Published in final edited form as:

Mini Rev Med Chem. 2017 ; 17(3): 258–267.

LHRH-Targeted Drug Delivery Systems for Cancer Therapy

Xiaoning Li^a, Oleh Taratula^{*,a}, Olena Taratula^a, Canan Schumann^a, Tamara Minko^b

^aDepartment of Pharmaceutical Sciences, College of Pharmacy, Oregon State University, Portland, OR 97201, United States;

^bDepartment of Pharmaceutics, Ernest Mario School of Pharmacy, Rutgers, the State University of New Jersey, Piscataway, NJ 08854, United States

Abstract

Targeted delivery of therapeutic and diagnostic agents to cancer sites has significant potential to improve the therapeutic outcome of treatment while minimizing severe side effects. It is widely accepted that decoration of the drug delivery systems with targeting ligands that bind specifically to the receptors on the cancer cells is a promising strategy that may substantially enhance accumulation of anticancer agents in the tumors. Due to the transformed cellular nature, cancer cells exhibit a variety of overexpressed cell surface receptors for peptides, hormones, and essential nutrients, providing a significant number of target candidates for selective drug delivery. Among others, luteinizing hormone-releasing hormone (LHRH) receptors are overexpressed in the majority of cancers, while their expression in healthy tissues, apart from pituitary cells, is limited. The recent studies indicate that LHRH peptides can be employed to efficiently guide anticancer and imaging agents directly to cancerous cells, thereby increasing the amount of these substances in tumor tissue and preventing normal cells from unnecessary exposure. This manuscript provides an overview of the targeted drug delivery platforms that take advantage of the LHRH receptors overexpression by cancer cells.

Keywords

cancer; drug delivery systems; GnRH; LHRH; nanoparticles; targeted delivery

1. INTRODUCTION

Cancer is one of the most challenging problems in modern medicine and remains a leading cause of death worldwide [1]. The main reasons for the high mortality rate among cancer patients are related to the poor accessibility of the available therapeutic and imaging agents to cancer cells, their lack of selectivity, fast clearance from the blood circulation, and toxicity on healthy organs [2–5]. Therefore, a targeted and selective to cancer cells drug

^{*}Department of Pharmaceutical Sciences, College of Pharmacy, Oregon State University, Portland, OR 97201, United States; Tel: +1-503-346-4704; Oleh.Taratula@oregonstate.edu.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

delivery approach holds immense potential to improve the efficacy of cancer diagnosis and treatment [2–5].

It is widely recognized that specific delivery of the anticancer agents to the cancer sites can be achieved by two major approaches, passive and active targeting [4–7]. Passive targeting relies on the ability of large molecules and nanoparticles ranging from 10 nanometers up to several hundred nanometers in size, to accumulate specifically in tumor microenvironment by the escape from systemic circulation into the tumor interstitium through leaky tumor blood vessels [4–7]. Furthermore, impaired lymphatic drainage is responsible for the retention of penetrated macromolecules inside of cancer tissues. Active targeting of cancer cells represents another strategy which is based on the modification of anticancer agents and/or drug-loaded nanoparticles with targeting ligands that bind specifically to the receptors preferentially expressed or highly overexpressed by cancer cells [4–7]. Due to the transformed cellular nature, many cancer cells exhibit a variety of overexpressed cell surface receptors for peptides, hormones, and essential nutrients [3, 5, 8–11], providing a significant number of target candidates for active drug targeting to cancer cells. This review is focused on the recent developments and advances in drug delivery systems with active targeting features that take advantage of a luteinizing hormone-releasing hormone (LHRH) receptor. The LHRH-targeted drug delivery systems (DDS), discussed in this article, are summarized in Figure 1.

2. LHRH

LHRH, also known as gonadotropin-releasing hormone (GnRH), is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂) that is crucial in the control of reproductive functions [12]. Under the stimulation of LHRH, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are released from the pituitary [13], and in turn, regulate gonadal sex steroid production in both males and females [14, 15]. Binding of LHRH to its receptors (LHRH-R) appears to lead to receptor microaggregation and internalization of the peptide [16]. It has been well established that LHRH-R are not only expressed in the pituitary but also in cancer tissues [17, 18]. Although the precise biological role of LHRH-R in cancer tumors is not well documented yet, the various studies suggest that the LHRH peptides could function as local regulators of tumor growth [19–21]. The LHRH-R overexpression was detected in both hormone-dependent cancer tissues, such as breast cancer [22], endometrial cancer [23], ovarian cancer [17, 24, 25], and prostate cancer [26], and hormone-independent tissues, such as pancreatic cancer [27, 28], lung cancer [29], melanoma [30], and glioblastoma [31]. Moreover, the incidence of LHRH-R expression in various cancers was reported to be high. For example, LHRH-R are expressed in about 86% of prostate cancer, 80% of human endometrial and ovarian cancers, 80% of renal cancer, 50% of breast cancers, and 32–50% of pancreatic cancers [18, 32–35]. The expression of LHRH-R in these various cancers is significantly higher than in normal tissues, including tissues of the reproductive organs [17, 18, 25, 36]. For example, the Minko group reported that some expression of LHRH-R was detected in the ovary [17, 25]. However, it was at least 80% lower than in ovarian cancer cells taken from the same patient. The previous studies also demonstrated that LHRH-R expression in the lymph node metastases was as strong as or stronger than the expression in the primary cancer tumors [35, 37]. Finally, the use of

LHRH agonists' therapy for treatment of advanced prostate cancer, which usually results in the downregulation of pituitary LHRH receptors, had no significant effect on receptor expression on the cancer cells, as noted in specimens from prostate cancer patients who received neoadjuvant LHRH agonist therapy [37]. These facts suggest that LHRH targeting peptide, or its analogues, can be used to efficiently guide anticancer and imaging agents directly to cancerous cells, thus increasing the amount of the drugs in tumor tissues and avoiding the unnecessary exposure of normal cells.

3. LHRH-DRUG CONJUGATES

A significant number of studies on LHRH-based targeting therapy are focused on linking therapeutic and imaging agents to LHRH or an LHRH analog, following the principle that the conjugates would specifically deliver these agents to the high-LHRH-R-expressing cancer cells rather than the non-expressing or low-expressing normal cells. In most of these studies, [D-Lys⁶]-LHRH, a degradation-resistant LHRH analog, was used due to its high binding affinity to LHRH-R as well as its reactive site at position 6.

3.1. Chemotherapeutic drugs

Schally's group first conjugated chemotherapeutic drugs such as cisplatin [38] and melphalan [39] to [D-Lys⁶]-LHRH and showed high affinity for cancer cells as well as cytotoxic effects on cancer cells. Later on, the same group developed more efficient conjugates by attaching the drug doxorubicin (DOX) or its analog 2-pyrrolino-DOX to the ϵ -amino group of [D-Lys⁶]-LHRH. These two conjugates, known as AN-152 (DOX) and AN-207 (2-pyrrolino-DOX), bound to LHRH-R with high affinity and showed excellent anticancer profiles [40]. The high efficiency of AN-152 has been demonstrated in many different cancer models, as well as in clinical trials [40–47]. Promising results have been reported from both phase I and II clinical trials, and a phase III was initiated in 2013 with an estimated primary completion by the end of 2015 [46, 47]. The clinical investigation and progress of this conjugate have been extensively reviewed elsewhere [8, 26, 48–50]. Of note, Zoptarelin doxorubicin acetate, AEZS-108, (formerly AN-152) has been granted orphan drug status for endometrial and ovarian cancer by the U.S. Food and Drug Administration (FDA).

Other types of anticancer drugs have also been conjugated to [D-Lys⁶]-LHRH for targeted cancer therapy. Aggarwal and co-workers linked curcumin (a diarylheptanoid oxidant found in turmeric) to [D-Lys⁶]-LHRH and reported growth inhibitory effects on pancreatic cancer cells *in vitro* and *in vivo* [51]. In a recent study, gemcitabine-[D-Lys⁶]-LHRH bioconjugates were developed, and their cytotoxicity on prostate cancer cells as well as tumor growth inhibition in mice was evaluated by Karamelas and co-workers [52].

