rich peptides such as protamine and the synthetic ones were examined for the delivery of siRNA as well [59].

Examples of the protein- or peptide-based nanocarrier systems used for delivery of siRNA in ovarian cancer are listed in **Table 2**.

4.3.4 *Synthetic polymer- and copolymer-based delivery systems*

A variety of synthetic polymers such as polyethylenimine (PEI) have been investigated for use as siRNA carrier [60]. The structures of cationic polymers are very different, which include the linear polymers such as linear PEI, the branched polymers such as branched PEI, the circle-like polymers such as cyclodextrin, the network-type polymers such as cross-linked poly (amino acid) (PAA) and the dendrimers [61]. Many cationic polymers are highly efficient in siRNA delivery [62]. However, anionic serum proteins can interact with net positively charged siRNA/polycation complexes and cause aggregation or decomplexation [43].

Polymer nanocarriers are prepared from polycation copolymers, including PEI, PLL and a highly hydrophilic polymer such as PEG or dextran. The nanoparticles are formed spontaneously after mixing the copolymer with the nucleic acid and the electrostatic interactions between the nucleic acid and the positively charged block of the copolymer occurred [43].

Polymeric nanocarrier constructions such as poly-alkylcyanoacrylate (PACA) [43] and poly (D, L-lactide-co-glycolide) (PLGA) were prepared to adsorb the nucleic acids on their surface [63]. They were also used for the preparation of nanocapsules (vesicular-type polymer carriers) to entrap nucleic acids. For another construct like nanogels, polycations such as PLL were used to load oligonucleotides inside the matrix of the nanogel [43].

Di-block or tri-block copolymers (synthetic block polymers) have been widely explored as materials for siRNA delivery. They can form nanocarriers with useful properties for delivery of siRNA. These nanocarriers typically result from cooperative electrostatic interactions between the genetic material and a cationic di-block copolymer. Upon complexation, the charge-compensated nucleic acid/cationic chains self-assemble into a micellar core while the hydrophilic segments form a protective corona [64]. For hydrophilic block, mostly, PEG is used. The polymers used for delivery of siRNA in ovarian cancer are listed in **Table 2**.

4.3.5 *siRNA delivery systems based on inorganic nanoparticles*

Inorganic nanoparticles are subjected to some modifications to become biocompatible and suitable for delivery of siRNA. Subsequently, they can be used to form complex

between the carrier and nucleic acid and targeted to the specific cells [65].

Layered double hydroxide (LDH) nanoparticles are a family of inorganic crystals that can tightly bind, protect and release siRNA molecules efficiently to mammalian cells *in vitro*. The uptake of siRNA-loaded LDH nanoparticles occurs via endocytosis, whereby the nanoparticles dissolve due to the low pH in the endosome, thereby aiding endosomal escape into the cytoplasm [66]. Magnetic nanoparticles (MNPs) typically composed of iron oxide have also been investigated as carriers for the delivery of siRNA [67,68,111]. It has been reported that hydroxyapatite (HAp), the crystalline form of calcium phosphate (CaP), exhibited binding affinity toward nucleic acids. Hence, CaP particles could be potentially useful for the systemic delivery of siRNA [69-71].

There are several reports on siRNA delivery using gold (Au) nanoparticles [72-74]. Due to the presence of a negative charge on the surface of AuNPs, they are highly reactive, which helps to modify the surface of AuNPs using several biomolecules. Due to the strong interaction between the gold surface and thiol/amine-containing molecules (organic molecules, DNA, protein, enzyme etc.), the surface of AuNPs can be easily modified [75,76]. Mesoporous silicon is being extensively studied as an emerging material for use in biomedical applications, including drug delivery. Mesoporous silicon efficiently delivered siRNA to ovarian tumors in a sustained mode [77,78] and also targeted to luciferase and glyceraldehyde 3-phosphate dehydrogenase [79].

Carbon nanotubes (CNTs) are well-ordered, all-carbon hollow graphitic nanomaterials with very high aspect ratios, lengths from several hundred nanometers to several micrometers and diameter of 0.4 – 2 nm for single-walled carbon nanotubes (SWNTs) and 2 – 100 nm for multiwalled carbon nanotubes (MWNTs). Due to their large surface areas, unique surface properties and needle-like shape, they can deliver siRNAs, to the target disease sites [80].

Knockdown of cyclin B1 gene with only 10 nM of siRNA delivered by carbonate apatite has been reported [81].