LHRH-III (pGlu-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH₂) is an isoform of human LHRH isolated from sea lamprey [53]. This decapeptide isoform can specifically bind to LHRH-R in cancer cells with strong antiproliferative effect [54], whereas the potency in its release of LH and FSH is insignificant in mammals [55]. These advantages, therefore, has promoted LHRH-III as a promising targeting moiety in the replacement of [D-Lys⁶]-LHRH in recent studies [56]. For example, a chemotherapeutic agent, daunorubicin, was attached to

LHRH-III at Lys⁸ via an oxime bond, and these daunorubicin-LHRH-III conjugates showed significant cytostatic effect on cancer cells in both *in vitro* and *in vivo* experiments [57, 58]. To further increase the cytostatic effect, LHRH-III derivatives were designed and synthesized to incorporate more than one conjugation site for drugs, yielding multifunctional bioconjugates that exert higher efficiency [59, 60].

3.2. Imaging agents

Several studies employed LHRH peptides to achieve targeted delivery of radionucleotides as imaging agents for positron emission tomography (PET) and single photon emission computed tomography (SPECT) [61–64]. The radionucleotides were attached to the LHRH with the help of chelating agents such as 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) [61, 62]. For example, Guo *et al.* chemically conjugated the DOTA to the D-Lys⁶ of LHRH via an aminohexanoic acid (Ahx) linker and used this conjugate for targeting of ¹¹¹In to breast and prostate cancer tissues [61, 62]. It was indicated that lipophilicity of the Ahx linker significantly increases the binding affinity of the LHRH peptide to the receptor. The developed LHRH conjugate demonstrated rapid accumulation in both breast and prostate cancer tumors and specific binding to the LHRH receptor, which provided for an efficient visualization of cancer lesions by SPECT [61, 62].

3.3. Photosensitizers

To enhance the selectivity of photodynamic therapy toward LHRH-positive cancer tissues, a few attempts were undertaken in order to develop LHRH-targeted photosensitizers [65, 66]. In two separate studies, LHRH analogs were directly conjugated to carboxylic groups of either protoporphyrin IX (PpIX) or Zinc phthalocyanine (ZnPc) [65, 66]. Both reports revealed that the targeted photosensitizers demonstrated high selectivity and internalization efficiency toward LHRH-positive cancer cells. Xu *et al.* also indicated that conjugation of a LHRH peptide to ZnPc does not influence the ROS generation properties of the photosensitizer [66]. However, the *in vivo* efficiency of the developed LHRH-targeted photosensitizers was not reported and therefore, their selectivity toward cancer tissues is unknown.

4. LHRH-DDS

Recent research efforts in LHRH-targeted cancer therapy have been devoted to nanometer-sized DDS coupled with LHRH targeting moieties [17, 25, 67–72]. The introduction of DDS into cancer treatment is aimed to improve the performance of anticancer drugs and imaging agents by increasing aqueous solubility, enhancing stability, prolonging circulation time, modifying biodistribution, etc.

When conjugated with targeting moiety such as LHRH, DDS can preferentially accumulate in tumors, release encapsulated or conjugated anticancer compounds, limit adverse side effects on normal tissues, and substantially enhance anticancer drug efficacy [17, 25, 67–72]. The most effective of these DDS consist of three components: the targeting moiety-LHRH analogs, nanocarriers, and anticancer agents (chemotherapeutics, proteins, DNA, siRNA, and imaging agents). Nanocarriers can be constructed using polymers (linear polymers,

branched dendrimers, polymeric nanogel, and micelles), lipid-based, and inorganic nanoparticles. The different chemical and physical characteristics of materials cause variations in nanocarrier properties, including pharmacokinetics, biodistribution, and cell-penetration capability. Therefore, the selection of nanocarrier materials is mainly determined by the desired therapeutic goal, toxicity/safety profile, type of anticancer drugs, and route of administration. The LHRH-targeted nano DDS discussed in this article, are summarized in Table 1.

4.1. Polymers

One widely used candidate for linear polymer-based nanocarriers is polyethylene glycol (PEG), a non-ionic water-soluble polymer, which has been approved for pharmaceutical applications by the FDA [73]. Minko and co-workers conjugated LRHR analog and an anticancer drug camptothecin (CPT) to the opposite ends of PEG (LHRH-PEG-CPT) [67]. The conjugation was shown to significantly enhance cytotoxicity to human ovarian carcinoma cells when compared to free CPT and PEG-CPT conjugates. The LHRH-PEG-CPT conjugate was also tested on mice with xenografts of human ovarian carcinoma [17]. LHRH-PEG-CPT decreased the tumor size significantly when compared with CPT alone, and PEG-CPT. Analysis of biodistribution revealed that the targeting polymer (LHRH-PEG) accumulated predominately in the ovaries (endogenous LHRH-R) of mice without tumors and in the cancer tissues (specific targeting) of mice bearing human ovarian carcinoma xenografts, whereas non-targeting conjugate (PEG) accumulated in the liver and in the tumor (enhanced permeability retention effect of nanocarriers). The level of the accumulation of tumor targeted polymer was almost twice that of PEG alone, demonstrating the specific targeting effect and enhanced activity of the anticancer drug in this LRHR-targeting DDS. The Minko group then proposed that the “active targeting” and anticancer activity could be further improved by increasing local concentrations of the targeting moiety and the drug, respectively. Therefore, more than one molecule of the targeting peptide as well as anticancer drugs were covalently conjugated with bis(2-carboxyethyl)-PEG with citric acid as a multivalent spacer [74]. The *in vitro* cytotoxicity and *in vivo* antitumor activity were tested on conjugates with up to three copies of the LHRH targeting moiety and the anticancer drug, and the authors reported amplified *in vitro* and *in vivo* activity of conjugates containing numerous copies of the targeting molecule and drug. Furthermore, a multifunctional DDS was developed based on this PEG-multivalent spacer system to address the treatment challenge of a drug-resistant ovarian tumor. To suppress cellular antiapoptotic defense primarily responsible for ovarian cancer cell drug resistance, one or several copies of the BCL2 Homology 3 domain (BH3) peptide were attached to PEG via a citric acid spacer, in addition to the anticancer drug CPT and LHRH targeting moiety [75]. In human ovarian cancer cell lines, this multifunctional DDS triggered a strong apoptotic response, and in nude mice with the human ovarian cancer xenograft, multiple treatments with DDS led to nearly complete regression of the primary tumor and prevented growth of malignant ascites.

To investigate and compare the influence of different LHRH-targeted nanocarriers on the efficacy of chemotherapy, the Minko group evaluated three commonly used nanocarriers: linear polymer (PEG), dendrimer (fourth generation polyamidoamine (PAMAM)), and

liposome (PEGylated liposome) [76]. The average size of PEG polymer, dendrimers, and liposomes were about 30, 5, and 100 nm respectively. All of the targeting DDS in this study consisted of the LHRH analog, paclitaxel (TAX) anticancer drug, and near-infrared cyanine Cy5.5 imaging agent. The result of this study revealed that attaching LHRH peptide to DDS augmented their anticancer efficacy to an extremely high level comparable for all three types of nanocarriers. Therefore, the authors suggested that tumor targeting minimizes the differences between DDS of various architecture, size, molecular mass, and composition in terms of anticancer efficacy and adverse side effects on healthy tissues. Notably, *in vivo* biodistribution of these three nanocarriers differed, though the primary accumulation site was a tumor in all cases. Additionally, the size difference of these nanocarriers was intrinsic from their composition, which should be taken into consideration. Overall, this study provided influential insight into targeted drug delivery by concluding that tumor-specific-receptor targeting of DDS is the most crucial factor in DDS design for high therapeutic activity.

The efficiency of the LHRH targeting approach was also confirmed for several other drug-loaded polymeric nanoparticles [77, 78]. Nukolova *et al.* developed block copolymer nanogels with an interior reservoir for cisplatin encapsulation [77]. The LHRH peptide was further conjugated to the nanogels surface via heterobifunctional PEG linker. *In vivo* antitumor activity studies revealed that LHRH-nanogels loaded with cisplatin substantially suppress the ovarian cancer tumor growth compared to non-targeted counterparts. Moreover, the tumor amount of cisplatin in mice treated with LHRH-nanogels was 23% greater than in animals injected with non-targeted nanogels. In another study, Li *et al.* designed LHRH-targeted cisplatin-loaded dextran nanoparticles and evaluated them in the orthotopic breast cancer metastasis model [78]. In agreement with the above discussed studies, LHRH-conjugated dextran nanoparticles significantly increased the accumulation of cisplatin in the primary and metastatic tumors, reduced drug delivery to kidneys, and improved its anticancer activity.

Biopolymers such as human serum albumin (HSA) have also been fabricated as nanocarriers for targeted delivery. Methotrexate-HSA conjugates were functionalized by LHRH as targeting moieties via a cross-linker [79]. The targeted conjugates exhibited significantly improved internalization of the anticancer drug and its antitumor activity in LHRH-R positive breast cancer cells.

4.2. Dendrimers

Dendrimers are three dimensional, hyperbranched polymeric architectures possessing a combination of peripheral functional groups and multiple interior cavities [80]. These distinctive attributes have made them attractive nanocarriers in the field of drug delivery [80]. Remarkably, targeting of the PEGylated Poly (propyleneimine) (PPI) dendrimers to the cancer cells by the LHRH peptide dramatically enhanced their accumulation in the tumor and substantially limited dendrimer accumulation in healthy organs after systemic administration [36, 71]. Therefore, the Minko and Taratula groups widely exploited LHRH-targeted PPI dendrimers for the delivery of siRNA, chemotherapeutic, imaging and phototherapeutic agents specifically to cancer tumors [36, 71, 81–83]. PPI dendrimers, for

example, have been mixed with siRNA to form nanoparticles via electrostatic interaction, caged with a dithiol cross-linker to improve stability, coated with PEG to diminish toxicity, and linked to LHRH-peptide at the distal end of PEG for targeted delivery to cancer cells [36, 71]. The siRNA-PPI complexes were found to maintain stability in plasma and were specifically uptaken by LHRH-positive tumor cells with consequent efficient gene silencing [71]. An *in vivo* body distribution study also demonstrated the high specificity of tumor accumulation of the complexes [36, 71]. The developed siRNA-PPI complexes were further employed to enhance efficacy of either chemotherapy or photodynamic therapy in the treatment of ovarian cancer through the suppression of the cancer cells resistance to these therapeutic modalities with the delivered siRNA [36, 81]. Thus, the LHRH-targeted PPI dendrimer-based complexes loaded with the *CD44* siRNA were used in tandem with paclitaxel, also chemically attached to the LHRH-targeted PPI dendrimer, for combinatorial treatment of ovarian cancer [36]. The paclitaxel was conjugated to the primary amines on the dendrimer surface via a pH sensitive linker, while the LHRH peptide was bound to the distal end of PEG that surrounds PPI-paclitaxel conjugates. The delivered *CD44* siRNA molecules and paclitaxel by the LHRH-equipped dendrimers resulted in the downregulation of the targeted protein, tumor eradication and prevention of adverse side effects of chemotherapeutic agent on healthy organs. Furthermore, the LHRH-targeted PPI dendrimers loaded with the *DJ-1* siRNA have been also used to overcome the antioxidant defense mechanism in ovarian cancer cells, which in turn significantly enhanced the therapeutic effect of photodynamic therapy [81]. The ovarian cancer tumors exposed to a single dose of this combinatorial therapy were completely eradicated from the mice and the treated animals showed no evidence of cancer recurrence.

In addition to the PPI dendrimers, a siRNA delivery system based on the LHRH targeted polyamidoamine (PAMAM) dendrimer was also developed [68, 84]. Thus, the internally cationic and surface neutral PAMAM was synthesized to enhance siRNA complexation efficiency and eliminate dendrimer toxicity associated with the surface positive amines. The targeted PAMAM dendrimers were prepared by the conjugation of a LHRH peptide to the PAMAM dendrimer surface via a succinic anhydride linker. The resulting LHRH-PAMAM conjugates efficiently complexed siRNA into spherical nanoparticles and only the targeted complexes significantly downregulated the targeted gene in LHRH-positive cancer cells.

A new generation of theranostic agent molecules, phthalocyanines (Pc) and derivatives, which possess outstanding near-infrared (NIR) photo physical properties, have also been encapsulated in PPI dendrimers for fluorescence-guided drug delivery and non-invasive treatment of cancer tumors by photodynamic and photothermal therapies [81–83, 85]. The Pc-PPI complexes were coated with PEG for biocompatibility and conjugated with a LHRH peptide for tumor-targeted delivery [81–83]. The Pc carrying DDS displayed a distinct NIR absorption (~700 nm) and fluorescence emission (~710 nm), required for efficient photodynamic therapy and fluorescence imaging. *In vitro* and *in vivo* imaging experiments demonstrated subcellular localization and organ distribution, validating the role of Pc as an imaging agent. It was also revealed that the developed LHRH-targeted DDS were effectively internalized into cancer cells and accumulated at the tumor site after intravenous administration into mice. The prepared formulation showed a low cytotoxicity under dark conditions but increased to a near 30-fold cytotoxicity with light irradiation via excessive

generation of toxic ROS [83]. The encapsulation of Pc and targeting modification of PPI addressed Pc's issues in clinical applications, such as aggregation, poor water solubility, and insufficient selectivity for cancer cells, presenting a significant potential of the designed DDS as a NIR theranostic agent. In addition, the LHRH-targeted, Pc-loaded PPI dendrimer was also conjugated with low-oxygen graphene nanosheets as a photothermal agent [82]. Notably, a low-power near-infrared irradiation of a single wavelength was used for both heat generations by the graphene nanosheets, and for reactive oxygen species (ROS)-production by Pc. The combinatorial phototherapy resulted in an enhanced destruction of ovarian cancer cells, with a killing efficacy of 90–95% [82].

4.3. Micelles

Polymeric micelles represent another type of promising polymeric LHRH-targeted DDS reported in several studies. Park and coworkers developed polyelectrolyte complex micelles by mixing poly (ethylenimine) (PEI) with siRNA-PEG-LHRH conjugates for targeted siRNA delivery to ovarian cancer cells [86]. A common approach for constructing polymeric micelles is based on core-shell architecture using amphiphilic block copolymers. For example, Jing and coworkers designed and synthesized a triblock copolymer, poly (ethylene oxide)-block-poly (allyl glycidyl ether)-block-poly (DL-lactide) (mPEG-b-PAGE-b-PLA) [87]. LHRH targeting moiety was conjugated to the allyl group on the PAGE block. This conjugate self-assembled into micelles loaded with Dox with 15–40 nm diameters and LHRH moieties in the hydrophilic corona. It was shown that these micelles were uptaken more effectively into LHRH-R positive cells (HeLa cells) than LHRH-R negative cells (HepG-2 cells), and *in vivo* biodistribution analysis showed preferred accumulation of these micelles in tumor sites at 24 hours post-injection. Mahato and coworkers assembled LHRH-conjugated micelles to deliver antiandrogen for prostate cancer treatment using polyethylene glycol-b-poly (carbonate-co-lactide) (PEG-b-p(CB-coLA)) [88]. The authors reported that an antiandrogen, CBDIV17-loaded LHRH micelles exhibited higher cellular uptake, cytotoxicity, apoptosis, and more efficient inhibition of the proliferation of tumor cells *in vitro* and tumor growth *in vivo*. Wu and coworkers developed multifunctional pH-sensitive micelles using LHRH modified poly (ethylene glycol)-poly (L-histidine)-doxorubicin (LHRH-PEG-PHIS-DOX) [89]. The micelle stability was improved through the bonding of hydrophobic Dox to the polymer backbone. These pH-sensitive micelles were used to deliver Dox-TAT (HIV-1 transactivator of transcription peptide) conjugate, which could be released following micelle dissociation at acidic tumor extracellular pH (5.0 to 6.8). The study suggested that released Dox-TAT could be delivered into the cytosol by TAT mediated pathway while undissociated micelles could be uptaken into the cells by an LHRH-R-mediated endocytosis process. Results in this study showed enhanced cytotoxicity of the constructed system against LHRH-positive A2780/Dox^R cells. Another study utilized carboxylic ligand functionalized dextran (dextran-succinic acid, Dex-SA) to form micelles, followed by the conjugation of LHRH onto the surface of micelle surface for targeted delivery of cisplatin [46]. Compared to non-targeted dextran micelles, the LHRH-targeted micelles caused substantially higher cellular internalization in MCF-7 tumor cells *in vitro* and greater accumulation in MCF-7 xenograft tumors *in vivo*. Systemic delivery of the LHRH-targeted micelles encapsulated with cisplatin displayed enhanced tumor suppression in MCF-7 tumor bearing mice as well.