5. Potential nanocarriers for siRNA delivery

Biopolymers are promising systems for delivery of siRNA in ovarian cancer. Among them, chitosan, PLGA and gelatine are more promising as they are biocompatible and nontoxic. Chitosan has been shown to be biocompatible [82], non-inflammatory, nontoxic and biodegradable. The protonated amine groups allow transport across cellular membranes and subsequent endocytosis into cells. Moreover, the positively charged amines (under slightly acidic conditions) allow electrostatic interaction with phosphate-bearing nucleic acids to form polyelectrolyte complexes. Numerous studies on DNA delivery with chitosan as a carrier biomaterial have shown effective expression of reporter genes *in vitro* and *in vivo*, promoting chitosan as an attractive candidate for siRNA delivery [31,83-85]. Although Chitosan has cationic and biocompatible properties, the nucleic acid transfection efficiency of chitosan has been known to be lower as compared with other cationic molecules such as PEI and cationic dendrimers. To enhance the cationic charge of chitosan, the additional cationic moieties such as polyarginine were linked to chitosan [86]. PLGA is also another poly-cation for combination with chitosan [87-89]. There are also reports of adding anionic polysaccharides such as cyclodextrin [90] and neutral polysaccharides such as PEG to chitosan to improve its properties. PEG conjugation with chitosan enhances particle stability with minimum aggregation without sacrificing transfection efficiency. PEGylation of chitosan elongates the plasma circulation time and prolongs gene transfer for sustained delivery of DNA. Blend microspheres of PEG-grafted chitosan with PLGA also result in sustained release of DNA for up to 9 weeks [91]. The reason for addition of an anionic polymer to chitosan prior to addition of nucleic acid is to achieve a more stable polyplex formation and smaller nanoparticles: alginate [92] or an alginate derivative named polyguluronate [54] and hyaluronic acid were used for this purpose [91,92]. The attachment of targeting ligands that provide specific nanoparticle–cell surface interactions thus has an important effect on the ultimate location of nanoparticles. In the case of chitosan, RGD peptide [93] and folate [94,95] ligands have been used successfully for targeting ovarian cancer cells.

PLGA is also an attractive polymer for delivery of biopharmaceuticals owing to its biocompatibility, biodegradability and outstanding controlled-release characteristics. PLGA has been approved by the FDA for certain human clinical uses [96]. However, it is difficult to load smaller nucleic acid molecules such as siRNA into PLGA nanoparticles because siRNA easily leaks from the inner water phase into the outer water phase during preparation due to its relatively low molecular weight, its hydrophilic character and the electrostatic repulsion forces present between the phosphate groups in the siRNA backbone and the anionic acid groups in the PLGA polymer. As a result, siRNA is usually incorporated into the PLGA nanoparticles with very low encapsulation efficiency and a limited loading percent, which represents a problem in further development of PLGA nanoparticles for siRNA delivery. A common strategy to increase the loading is to complex siRNA with cationic excipients [97,98]. As described before, one promising approach could be co-encapsulation with chitosan [96,98,99].

Gelatin, the denatured form of collagen, has also been demonstrated as a viable gene delivery vehicle [96]. The advantage to using gelatin as a carrier for controlled release is that polyion complexation can be used to load the therapeutic agent into the matrix under mild conditions [99]. Furthermore, nanoparticulate colloidal systems based on gelatine are safe and simple delivery systems with potential for i.v. application [100] and their biosafety have been proven [101]. Besides, they have had much success as drug delivery carriers especially with DNA oligonucleotides. Oligonucleotides had been adsorbed on the surface of gelatin or modified gelatine

nanoparticles, and usually this process is carried out after the preparation of the nanoparticles [102].

Among the siRNA therapeutic in clinical trials, SNALP vehicles (stable nucleic acid lipid particle) have been used for delivery by intravenous injection [3]. So the lipid-based systems are currently the only carriers used in clinical trials in spite of the safety and lower cost of biopolymers.

6. Conclusion

Gene silencing therapy represents a possible opportunity for treatment of ovarian cancer patients. Gene silencing therapy based on siRNA requires the selection of targeted genes and development of a strategy for delivery of genetic drugs. Clinical application of siRNA may rely on a combination of identifying optimal siRNAs against optimal target genes and developing an efficient system for siRNA delivery into the cancer cells. Given the current difficulties of treating women with ovarian cancer, any new treatment with improved clinical outcomes would be of enormous benefit for patients.

7. Expert opinion

Ovarian cancer is a leading gynecologic cause of death in women around the world and that is due to difficulties in early stage diagnosis and lack of durability of chemotherapies. Bionanotechnology based on siRNA nanocarriers offer many promising solutions for the challenges associated with the current ovarian cancer therapy. Because of their unique physical and biological properties, nanocarriers can selectively deliver (siRNA) to specific tissues and cells and enhance targeting by active and passive routes thus reduce toxicity and improve the therapeutic activity. Nanocarriers have additional advantages such as ability to control the release of their contents, biodegradability and biocompatibility properties. Despite of all these advantages, treatment of ovarian cancer with siRNA nanocarriers is still at its infancy and faces many challenges. The main challenge of the delivery of siRNA to ovarian cancer using nanocarriers is related to their targeting properties. The internal systems are very complex and there are many barriers that prevent siRNA from reaching the target site. A wide range of nanocarriers have been investigated for effective delivery of siRNA including liposomes, nanogels, dendrimers, gold and chitosan nanoparticles. However, minimal success has been achieved in this field. Development of nanocarriers based on knowledge of their *in vivo* behavior such as interactions with target cells, receptors and molecular mechanisms could be more effective for achieving delivery. In addition, understanding the barriers to siRNA delivery will help in the design of systems with different targeting strategies that ensure the effectiveness of this therapeutic modality.

Another limitation for the use of siRNA nanocarriers in ovarian cancers is their safety. As these systems will be developed for *in vivo* administration, therefore, their biocompatibility is crucial. Our literature research revealed that only one siRNA nanocarrier system has made it to Phase I clinical trial for safety. Future studies, therefore, need to focus on evaluating the toxicities of these systems to facilitate their use in treatment ovarian cancer. Other challenges include the complexity of these systems and the difficulties in controlling their physicochemical properties, which make mass production costly and non-reproducible.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