4.4. Liposomes and lipid-based nanoparticles

Liposomes are another type of efficient and well characterized drug delivery carrier. They are reported to be biodegradable, biologically inert, and have limited intrinsic toxicity [90]. Song and coworkers constructed an LHRH-targeted liposome system for the delivery of an anticancer drug mitoxantrone (Mit) [91]. A peptide analog of LHRH, gonadorelin, was attached to PEGylated liposomes via a thioether bond. The loading of MIT reached 98% at a concentration of 1.0 mg/mL. *In vitro* studies on LHRH-R high expressing MCF-7 cells revealed that targeted liposomes showed higher internalization and cytotoxicity than non-targeted ones. This improved performance, however, was Mit dose-dependent, and at a certain concentration range the difference between targeted and non-targeted liposomes may not be profound. This study also pointed out that the investigated formulations of targeted liposome carrying Mit did not reach the toxicity level of free Mit. The authors suggested that the release of encapsulated Mit from endocytosed liposomes and further escape from endosomes or lysosomes was lower than free Mit, in accordance with previous studies [92].

Nanostructured Lipid Carriers (NLCs) are the new generation of lipid nanoparticles combined with advantages from different nanocarriers including liposomes [93]. A multifunctional NCL DDS was developed by the Minko group for pulmonary co-delivery of an anticancer drug and siRNA by inhalation [94]. This nanostructured lipid carrier contains an analog of LHRH as a targeting moiety on the surface, encapsulated anticancer drug (doxorubicin or paclitaxel), and electrostatically bound siRNAs targeted to *MRP1* mRNA as a suppressor of drug efflux pumps (pump drug resistance), as well as siRNA targeted to *BCL2* mRNA as a suppressor of antiapoptotic defense (nonpump drug resistance). In studies on mouse orthotopic models of human lung cancer, after inhalation, the proposed NCL effectively delivered its payload to lung cancer cells, whereas healthy lung tissues and organs were significantly less exposed when compared with intravenous injection. Results displayed efficient suppression of tumor growth and prevention of adverse side effects on healthy organs, demonstrating high efficiency of this NCL DDS for tumor-targeted delivery by inhalation of anticancer drugs and siRNA mixtures specifically to lung cancer cells.

4.5. Inorganic nanoparticles

Inorganic nanocarriers have also been constructed for LHRH-targeted DDS. Mesoporous silica nanoparticles (MSN) possess large pore volume and surface area, which are ideal for loading a large amount of drugs inside the pores and conjugation of other active components on the surface. In a recent study, the MSN surface was modified with conjugation of the LHRH peptide via PEG spacer, and loaded with anticancer drugs (doxorubicin and cisplatin), in combination of two types of siRNA as suppressors of pump and nonpump cellular resistance [95]. The experiment data confirmed enhanced cytotoxicity on human lung adenocarcinoma cell lines. In a mouse orthotopic model, the local delivery of this system by inhalation led to preferential accumulation of nanocarriers in the lung, and minimized the escape of MSN into the systemic circulation and their accumulation in other organs. Another study employed superparamagnetic iron oxide nanoparticles (SPION) to combine with the targeted delivery of chemotherapeutic agents and mild hyperthermia for synergistic therapy [83]. SPION were coated with oleic acid, poly (maleic anhydride-alt-1-octadecene), and poly (ethylene-imine) (PEI) loading of the anticancer drug Dox and the

conjugation of the LHRH peptide as the targeting moiety. This DDS displayed faster drug release in acidic conditions mimicking the tumor environment and efficient internalization by drug resistant ovarian cancer cells. Application of mild hyperthermia (40 °C) generated by SPION under exposure to an alternating magnetic field was shown to improve Dox anticancer activity. In addition, the LHRH-targeted SPION were also investigated for siRNA delivery to the lung cancer tumors [96]. The positively charged SPION in tandem with PPI dendrimers were employed to complex *BCL2* siRNA via electrostatic interaction. The resulting complexes were further modified with PEG and LHRH peptide. The developed siRNA delivery system sufficiently enhanced *in vivo* antitumor activity of cisplatin.

As inorganic/metallic nanoparticles revealed strong potential toward the development of new targeted carriers for cancer therapy, some were found to be highly cytotoxic [97]. Sun and coworkers proposed that these NPs could be utilized as self-therapeutic agents as well as nanocarriers, circumventing multistep conjugation of drugs onto the NPs [98]. Therefore, chemically disordered face-centered cubic (fcc) FePt NPs were functionalized with an LHRH peptide via phospholipid. The LHRH-fcc-FePt NPs were found to bind preferentially, and were highly toxic to the human ovarian cancer cell line. It was concluded that their cytotoxicity resulted from the release of Fe from fcc-NPs in low pH solution, which could further catalyze H₂O₂ decomposition into ROS within cells, causing fast oxidation and deterioration of cellular membranes. This work provided a new type of DDS with the dual roles of a carrier and an anticancer drug for targeted cancer therapy.

The LHRH-targeted inorganic nanoparticles have been also studied as contrast agents for PET and magnetic resonance imaging (MRI) [69]. Water soluble manganese oxide (Mn₃O₄) nanoparticles were synthesized by coating hydrophobic Mn₃O₄ nanocrystals with lipid-PEG molecules [69]. In the next step, the targeted Mn₃O₄ nanoparticles were prepared by the coupling of maleimide moiety on the lipid-PEG molecules with a cysteine residue on the LHRH peptide. The prepared nanoparticles exhibited specific accumulation in lung, ovarian, and melanoma tumors after systemic administration and substantially enhanced MRI signal in the cancer masses. Several studies also reported the development of LHRH-targeted SPION for MRI application [99]. The targeted SPION-based contrast agents were prepared by linking of the carboxylated LHRH peptide to the amine groups on the SPION surface. LHRH peptide increased accumulation of SPION in cancer cells by nine times when compared to non-targeted nanoparticles [99]. Furthermore, *in vivo* studies demonstrated that LHRH-SPION selectively accumulated in primary breast cancer tumors and lung metastasis. Gao *et al.* developed a chelator-free [⁶⁴Cu]Cu nanoclusters as radioactive tracers for PET imaging by employing LHRH modified bovine serum albumin as a scaffold [100]. The tumor uptake of the LHRH-equipped nanoclusters was four times higher when compared to their non-targeted counterparts after tail vein injection. The developed cluster significantly improved PET imaging of orthotopic lung cancer.

5. SAFETY OF LHRH-TARGETED DDS

Because LHRH receptors are expressed in the pituitary gland, theoretically there is a probability that DDS targeted to these receptors could also accumulate in the pituitary gonadotrophic cells. However, the previous study demonstrated that LHRH-targeted DDS do

not breach the pituitary barriers from the systemic circulation and the normal functions of this organ were not detectably impaired [17]. The Minko group revealed that LHRH-targeted DDS (LHRH-PEG-Camptothecin) do not influence the plasma concentration of luteinizing hormone (LH) following systemic administration. Moreover, the female mice, treated four times within a two-week period with LHRH-PEG- Camptothecin (2.5 mg/kg), had healthy viable offspring. The number and viability of offspring per mouse, their behavior, and weight change during the following four weeks did not vary from those of the control mice [17]. In addition, an FDA approved orphan drug, AEZS-108 (LHRH peptide linked to doxorubicin) showed a favorable safety profile in Phase I and II clinical trials, and no permanent impairment of the pituitary gland function was reported [46, 101].