1. Rao DD, Vorhies JS, Senzer N, et al. SiRNA vs.shRNA: similarities and differences. Adv Drug Deliv Rev 2009;61:746-59
2. Numnum TM, Makhija S, Lu B, et al. Improved anti-tumor therapy based upon infectivity-enhanced adenoviral delivery of RNA interference in ovarian carcinoma cell lines. Gynecol Oncol 2008;108:34-41
3. Burnett JC, Rossi JJ, Tiemann K. Current progress of siRNA/shRNA therapeutics in clinical trials. Biotechnol J 2011;6:1130-46
4. Garson K, Shaw TJ, Clark KV, et al. Models of ovarian cancer-Are we there yet? Mol Cell Endocrinol 2005;239:15-26
5. Gorringe KL, Campbella IG. Large-scale genomic analysis of ovarian carcinomas. Mol Oncol 2009;3:157-64
6. Vaughan S, Coward JI Jr, Bast RC, et al. Rethinking ovarian cancer: recommendations for improving outcomes. Nat Rev Cancer 2011;11:719-25
7. Kurman RJ, Shih IM. The Origin and pathogenesis of epithelial ovarian cancer-a proposed unifying theory. Am J Surg Pathol 2010;34:433-43
8. Despierre E, Lambrechts D, Neven P, et al. The molecular genetic basis of ovarian cancer and its roadmap towards a better treatment. Gynecol Oncol 2010;117:358-65
9. The Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature 2011;474:609-15
10. Vanderhyden BC, Dorward AM. Ovarian cancer and the environment: rodent models. In: McQueen CA. editor. Comprehensive Toxicology. Volume 11 2nd edition. Elsevier Ltd; Elsevier: 2010. p. 483-98
11. Ashworth A, Balkwill F, Bast RC, et al. Opportunities and challenges in ovarian cancer research, a perspective from the 11th Ovarian cancer action/HHMT Forum. Gynecol Oncol 2008;108:652-7
12. Maloney A, Clarke PA, Naaby-Hansen S, et al. Gene and protein expression profiling of human ovarian cancer cells treated with the heat shock protein 90 inhibitor 17-allylamino-17-demethoxygeldanamycin. Cancer Res 2007;67:3239-53
13. Chon HS, Lancaster JM. Microarray-based gene expression studies in ovarian cancer. Cancer Control 2011;18:8-15
14. Agarwal R, Linch M, Kaye SB. Novel therapeutic agents in ovarian cancer. Eur J Surg Oncol 2006;32:875-86
15. Disaia PJ, Bloss JD. Treatment of ovarian cancer:new strategies. Gynecol Oncol 2003;90:S24-32
16. Kieback DJ, Hasenburg A, Runnebaum IB, et al. Gene therapy of ovarian cancer-state of the art and future perspectives. In: Altchek A, Deligdisch L, Kase NG, editors. Diagnosis and Management of Ovarian Disorders. 2nd edition. Elsevier Science; USA: 2003. p. 277-304
17. Sawicki JA, Anderson DG, Langer R. Nanoparticle delivery of suicide DNA for epithelial ovarian cancer therapy. Adv Exp Med Biol 2008;622:209-19
18. Singh P, Joshi S, Russell P, et al. Purine nucleoside phosphorylase mediated molecular chemotherapy and conventional chemotherapy: a tangible union against chemoresistant cancer. BMC Cancer 2011;11:368
19. Dachs GU, Tupper J, Tozer GM. From bench to bedside for gene-directed enzyme prodrug therapy of cancer. Anticancer Drugs 2005;16:349-59
20. Imamura O, Okada H, Takashima Y, et al. SiRNA-mediated Erc gene silencing suppresses tumor growth in Tsc2 mutant renal carcinoma model. Cancer Lett 2008;268:278-85
21. Kenny GD, Kamaly N, Kalber TL, et al. Novel multifunctional nanoparticle mediates siRNA tumour delivery, visualisation and therapeutic tumour reduction in vivo. J Control Release 2011;149:111-16
22. Guo P, Coban O, Snead NM, et al. Engineering RNA for Targeted siRNA delivery and medical application. Adv Drug Deliv Rev 2010;62:650-66
23. Akhtar S, Benter I. Toxicogenomics of non-viral drug delivery systems for RNAi: potential impact on siRNA-mediated gene silencing activity and specificity. Adv Drug Deliv Rev 2007;59:164-82
24. Tanaka K, Kanazawa T, Ogaw T, et al. Disulfide crosslinked stearoyl carrier peptides containing arginine and histidine enhance siRNA uptake and gene silencing. Int J Pharm 2010;398:219-24
25. Bhaskar S, Tian F, Stoeger T, et al. Multifunctional nanocarriers for diagnostics, drug delivery and targeted treatment across blood-brain barrier: perspectives on tracking and neuroimaging. Part Fibre Toxicol 2010;7:3
26. Torchilin VP. Nanocarriers for drug delivery: needs and requirements. In: Torchilin VP, editor. Nanoparticulates as Drug Carriers. Imperial College Press; World Scientific: 2006. p. 1-6
27. Cho K, Wang X, Nie S, et al. Therapeutic nanoparticles for drug delivery in cancer. Clin Cancer Res 2008;14:1310-16
28. Whitehead KA, Langer R, Anderson DG. Knocking down barriers: advances in siRNA delivery. Nat Rev Drug Discov 2009;8:129-38
29. David S, Pitard B, Benoit JP, et al. Non-viral nanosystems for systemic siRNA delivery. Pharmacol Res 2010;62:100-14
30. He X, Arslan AD, Pool MD, et al. Knockdown of splicing factor SRp20 causes apoptosis in ovarian cancer cells and its expression is associated with malignancy of epithelial ovarian cancer. Oncogene 2011;30:356-65
31. Liua X, Howard KA, Dong M, et al. The influence of polymeric properties on chitosan/siRNA nanoparticle formulation and gene silencing. Biomaterials 2007;28:1280-8
32. Li S, Huang L. Non-viral is superior to viral gene delivery. J Control Release 2007;123:181-3
33. Reischl D, Zimmer A. Drug delivery of siRNA therapeutics: potentials and limits of nanosystems. Nanomed Nanotechnol Biol Med 2009;5:8-20
34. Chen Y, Huang L. Tumor-targeted delivery of siRNA by non-viral vector: safe and effective cancer therapy. Expert Opin Drug Deliv 2008;5:1301-11

35. Jain NK, Nahar M. PEGylated nanocarriers for systemic delivery. Methods Mol Biol 2010;624:221-34
36. Kim PS, Djazayeri S, Zeineldin R. Novel nanotechnology approaches to diagnosis and therapy of ovarian cancer. Gynecol Oncol 2011;120:393-403
37. Jain RK. Delivery of molecular and cellular medicine to solid tumors. Adv Drug Deliv Rev 2001;46:149-68
38. Shim MS, Kwon YJ. Efficient and targeted delivery of siRNA in vivo. FEBS J 2010;277:4814-27
39. Danhier F, Feron O, Preat V. To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. J Control Release 2010;148:135-46
40. Nie S. Understanding and overcoming major barriers in cancer nanomedicine. Nanomedicine(Lond) 2010;5:523-8
41. Hong S, Leroueil PR, Majoros IJ. The binding avidity of a nanoparticle-based multivalent targeted drug delivery platform. Chem Biol 2007;14:107-15
42. Wang M, Thanou M. Targeting nanoparticles to cancer. Pharmacol Res 2010;62:90-9
43. Martimprey H, Vauthier C, Malvy C, et al. Polymer nanocarriers for the delivery of small fragments of nucleic acids: oligonucleotides and siRNA. Eur J Pharm Biopharm 2009;71:490-504
44. Tarapore P, Shu Y, Guo P, et al. Application of phi29 motor pRNA for targeted therapeutic delivery of siRNA silencing metallothionein-IIA and surviving in ovarian cancers. Mol Ther 2011;19:386-94
45. Chen Q, Butler D, Querbes W, et al. Lipophilic siRNAs mediate efficient gene silencing in oligodendrocytes with direct CNS delivery. J Control Release 2010;144:227-32
46. Cao A, Briane D, Coudert R. Cationic liposomes as transmembrane carriers of nucleic acids. Adv Planar Lipid Bilayer Liposom 2006;4:136-82
47. Kim HK, Davaa E, Myung CS, et al. Enhanced siRNA delivery using cationic liposomes with new polyarginine-conjugated PEG-lipid. Int J Pharm 2010;392:141-7
48. Pozo-Rodriguez A, Delgado D, Angeles Solinis M, et al. Solid lipid nanoparticles as potential tools for gene therapy: in vivo protein expression after intravenous administration. Int J Pharm 2010;385:157-62
49. Mevel M, Kamaly N, Carmona S, et al. DODAG; a versatile new cationic lipid that mediates efficient delivery of pDNA and siRNA. J Control Release 2010;143:222-32
50. Xue HY, Wong HL. Tailoring nanostructured solid-lipid carriers for time-controlled intracellular siRNA kinetics to sustain RNAi-mediated chemosensitization. Biomaterials 2011;32:2662-72
51. Gao Y, Liu XL, Li XR. Research progress on siRNA delivery with nonviral carriers. Int J Nanomed 2011;6:1017-25
52. Liu Z, Jiao Y, Wang Y, et al. Polysaccharides-based nanoparticles as drug delivery systems. Adv Drug Deliv Rev 2008;60:1650-62
53. Oh JK, Lee DI, Park JM. Biopolymer-based microgels/nanogels for drug delivery applications. Prog Polym Sci 2009;34:1261-82
54. Lee DW, Yun K, Ban H, et al. Preparation and characterization of chitosan/polyguluronate nanoparticles for siRNA delivery. J Control Release 2009;139:146-52
55. Cohen JL, Schubert S, Wich PR, et al. Acid-degradable cationic dextran particles for the delivery of siRNA therapeutics. Bioconjug Chem 2011;22:1056-65
56. Singh HD, Wang G, Uludag H, et al. Poly-L-lysine-coated albumin nanoparticles: stability, mechanism for increasing in vitro enzymatic resilience, and siRNA release characteristics. Acta Biomater 2010;6:4277-84
57. Eguchi A, Dowdy SF. SiRNA delivery using peptide transduction domains. Trends Pharmacol Sci 2009;30(7):341-5
58. Lochmann D, Jauk E, Zimmer A. Drug delivery of oligonucleotides by peptides. Eur J Pharm Biopharm 2004;58:237-51
59. Choi Y, Lee JY, Suh JS, et al. The systemic delivery of siRNAs by a cell penetrating peptide, low molecular weight protamine. Biomaterials 2010;31:1429-43
60. Hobel S, Loos A, Appelhans D, et al. Maltose- and maltotriose-modified, hyperbranched poly(ethylene imine)s (OM-PEIs): physicochemical and biological properties of DNA and siRNA complexes. J Control Release 2011;149:146-58
61. Zhu L, Mahato RI. Lipid and polymeric carrier-mediated nucleic acid delivery. Expert Opin Drug Deliv 2010;7:1209-26
62. Convertine A, Benoit D, Duvall C, et al. Development of a novel endosomolytic diblock copolymer for siRNA delivery. J Control Release 2009;133:221-9
63. Kumar MR, Bakowsky U, Lehr CM. Preparation and characterization of cationic PLGA nanospheres as DNA carriers. Biomaterials 2004;25:1771-7
64. Felber AE, Castagner B, Elsabahy M, et al. SiRNA nanocarriers based on methacrylic acid copolymers. J Control Release 2011;152:159-67
65. Xu ZP, Zeng QH, Lu GQ, et al. Inorganic nanoparticles as carriers for efficient cellular delivery. Chem Eng Sci 2006;61:1027-40
66. Ladewig K, Niebert M, Xu Z, et al. Efficient siRNA delivery to mammalian cells using layered double hydroxide nanoparticles. Biomaterials 2010;31:1821-9
67. Sun C, Lee J, Zhang M. Magnetic nanoparticles in MR imaging and drug delivery. Adv Drug Deliv Rev 2008;60:1252-65
68. Figuerola A, Di Corato R, Manna L, et al. From iron oxide nanoparticles towards advanced iron-based inorganic materials designed for biomedical applications. Pharmacol Res 2010;62:126-43
69. Li J, Chen Y, Tseng Y, et al. Biodegradable calcium phosphate nanoparticle with lipid coating for systemic siRNA delivery. J Control Release 2010;142:416-21
70. Zhang M, Ishii A, Nishiyama N, et al. PEGylated calcium phosphate nanocomposites as smart environment-sensitive carriers for siRNA delivery. Adv Mater 2009;21:3520-5
71. Park K. Systemic siRNA delivery using biocompatible calcium phosphate nanoparticles. J Control Release 2010;142:295
72. Kim D, Kim J, Park M, et al. Modulation of biological processes in the nucleus by delivery of DNA oligonucleotides conjugated with