CONCLUSIONS AND FUTURE PERSPECTIVES

The overexpression of LHRH receptors has been discovered in many types of cancer. Therefore, many drug delivery strategies have been developed for cancer-specific therapy based on LHRH targeting. Extensive studies exploited the LHRH peptide or its analogs as a targeting ligand for potent chemotherapeutic drugs, demonstrating the efficiency of the specific binding between LHRH peptide/analog-based carriers and LHRH-R in cancer cells/tumors. Many *in vitro* and *in vivo* experiments also reported enhanced internalization of the drugs into cancer cells and accumulation in tumor site, confirming encouraging effectiveness of the LHRH-targeted delivery. Importantly, the entrance of [D-Lys⁶]-LHRH-Dox (AEZS-108) into phase III clinical trial and its FDA-granted orphan drug status for endometrial and ovarian cancers demonstrate significant potential of the LHRH-based targeting strategy. Recent focus and efforts have also been put into the design and development of novel nano drug delivery systems using LHRH peptide/analog as targeting moieties. Studies also revealed positive results on many types of LHRH-DDS in terms of binding, accumulation, and treatment efficacy. Furthermore, several newly constructed DDS were multifunctional and were able of delivering not only conventional chemotherapeutic drugs but also oligonucleotides for gene therapy, photoactive drugs for PDT, and imaging agents. While current and future research on the development of innovative platforms for LHRH-based DDS is needed to broaden treatment options for LHRH-R expressing cancers, additional preclinical and clinical studies are also in urgent demand to provide detailed knowledge to guide the development of pharmacokinetics, cell/tissue penetration ability, and acute and long-term safety.

ACKNOWLEDGEMENTS

The authors are grateful for funding support provided by the OSU College of Pharmacy, OSU Venture Development Fund, OSU General Research Fund and in part by the R01 CA175318 grant from the National Cancer Institute.

REFERENCES

- [1]. 2015 Cancer facts and figures. American Cancer Society. <http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2014/>.
- [2]. Minko T Nanotechnology and drug resistance. *Adv. Drug Deliv. Rev.* 2013, 65, 1665–1666. [PubMed: 24177352]

- [3]. Minko T; Rodriguez-Rodriguez L; Pozharov V Nanotechnology approaches for personalized treatment of multidrug resistant cancers. *Adv. Drug Deliv. Rev.* 2013, 65, 1880–1895. [PubMed: 24120655]
- [4]. Bae YH; Park K Targeted drug delivery to tumors: Myths, reality and possibility. *J. Control. Release*, 2011, 153, 198–205. [PubMed: 21663778]
- [5]. Torchilin VP Passive and active drug targeting: Drug delivery to tumors as an example. *Handb. Exp. Pharmacol.* 2010, 197, 3–53.
- [6]. Bertrand N; Wu J; Xu X; Kamaly N; Farokhzad OC Cancer nanotechnology: The impact of passive and active targeting in the era of modern cancer biology. *Adv. Drug Deliv. Rev.* 2014, 66, 2–25. [PubMed: 24270007]
- [7]. 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, 135–146. [PubMed: 20797419]
- [8]. Limonta P; Montagnani Marelli M; Mai S; Motta M; Martini L; Moretti RM GnRH receptors in cancer: From cell biology to novel targeted therapeutic strategies. *Endocr. Rev.* 2012, 33, 784–811. [PubMed: 22778172]
- [9]. Houshmand P; Zlotnik A Targeting tumor cells. *Curr. Opin. Cell Biol.* 2003, 15, 640–644. [PubMed: 14519400]
- [10]. Reubi JC Peptide receptors as molecular targets for cancer diagnosis and therapy. *Endocr. Rev.* 2003, 24, 389–427. [PubMed: 12920149]
- [11]. Stevanovic S Identification of tumour-associated t-cell epitopes for vaccine development. *Nat. Rev. Cancer*, 2002, 2, 514–520. [PubMed: 12094237]
- [12]. Ferro VA; Stimson WH Anti-gonadotropin releasing hormone vaccines and their potential use in the treatment of hormone-responsive cancers. *Biodrugs*, 1999, 12, 1–12. [PubMed: 18031157]
- [13]. Schally AV; Arimura A; Kastin AJ Hypothalamic regulatory hormones. *Science*, 1973, 179, 341–350. [PubMed: 4345570]
- [14]. Conn PM; Crowley WF Gonadotropin-releasing hormone and its analogs. *Ann. Rev. Med.* 1994, 45, 391–405. [PubMed: 8198390]
- [15]. Conn PM; Huckle WR; Andrews WV; McArdle CA The molecular mechanism of action of gonadotropin releasing hormone (GnRH) in the pituitary. *Recent Prog. Horm. Res.* 1987, 43, 29–68. [PubMed: 2819996]
- [16]. Schally AV; Nagy A Chemotherapy targeted to cancers through tumoral hormone receptors. *Trends Endocrinol. Metab.* 2004, 15, 300–310. [PubMed: 15350601]
- [17]. Dharap SS; Wang Y; Chandna P; Khandare JJ; Qiu B; Gunaseelan S; Sinko PJ; Stein S; Farmanfarmaian A; Minko T Tumor-specific targeting of an anticancer drug delivery system by lhrh peptide. *Proc. Natl. Acad. Sci. U S A*, 2005, 102, 12962–12967. [PubMed: 16123131]
- [18]. Nagy A; Schally AV Targeting of cytotoxic luteinizing hormone-releasing hormone analogs to breast, ovarian, endometrial, and prostate cancers. *Biol. Reprod.* 2005, 73, 851–859. [PubMed: 16033997]
- [19]. Emons G; Weiss S; Ortmann O; Grundker C; Schulz KD Lhrh might act as a negative autocrine regulator of proliferation of human ovarian cancer. *Eur. J. Endocrinol.* 2000, 142, 665–670. [PubMed: 10832105]
- [20]. Irmner G; Burger C; Muller R; Ortmann O; Peter U; Kakar SS; Neill JD; Schulz KD; Emons G Expression of the messenger rnas for luteinizing hormone-releasing hormone (LHRH) and its receptor in human ovarian epithelial carcinoma. *Cancer Res.* 1995, 55, 817–822. [PubMed: 7850795]
- [21]. Schally AV; Comaru-Schally AM Mode of action of LHRH analogs Holland-Frei Cancer Medicine. 6th edition, 2003.
- [22]. Cuzick J; Ambroisine L; Davidson N; Jakesz R; Kaufmann M; Regan M; Sainsbury R Use of luteinising-hormone-releasing hormone agonists as adjuvant treatment in premenopausal patients with hormone-receptor-positive breast cancer: A meta-analysis of individual patient data from randomised adjuvant trials. *Lancet*, 2007, 369, 1711–1723. [PubMed: 17512856]

- [23]. Gründker C; Günthert AR; Millar RP; Emons G Expression of gonadotropin-releasing hormone II (GnRH-II) receptor in human endometrial and ovarian cancer cells and effects of GnRH-II on tumor cell proliferation. *J. Clin. Endocrinol. Metab.*, 2002, 87, 1427–1430. [PubMed: 11889221]
- [24]. Gründker C; Emons G Role of gonadotropin-releasing hormone (GnRH) in ovarian cancer. *Reprod. Biol. Endocrinol.*, 2003, 1, 65. [PubMed: 14594454]
- [25]. Dharap SS; Minko T Targeted proapoptotic LHRH-BH3 peptide. *Pharm. Res.*, 2003, 20, 889–896. [PubMed: 12817893]
- [26]. Limonta P; Marelli MM; Mai S; Motta M; Martini L; Moretti RM GnRH receptors in cancer: From cell biology to novel targeted therapeutic strategies. *Endocrine Rev.*, 2012, 33, 784–811. [PubMed: 22778172]
- [27]. Friess H; Büchler M; Kiesel L; Krüger M; Beger HG LH-RH receptors in the human pancreas. Basis for antihormonal treatment in ductal carcinoma of the pancreas. *Int. J. Pancreatol.*, 1991, 10, 151–159. [PubMed: 1660910]
- [28]. Szende B; Srkalovic G; Timar J; Mulchahey JJ; Neill JD; Lapis K; Csikos A; Szepeshazi K; Schally AV Localization of receptors for luteinizing hormone-releasing hormone in pancreatic and mammary cancer cells. *Proc. Natl. Acad. Sci. U S A*, 1991, 88, 4153–4156. [PubMed: 1851995]
- [29]. Koushik K; Bandi N; Sundaram S; Kompella UB Evidence for LHRH-receptor expression in human airway epithelial (Calu-3) cells and its role in the transport of an LHRH agonist. *Pharm. Res.*, 2004, 21, 1034–1046. [PubMed: 15212170]
- [30]. Moretti RM; Montagnani Marelli M; Van Groeninghen JC; Limonta P Locally expressed lhrh receptors mediate the oncostatic and antimetastatic activity of lhrh agonists on melanoma cells. *J. Clin. Endocrinol. Metab.*, 2002, 87, 3791–3797. [PubMed: 12161512]
- [31]. Montagnani Marelli M; Moretti RM; Mai S; Müller O; Van Groeninghen JC; Limonta P Novel insights into gnrh receptor activity: Role in the control of human glioblastoma cell proliferation. *Oncol. Rep.*, 2009, 21, 1277–1282. [PubMed: 19360304]
- [32]. Gründker C; Ernst J; Reutter MD; M. GB; G. E Effective targeted chemotherapy using AEZS-108 (AN-152) for lhrh receptor-positive pancreatic cancers. *Oncol. Rep.*, 2011, 26, 629–635. [PubMed: 21667032]
- [33]. Engel J; Emons G; Pinski J; Schally AV Aezs-108: A targeted cytotoxic analog of LHRH for the treatment of cancers positive for LHRH receptors. *Exp. Opin. Investig. Drugs*, 2012, 21, 891–899.
- [34]. Grundker C; Volker P; Griesinger F; Ramaswamy A; Nagy A; Schally AV; Emons G Antitumor effects of the cytotoxic luteinizing hormone-releasing hormone analog an-152 on human endometrial and ovarian cancers xenografted into nude mice. *Am. J. Obstet. Gynecol.*, 2002, 187, 528–537. [PubMed: 12237622]
- [35]. Straub B; Muller M; Krause H; Schrader M; Goessl C; Heicappell R; Miller K Increased incidence of luteinizing hormone-releasing hormone receptor gene messenger rna expression in hormone-refractory human prostate cancers. *Clin. Cancer Res.*, 2001, 7, 2340–2343. [PubMed: 11489810]
- [36]. Shah V; Taratula O; Garbuzenko OB; Taratula OR; Rodriguez-Rodriguez L; T., M. Targeted nanomedicine for suppression of CD44 and simultaneous cell death induction in ovarian cancer: An optimal delivery of sirna and anticancer drug. *Clin. Cancer Res.*, 2013, 19, 6193–6204. [PubMed: 24036854]
- [37]. Liu SV; Schally AV; Hawes D; Xiong S; Fazli L; Gleave M; Cai J; Groshen S; Brands F; Engel J; Pinski J Expression of receptors for luteinizing hormone-releasing hormone (LH-RH) in prostate cancers following therapy with LH-RH agonists. *Clin. Cancer Res.*, 2010, 16, 4675–4680. [PubMed: 20670943]
- [38]. Bajusz S; Janaky T; Csernus VJ; Bokser L; Fekete M; Srkalovic G; Redding TW; Schally AV Highly potent metalloptide analogues of luteinizing hormone-releasing hormone. *Proc. Natl. Acad. Sci. U S A*, 1989, 86, 6313–6317. [PubMed: 2548206]
- [39]. Bajusz S; Janaky T; Csernus VJ; Bokser L; Fekete M; Srkalovic G; Redding TW; Schally AV Highly potent analogues of luteinizing hormone-releasing hormone containing D-phenylalanine

nitrogen mustard in position 6. *Proc. Natl. Acad. Sci. U S A*, 1989, 86, 6318–6322. [PubMed: 2548207]

- [40]. Nagy A; Schally AV; Armatis P; Szepeshazi K; Halmos G; Kovacs M; Zarandi M; Groot K; Miyazaki M; Jungwirth A; Horvath J Cytotoxic analogs of luteinizing hormone-releasing hormone containing doxorubicin or 2-pyrrolinodoxorubicin, a derivative 500–1000 times more potent. *Proc. Natl. Acad. Sci. U S A*, 1996, 93, 7269–7273. [PubMed: 8692981]
- [41]. Arencibia JM; Schally AV; Krupa M; Bajo AM; Nagy A; Szepeshazi K; Plonowski A Targeting of doxorubicin to ES-2 human ovarian cancers in nude mice by linking to an analog of luteinizing hormone-releasing hormone improves its effectiveness. *Int. J. Oncol*, 2001, 19, 571–577. [PubMed: 11494038]
- [42]. Engel JB; Keller G; Schally AV; Nagy A; Chism DD; Halmos G Effective treatment of experimental human endometrial cancers with targeted cytotoxic luteinizing hormone-releasing hormone analogues an-152 and an-207. *Fertil. Steril*, 2005, 83 Suppl 1, 1125–1133. [PubMed: 15831285]
- [43]. Halmos G; Nagy A; Lamharzi N; Schally AV Cytotoxic analogs of luteinizing hormone-releasing hormone bind with high affinity to human breast cancers. *Cancer Lett*, 1999, 136, 129–136. [PubMed: 10355741]
- [44]. Krebs LJ; Wang X; Nagy A; Schally AV; Prasad PN; Liebow C A conjugate of doxorubicin and an analog of luteinizing hormone-releasing hormone shows increased efficacy against oral and laryngeal cancers. *Oral. Oncol*, 2002, 38, 657–663. [PubMed: 12353491]
- [45]. Letsch M; Schally AV; Szepeshazi K; Halmos G; Nagy A Preclinical evaluation of targeted cytotoxic luteinizing hormone-releasing hormone analogue AN-152 in androgen-sensitive and insensitive prostate cancers. *Clin. Cancer Res*, 2003, 9, 4505–4513. [PubMed: 14555524]
- [46]. Emons G; Gorchev G; Sehouli J; Wimberger P; Stähle A; Hanker L; Hilpert F; Sindermann H; Gründker C; Harter P Efficacy and safety of AEZS-108 (INN: Zoptarelin doxorubicin acetate) an LHRH agonist linked to doxorubicin in women with platinum refractory or resistant ovarian cancer expressing LHRH receptors: A multicenter phase ii trial of the ago-study group (AGO GYN 5). *Gynecol. Oncol*, 2014, 133, 427–432. [PubMed: 24713545]
- [47]. Liu SV; Tsao-Wei DD; Xiong S; Groshen S; Dorff TB; Quinn DI; Tai YC; Engel J; Hawes D; Schally AV; Pinski JK Phase I, dose-escalation study of the targeted cytotoxic LHRH analog AEZS-108 in patients with castration- and taxane-resistant prostate cancer. *Clin. Cancer Res*, 2014, 20, 6277–6283. [PubMed: 25278449]
- [48]. Mezo G; Manea M Receptor-mediated tumor targeting based on peptide hormones. *Expert Opin. Drug Deliv*, 2010, 7, 79–96. [PubMed: 19947889]
- [49]. Engel JB; Schally AV; Dietl J; Rieger L; Hönig A Targeted therapy of breast and gynecological cancers with cytotoxic analogues of peptide hormones. *Mol. Pharm*, 2007, 4, 652–658. [PubMed: 17705441]
- [50]. Schally AV; Nagy A New approaches to treatment of various cancers based on cytotoxic analogs of LHRH, somatostatin and bombesin. *Life Sci*, 2003, 72, 2305–2320. [PubMed: 12639697]
- [51]. Aggarwal S; Ndinguri MW; Solipuram R; Wakamatsu N; Hammer RP; Ingram D; Hansel W [dlys(6)]-luteinizing hormone releasing hormone-curcumin conjugate inhibits pancreatic cancer cell growth in vitro and in vivo. *Int. J. Cancer*, 2011, 129, 1611–1623. [PubMed: 21484797]
- [52]. Karampelas T; Argyros O; Sayyad N; Spyridaki K; Pappas C; Morgan K; Kolios G; Millar RP; Liapakis G; Tzakos AG; Fokas D; Tamvakopoulos C GnRH-gemcitabine conjugates for the treatment of androgen-independent prostate cancer: Pharmacokinetic enhancements combined with targeted drug delivery. *Bioconjug. Chem*, 2014, 25, 813–823. [PubMed: 24661240]
- [53]. Sower SA; Chiang YC; Lovas S; Conlon JM Primary structure and biological activity of a third gonadotropin-releasing hormone from lamprey brain. *Endocrinology*, 1993, 132, 1125–1131. [PubMed: 8440174]
- [54]. Lovas S; Pályi I; Vincze B; Horváth J; Kovács M; Mezö I; Tóth G; Teplán I; Murphy RF Direct anticancer activity of gonadotropin-releasing hormone-III. *J Pept Res*, 1998, 52, 384–389. [PubMed: 9894843]