gold nanoparticles. Biomaterials 2011;32:2593-604

73. Duncan B, Kim C, Rotello VM. Gold nanoparticle platforms as drug and biomacromolecule delivery systems. J Control Release 2010;148:122-7
74. Pissuwan D, Niidome T, Cortie MB. The forthcoming applications of gold nanoparticles in drug and gene delivery systems. J Control Release 2011;149:65-71
75. Patra CR, Bhattacharya R, Mukhopadhyay D, et al. Fabrication of gold nanoparticles for targeted therapy in pancreatic cancer. Adv Drug Deliv Rev 2010;62:346-61
76. Ghosh P, Han G, De M, et al. Gold nanoparticles in delivery applications. Adv Drug Deliv Rev 2008;60:1307-15
77. Tanaka T, Godine B, Bhavane R, et al. In vivo evaluation of safety of nanoporous silicon carriers following single and multiple dose intravenous administrations in mice. Int J Pharm 2010;402:190-7
78. Tanaka T, Mangala LS, Vivas-Mejia PE, et al. Sustained small interfering RNA delivery by mesoporous silicon particles. Cancer Res 2010;70:3687-96
79. Bhattarai SR, Muthuswamy E, Wani A, et al. Enhanced gene and siRNA delivery by polycation-modified mesoporous silica nanoparticles loaded with chloroquine. Pharm Res 2010;27:2556-68
80. Cheung W, Pontoriero F, Taratula O, et al. DNA and carbon nanotubes as medicine. Adv Drug Deliv Rev 2010;62:633-49
81. Hossain S, Stanislaus A, Chua MJ, et al. Carbonate apatite-facilitated intracellularly delivered siRNA for efficient knockdown of functional genes. J Control Release 2010;147:101-8
82. Jayakumar R, Chennazhi KP, Muzzarelli RAA, et al. Chitosan conjugated DNA nanoparticles in gene therapy. Carbohydr Polym 2010;79:1-8
83. Rudzinski WE, Aminabhavi TM. Chitosan as a carrier for targeted delivery of small interfering RNA. Int J Pharm 2010;399:1-11
84. Lai W, Lin MC. Nucleic acid delivery with chitosan and its derivatives. J Control Release 2009;134:158-68
85. Kim T, Jiang H, Jere D, et al. Chemical modification of chitosan as a gene carrier in vitro and in vivo. Prog Polym Sci 2007;32:726-53
86. Noh SM, Park MO, Shim G, et al. Pegylated poly-L-arginine derivatives of chitosan for effective delivery of siRNA. J Control Release 2010;145:159-64
87. Nafee N, Taetz S, Schneider M, et al. Chitosan-coated PLGA nanoparticles for DNA/RNA delivery: effect of the formulation parameters on complexation and transfection of antisense oligonucleotides. Nanomed Nanotechnol Biol Med 2007;3:173-83
88. Tahara K, Sakai T, Yamamoto H, et al. Establishing chitosan coated PLGA nanosphere platform loaded with wide variety of nucleic acid by complexation with cationic compound for gene delivery. Int J Pharm 2008;354:210-16
89. Tan ML, Choong P, Dass CR. Cancer, chitosan nanoparticles and catalytic nucleic acids. J Pharm Pharmacol 2009;61:3-12
90. Teijeiro-Osorio D, Remunan-Lopez C, Alonso MJ. Chitosan/cyclodextrin nanoparticles can efficiently transfect the airway epithelium in vitro. Eur J Pharm Biopharm 2009;71:257-63
91. Duceppe N, Tabrizian M. Factors influencing the transfection efficiency of ultra low molecular weight chitosan/hyaluronic acid nanoparticles. Biomaterials 2009;30:2625-31
92. Gazori T, Haririan I, Fouladde S, et al. Inhibition of EGFR expression with chitosan/alginate nanoparticles encapsulating antisense oligonucleotides in T47D cell line using RT-PCR and immunocytochemistry. Carbohydr Polym 2010;80:1042-7
93. Urban-Klein B, Werth S, Abuharbeid S, et al. RNAi-mediated gene-targeting through systemic application of polyethylenimine (PEI)-complexed siRNA in vivo. Gene Ther 2005;12:461-6
94. Sudimack BAJ, Lee RJ. Targeted drug delivery via the folate receptor. Adv Drug Deliv Rev 2000;41:147-62
95. Leamon CP, Low PS. Folate-mediated targeting: from diagnostics to drug and gene delivery. Drug Discov Today 2001;6:44-51
96. Dang JM, Leong KW. Natural polymers for gene delivery and tissue engineering. Adv Drug Deliv Rev 2006;58:487-99
97. Cun D, Jensen DK, Maltesen MJ, et al. High loading efficiency and sustained release of siRNA encapsulated in PLGA nanoparticles: quality by design optimization and characterization. Eur J Pharm Biopharm 2011;77:26-35
98. Cun D, Foged C, Yang M, et al. Preparation and characterization of poly (dl-lactide-co-glycolide) nanoparticles for siRNA delivery. Int J Pharm 2010;390:70-5
99. Young S, Wong M, Tabata Y, et al. Gelatin as a delivery vehicle for the controlled release of bioactive molecules. J Control Release 2005;109:256-74
100. Zillies J, Coester C. Evaluating gelatine based nanoparticles as a carrier system for double stranded oligonucleotides. J Pharm Pharm Sci 2005;7:17-21
101. Kushibiki T, Nagata-Nakajima N, Sugai M, et al. Enhanced anti-fibrotic activity of plasmid DNA expressing small interference RNA for TGF-h type II receptor for a mouse model of obstructive nephropathy by cationized gelatin prepared from different amine compounds. J Control Release 2006;110:610-17
102. Ofokansi K, Winter G, Fricker G, et al. Matrix-loaded biodegradable gelatin nanoparticles as new approach to improve drug loading and delivery. Eur J Pharm Biopharm 2010;76:1-9
103. Zeineldin R, Muller CY, Stack MS, et al. Targeting the EGF receptor for ovarian cancer therapy. J Oncol 2010;2010:1-11
104. Tai W, Mahato R, Cheng K. The role of HER2 in cancer therapy and targeted drug delivery. J Control Release 2010;146:264-75
105. Krieger ML, Eckstein N, Schneider V, et al. Overcoming cisplatin resistance of ovarian cancer cells by targeted liposomes in vitro. Int J Pharm 2010;389:10-17
106. Han HD, Mangala LS, Lee JW, et al. Targeted gene silencing using RGD-labeled chitosan nanoparticles. Clin Cancer Res 2010;16:3910-22
107. Dickerson EB, Blackburn WH, Smith MH, et al. Chemosensitization of cancer cells by siRNA using targeted nanogel delivery. BMC Cancer 2010;10:1-11
108. Kim SH, Jeong JH, Lee SH, et al. LHRH receptor-mediated delivery of siRNA using polyelectrolyte complex micelles self-assembled from

siRNA-PEG-LHRH conjugate and PEI. Bioconjug Chem 2008;19:2156-62

109. Levy-Nissenbaum E, Radovic-Moreno AF, Wang AZ, et al. Nanotechnology and aptamers: applications in drug delivery. Trends Biotechnol 2008;26:442-9

110. Koolpe M, Dail M, Pasquale EB. An ephrin mimetic peptide that selectively targets the EphA2 receptor. J Biol Chem 2002;277:46974-9

111. Scarberry KE, Dickerson EB, McDonald JF, et al. Magnetic nanoparticle-peptide conjugates for in vitro and in vivo targeting and extraction of cancer cells. J Am Chem Soc 2008;130:10258-62