- [55]. Kovacs M; Seprodi J; Koppan M; Horvath JE; Vincze B; Teplan I; Flerko B Lamprey gonadotropin hormone-releasing hormone-III has no selective follicle-stimulating hormone-releasing effect in rats. *J. Neuroendocrinol*, 2002, 14, 647–655. [PubMed: 12153467]
- [56]. Mezo G; Manea M; Szabí I; Vincze B; Kovács M New derivatives of GnRH as potential anticancer therapeutic agents. *Curr. Med. Chem*, 2008, 15, 2366–2379. [PubMed: 18855666]
- [57]. Orbán E; Mezo G; Schlage P; Csík G; Kuli Z; Ansorge P; Fellingner E; Möller HM; Manea M In vitro degradation and antitumor activity of oxime bond-linked daunorubicin-GnRH-III bioconjugates and DNA-binding properties of daunorubicin-amino acid metabolites. *Amino Acids*, 2011, 41, 469–483. [PubMed: 20953647]
- [58]. Szabó I; Manea M; Orbán E; Csámpai A; Bosze S; Szabó R; Tejeda M; Gaál D; Kapuvári B; Przybylski M; Hudecz F; Mezo G Development of an oxime bond containing daunorubicin-gonadotropin-releasing hormone-III conjugate as a potential anticancer drug. *Bioconju. Chem*, 2009, 20, 656–665.
- [59]. Leurs U; Lajko E; Mezo G; Orban E; Ohlschlager P; Marquardt A; Kohidai L; Manea M GnRH-III based multifunctional drug delivery systems containing daunorubicin and methotrexate. *Eur. J. Med. Chem*, 2012, 52, 173–183. [PubMed: 22480495]
- [60]. Leurs U; Mezo G; Orban E; Ohlschlager P; Marquardt A; Manea M Design, synthesis, in vitro stability and cytostatic effect of multifunctional anticancer drug-bioconjugates containing GnRH-III as a targeting moiety. *Biopolymers*, 2012, 98, 1–10. [PubMed: 21509746]
- [61]. Guo H; Gallazzi F; Sklar LA; Miao Y A novel indium-111-labeled gonadotropin-releasing hormone peptide for human prostate cancer imaging. *Bioorg. Med. Chem. Lett*, 2011, 21, 5184–5187. [PubMed: 21821417]
- [62]. Guo H; Lu J; Hathaway H; Royce ME; Prossnitz ER; Miao Y Synthesis and evaluation of novel gonadotropin-releasing hormone receptor-targeting peptides. *Bioconjug. Chem*, 2011, 22, 1682–1689. [PubMed: 21749045]
- [63]. Barda Y; Cohen N; Lev V; Ben-Aroya N; Koch Y; Mishani E; Fridkin M; Gilon C Backbone metal cyclization: Novel 99m Tc labeled GnRH analog as potential SPECT molecular imaging agent in cancer. *Nucl. Med. Biol*, 2004, 31, 921–933. [PubMed: 15464394]
- [64]. Schottelius M; Berger S; Poethko T; Schwaiger M; Wester HJ Development of novel 68Ga- and 18F-labeled GnRH-I analogues with high GnRHR-targeting efficiency. *Bioconjug. Chem*, 2008, 19, 1256–1268. [PubMed: 18510351]
- [65]. Rahimipour S; Ben-Aroya N; Ziv K; Chen A; Fridkin M; Koch Y Receptor-mediated targeting of a photosensitizer by its conjugation to gonadotropin-releasing hormone analogues. *J. Med. Chem*, 2003, 46, 3965–3974. [PubMed: 12954050]
- [66]. Xu P; Chen J; Chen Z; Zhou S; Hu P; Chen X; Huang M Receptor-targeting phthalocyanine photosensitizer for improving antitumor photocytotoxicity. *PLoS One*, 2012, 7, e37051. [PubMed: 22693566]
- [67]. Dharap SS; Qiu B; Williams GC; Sinko P; Stein S; Minko T Molecular targeting of drug delivery systems to ovarian cancer by BH3 and lhrh peptides. *J. Control. Release*, 2003, 91, 61–73. [PubMed: 12932638]
- [68]. Patil ML; Zhang M; Taratula O; Garbuzenko OB; He H; Minko T Internally cationic polyamidoamine PAMAM-OH dendrimers for siRNA delivery: Effect of the degree of quaternization and cancer targeting. *Biomacromolecules*, 2009, 10, 258–266. [PubMed: 19159248]
- [69]. Savla R; Garbuzenko OB; Chen S; Rodriguez-Rodriguez L; Minko T Tumor-targeted responsive nanoparticle-based systems for magnetic resonance imaging and therapy. *Pharm. Res*, 2014, 31, 3487–3502. [PubMed: 24919932]
- [70]. Shah V; Taratula O; Garbuzenko OB; Taratula OR; Rodriguez-Rodriguez L; Minko T Targeted nanomedicine for suppression of CD44 and simultaneous cell death induction in ovarian cancer: An optimal delivery of sirna and anticancer drug. *Clin. Cancer Res*, 2013, 19, 6193–6204. [PubMed: 24036854]
- [71]. Taratula O; Garbuzenko OB; Kirkpatrick P; Pandya I; Savla R; Pozharov VP; He HX; Minko T Surface-engineered targeted PPI dendrimer for efficient intracellular and intratumoral siRNA delivery. *J. Control. Release*, 2009, 140, 284–293. [PubMed: 19567257]