112. Zhao Y, Zong Z, Xu H. RhoC expression level is correlated with the clinicopathological characteristics of ovarian cancer and the expression levels of ROCK-I, VEGF, and MMP9. Gynecol Oncol 2010;116:563-71

113. Merritt WM, Lin YG, Spannuth WA, et al. Effect of interleukin-8 gene silencing with liposome encapsulated small interfering RNA on ovarian cancer cell growth. J Natl Cancer Inst 2008;100:359-72

114. Landen Jr CN, Chavez-Reyes A, Bucana C, et al. Therapeutic EphA2 gene targeting in vivo using neutral liposomal small interfering RNA delivery. Cancer Res 2005;65:6910-08

115. Landen CN, Merritt WM, Mangala LS, et al. Intraperitoneal Delivery of liposomal siRNA for therapy of advanced ovarian cancer. Cancer Biol Ther 2006;5:1708-13

116. Mangala LS, Han HD, Berestein G, et al. Liposomal siRNA for Ovarian Cancer. Therapeutic Applications of RNAi: Methods and Protocols. Volume 555 Humana Press, a part of Springer Science Business Media, LLC; New York: 2009. p. 29-42

117. Halder J, Kamat AA, Landen CN Jr, et al. Focal adhesion kinase targeting using in vivo short interfering RNA delivery in neutral liposomes for ovarian carcinoma therapy. Clin Cancer Res 2006;12:4916-24

118. Rose SL, Kunnimalaiyaan M, Drenzek J, et al. Notch 1 signaling is active in ovarian cancer. Gynecol Oncol 2010;117:130-3

119. Goldberg MS, Xinga D, Ren Y, et al. Nanoparticle-mediated delivery of siRNA targeting Parp1 extends survival of mice bearing tumors derived from Brca1-deficient ovarian cancer cells. Proc Natl Acad Sci 2011;108:745-50

120. Spannuth WA, Mangala LS, Stone RL, et al. Converging evidence for efficacy from parallel EphB4-targeted approaches in ovarian carcinoma. Mol Cancer Ther 2010;9:2377-88

121. Landen CN Jr, Goodman B, Katre AA, et al. Targeting aldehyde dehydrogenase cancer stem cells in ovarian cancer. Mol Cancer Ther 2010;9:3186-99

122. Feo AD, Huang F, Sangodkar J, et al. KLF6-SV1 is a novel antiapoptotic protein that targets the BH3-only protein NOXA for degradation and whose inhibition extends survival in an ovarian cancer. Model Cancer Res 2009;69:4733-41

123. Ye S, Hao X, Zhou T, et al. Plexin-B1 silencing inhibits ovarian cancer cell migration and invasion. BMC Cancer 2010;10:611-22

124. Han HD, Mangala LS, Lee JW, et al. Targeted gene silencing using RGD-labeled chitosan nanoparticles. Clin Cancer Res 2010;16:3910-22

125. Sood AK, Mangala LS, Han H, et al. In vivo angiogenic gene silencing using chitosan nanoparticles in ovarian carcinoma. Gynecol Oncol 2009;112:S2-S185

126. Kim SW, Kim NY, Choi YB, et al. RNA interference in vitro and in vivo using an arginine peptide/siRNA complex system. J Control Release 2010;143:335-43

127. Patil ML, Zhang M, Minko T. Multifunctional triblock nanocarrier (PAMAM-PEG-PLL) for the efficient intracellular siRNA delivery and gene silencing. ACS Nano 2011;5:1877-87

128. Blackburn WH, Dickerson EB, Smith MH, et al. Peptide functionalized nanogels for targeted siRNA delivery. Bioconjug Chem 2009;20:960-8

129. Chen AM, Zhang M, Wei D, et al. Co-delivery of doxorubicin and Bcl-2 siRNA by mesoporous silica nanoparticles enhances the efficacy of chemotherapy inmultidrug-resistant cancer cells. Small 2009;5:2673-7

Affiliation

Eshrat Gharaei Fathabadi[1], Andrew N. Shelling[2] & Raida Al-Kassas[†1]
[†]Author for correspondence
[1]University of Auckland, School of Pharmacy, Faculty of Medical and Health Sciences, Auckland, New Zealand
Tel: +64 9 373 7599;
E-mail: r.al-kassas@auckland.ac.nz
[2]University of Auckland, Faculty of Medical and Health Sciences, Department of Obstetrics and Gynaecology, Auckland, New Zealand