- [72]. Taratula O; Schumann C; Naleway MA; Pang AJ; Chon KJ A multifunctional theranostic platform based on phthalocyanine-loaded dendrimer for image-guided drug delivery and photodynamic therapy. *Mol. Pharm*, 2013, 10, 3946–3958. [PubMed: 24020847]
- [73]. Greenwald RB Peg drugs: An overview. *J. Control. Release*, 2001, 74, 159–171. [PubMed: 11489492]
- [74]. Khandare JJ; Chandna P; Wang Y; Pozharov VP; Minko T Novel polymeric prodrug with multivalent components for cancer therapy. *J. Pharmacol. Experim. Ther*, 2006, 317, 929–937.
- [75]. Chandna P; Khandare JJ; Ber E; Rodriguez-Rodriguez L; Minko T Multifunctional tumor-targeted polymer-peptide-drug delivery system for treatment of primary and metastatic cancers. *Pharm. Res*, 2010, 27, 2296–2306. [PubMed: 20700631]
- [76]. Saad M; Garbuzenko OB; Ber E; Chandna P; Khandare JJ; Pozharov VP; Minko T Receptor targeted polymers, dendrimers, liposomes: Which nanocarrier is the most efficient for tumor-specific treatment and imaging? *J. Control. Release*, 2008, 130, 107–114. [PubMed: 18582982]
- [77]. Nukolova NV; Oberoi HS; Zhao Y; Chekhonin VP; Kabanov AV; Bronich TK LHRH-targeted nanogels as a delivery system for cisplatin to ovarian cancer. *Mol. Pharm*, 2013, 10, 3913–3921. [PubMed: 23957812]
- [78]. Li M; Tang Z; Zhang Y; Lv S; Li Q; Chen X Targeted delivery of cisplatin by LHRH-peptide conjugated dextran nanoparticles suppresses breast cancer growth and metastasis. *Acta Biomater*, 2015, 18, 132–143. [PubMed: 25735801]
- [79]. Taheri A; Dinarvand R; Atyabi F; Ahadi F; Nouri FS; Ghahremani MH; Ostad SN; Borougeni AT; Mansoori P Enhanced anti-tumoral activity of methotrexate-human serum albumin conjugated nanoparticles by targeting with luteinizing hormone-releasing hormone (LHRH) peptide. *Int. J. Mol. Sci*, 2011, 12, 4591–4608. [PubMed: 21845098]
- [80]. Kesharwani P; Jain K; Jain NK Dendrimer as nanocarrier for drug delivery. *Prog. Polymer Sci*, 2014, 39, 268–307.
- [81]. Schumann C; Taratula O; Khalimonchuk O; Palmer AL; Cronk LM; Jones CV; Escalante CA; Taratula O ROS-induced nanotherapeutic approach for ovarian cancer treatment based on the combinatorial effect of photodynamic therapy and DJ-1 gene suppression. *Nanomedicine*, 2015, S1549–9634(15)00148–3.
- [82]. Taratula O; Patel M; Schumann C; Naleway MA; Pang AJ; He H Phthalocyanine-loaded graphene nanoplateform for imaging-guided combinatorial phototherapy. *Int. J. Nanomedicine*, 2015, 10, 2347–2362. [PubMed: 25848255]
- [83]. Taratula O; Dani RK; Schumann C; Xu H; Wang A; Song H; Dhagat P Multifunctional nanomedicine platform for concurrent delivery of chemotherapeutic drugs and mild hyperthermia to ovarian cancer cells. *Int. J. Pharm*, 2013, 458, 169–180. [PubMed: 24091153]
- [84]. Minko T; Patil ML; Zhang M; Khandare JJ; Saad M; Chandna P; Taratula O LHRH-targeted nanoparticles for cancer therapeutics. *Methods Mol. Biol*, 2010, 624, 281–294. [PubMed: 20217603]
- [85]. Taratula O; Schumann C; Duong T; Taylor KL Dendrimer-encapsulated naphthalocyanine as a single agent-based theranostic nanoplateform for near-infrared fluorescence imaging and combinatorial anticancer phototherapy. *Nanoscale*, 2015, 7, 3888–3902. [PubMed: 25422147]
- [86]. Kim SH; Jeong JH; Lee SH; Kim SW; Park TG LHRH receptor-mediated delivery of siRNA using polyelectrolyte complex micelles self-assembled from siRNA-PEG-LHRH conjugate and PEI. *Bioconjug. Chem*, 2008, 19, 2156–2162. [PubMed: 18850733]
- [87]. Wang R; Hu XL; Yue J; Zhang WJ; Cai LY; Xie ZG; Huang YB; Jing XB Luteinizing-hormone-releasing-hormone-containing biodegradable polymer micelles for enhanced intracellular drug delivery. *J. Mater. Chem. B*, 2013, 1, 293–301.
- [88]. Wen D; Chitkara D; Wu H; Danquah M; Patil R; Miller DD; Mahato RI LHRH-conjugated micelles for targeted delivery of antiandrogen to treat advanced prostate cancer. *Pharm. Res*, 2014, 31, 2784–2795. [PubMed: 24789451]
- [89]. Yang TH; Li F; Zhang HT; Fan L; Qiao YB; Tan GG; Zhang HF; Wu H Multifunctional pH-sensitive micelles for tumor-specific uptake and cellular delivery. *Polymer Chem*, 2015, 6, 1373–1382.

- [90]. Allen TM; Cullis PR Drug delivery systems: Entering the mainstream. *Science*, 2004, 303, 1818–1822. [PubMed: 15031496]
- [91]. He YN; Zhang LH; Song CX Luteinizing hormone-releasing hormone receptor-mediated delivery of mitoxantrone using LHRH analogs modified with PEGylated liposomes. *Int. J. Nanomedicine*, 2010, 5, 697–705. [PubMed: 20957221]
- [92]. Beuttler J; Rothdiener M; Müller D; Frejd FY; Kontermann RE Targeting of epidermal growth factor receptor (EGFR)-expressing tumor cells with sterically stabilized affibody liposomes (SAL). *Bioconjug. Chem*, 2009, 20, 1201–1208. [PubMed: 19435362]
- [93]. Uner M Preparation, characterization and physico-chemical properties of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC): Their benefits as colloidal drug carrier systems. *Pharmazie*, 2006, 61, 375–386. [PubMed: 16724531]
- [94]. Taratula O; Kuzmov A; Shah M; Garbuzenko OB; Minko T Nanostructured lipid carriers as multifunctional nanomedicine platform for pulmonary co-delivery of anticancer drugs and siRNA. *J. Control. Release*, 2013, 171, 349–357.
- [95]. Taratula O; Garbuzenko OB; Chen AM; Minko T Innovative strategy for treatment of lung cancer: Targeted nanotechnology-based inhalation co-delivery of anticancer drugs and siRNA. *J. Drug Target*, 2011, 19, 900–914. [PubMed: 21981718]
- [96]. Taratula O; Garbuzenko O; Savla R; Wang YA; He H; Minko T Multifunctional nanomedicine platform for cancer specific delivery of siRNA by superparamagnetic iron oxide nanoparticles-dendrimer complexes. *Curr. Drug Deliv*, 2011, 8, 59–69. [PubMed: 21034421]
- [97]. Gao J; Liang G; Zhang B; Kuang Y; Zhang X; Xu B FePt@CoS(2) yolk-shell nanocrystals as a potent agent to kill HeLa cells. *J. Am. Chem. Soc*, 2007, 129, 1428–1433. [PubMed: 17263428]
- [98]. Xu CJ; Yuan ZL; Kohler N; Kim JM; Chung MA; Sun SH FePt nanoparticles as an Fe reservoir for controlled Fe release and tumor inhibition. *J. Am. Chem. Soc*, 2009, 131, 15346–15351. [PubMed: 19795861]
- [99]. Zhou J; Leuschner C; Kumar C; Hormes JF; Soboyejo WO Sub-cellular accumulation of magnetic nanoparticles in breast tumors and metastases. *Biomaterials*, 2006, 27, 2001–2008. [PubMed: 16280161]
- [100]. Gao F; Cai P; Yang W; Xue J; Gao L; Liu R; Wang Y; Zhao Y; He X; Zhao L; Huang G; Wu F; Chai Z; Gao X Ultrasmall [(64)Cu]Cu nanoclusters for targeting orthotopic lung tumors using accurate positron emission tomography imaging. *ACS Nano*, 2015, 9, 4976–4986. [PubMed: 25919205]
- [101]. Emons G; Kaufmann M; Gorchev G; Tsekova V; Grundker C; Gunthert AR; Hanker LC; Velikova M; Sindermann H; Engel J; Schally AV Dose escalation and pharmacokinetic study of AEZS-108 (AN-152), an LHRH agonist linked to doxorubicin, in women with LHRH receptor-positive tumors. *Gynecol. Oncol*, 2010, 119, 457–461. [PubMed: 20828803]

Figure 1.
LHRH-targeted drug delivery systems

Table 1.

Summary of LHRH-targeted nano-DDS

Delivery System	Carrier Composition	Cancer Type	Reference
Polymeric NP	PEG	Ovarian	67, 17, 74, 75,76
	PEG-PMAA	Ovarian	77
	Dextran	Breast	78
	HSA	Breast	79
Dendrimer	PPI	Ovarian	36, 71, 81–83, 85
	PAMAM	Ovarian	68, 76, 84
Micelle	PEI	Ovarian	86
	PEG-PAGE-PLA	Cervical	87
	PEG-b-p(CB-coLA)	Prostate	88
	PEG-PHIS	Ovarian	89
Liposome and Lipid-based NP	Liposome	Breast	76, 91
	NCL	Lung	94
Inorganic NP	MSN	Lung	95
	SPION	Ovarian	83
	SPION	Lung	96
	(fcc)FePt	Ovarian	98
	Mn ₃ O ₄	Lung, ovarian, melanoma	69
	SPION	Breast	99
	[⁶⁴ Cu]Cu	Lung	100