Expert Opinion

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Towards a targeted multi-drug delivery approach to improve therapeutic efficacy in breast cancer

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Importance of the field: Significant improvements in breast cancer treatments have resulted in a significant decrease in mortality. However, current breast cancer therapies, for example, chemotherapy, often result in high toxicity and nonspecific side effects. Other treatments, such as hormonal and antiangiogenic therapies, often have low treatment efficacy if used alone. In addition, acquired drug resistance decreases further the treatment efficacy of these therapies. Intra-tumor heterogeneity of the tumor tissue may be a major reason for the low treatment efficacy and the development of chemoresistance. Therefore, targeted multi-drug therapy is a valuable option for addressing the multiple mechanisms that may be responsible for reduced efficacy of current therapies.

Areas covered in this review: In this article, different classes of drugs for treating breast cancer, the possible reasons for the drug resistance in breast cancer, as well as different targeted drug delivery systems are summarized. The current targeting strategies used in cancer treatment are discussed.

What the reader will gain: This article considers the current state of breast cancer therapy and the possible future directions in targeted multi-drug delivery for treating breast cancer.

Take home message: A better understanding of tumor biology and physiological responses to nanoparticles, as well as advanced nanoparticle design, are needed to improve the therapeutic outcomes for treating breast cancer using nanoparticle-based targeted drug delivery systems. Moreover, selective delivery of multi-drugs to tumor tissue using targeted drug delivery systems may reduce systemic toxicity further, overcome drug resistances, and improve therapeutic efficacy in treating breast cancer.

Keywords: chemoresistance, multi-drug therapy, nanoparticles, targeted drug delivery

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1. Introduction

Breast cancer is the second leading cause of cancer-related death in American women. Despite decades of advances in breast cancer therapies, the mortality rate has been reduced only slightly in the US. Clinically, chemotherapy is often used as neoadjuvant therapy to decrease tumor size before breast surgery and/or radiation, or serves as an adjuvant therapy after surgery. However, given that most anticancer agents do not greatly differentiate between cancerous and normal cells, systemic toxicity and adverse effects [1] associated with these chemotherapeutics significantly limit their treatment efficacy. In addition, acquired drug resistance

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Article highlights.

- Low therapeutic efficacy in treating breast cancer is caused by multiple factors, and the paper discusses the classes of drugs used in breast cancer therapy, causes of low therapeutic efficacy of these drugs, and the targeted drug delivery systems that can be used to improve therapeutic efficacy and overcome drug resistance.
- Even though many different anticancer drugs from different classes have been developed recently, the drug resistance developed in many tumor cells often reduces their treatment efficacy significantly.
- Targeted drug delivery systems can potentially decrease systemic exposure of chemotherapeutics without sacrificing the therapeutic efficacy by selectively targeting therapeutic drugs to the diseased site by means of drug carriers. However, given the multiplicity of reasons for low treatment efficacy of current therapies, new targeting strategies are needed.
- An ideal multi-drug therapy, which includes targeted delivery of multiple agents from different drug classes to multiple targets in the tumor using diverse targeting approaches, will maximize the therapeutic efficacy, overcome drug resistance and reduce side effects.

This box summarizes key points contained in the article.

may further decrease the treatment efficacy of the chemotherapy and other adjuvant therapies. Although higher doses of chemotherapy may overcome drug resistance, and patients with high doses of chemotherapy had significantly fewer breast cancer-related events, the overall survival rate was not improved because of increased treatment toxicity [2]. Therefore, a treatment that could deliver a high dose of chemotherapy to patients with less systemic toxicity would be more effective in treating breast cancer. The heterogeneity of the breast cancer also restricts the efficacy of the cancer treatment. For this reason, multi-drug therapy using different classes of drugs to target different mechanisms may also provide better treatment efficacy.

Targeted drug delivery systems can potentially decrease systemic exposure of chemotherapeutics without sacrificing the therapeutic efficacy by selectively targeting therapeutic drugs to the diseased site by means of drug carriers. A nanocarrier conjugated with specific surface ligands may selectively deposit a high dose of anticancer drugs directly into the cancerous cell. Given the multiplicity of reasons for low treatment efficacy of current therapies, new strategies focusing on targeted delivery of multiple agents from different classes to multiple targets in the tumor are being developed, which may overcome drug resistance, improve therapeutic efficacy and reduce side effects.

In this review, the following are addressed: the classes of drugs that have been used in breast cancer therapy; causes of low therapeutic efficacy of these drugs; and the targeted drug delivery systems that can be used to improve therapeutic efficacy and overcome drug resistance.

2. Treatments

2.1 Drug classes

Breast cancer is commonly treated using a combination of any of the following: surgery, radiation, chemotherapy, and hormonal/biological therapies [3]. Although chemo- and hormonal/biological therapies generally serve as adjuvant or neoadjuvant treatments, secondary to radiation and surgery, these drugs play a critical role in the eradication of cancer cells from the body. Based on the treatment mechanisms, the anticancer drugs can be classified into different categories, including chemotherapeutic drugs, hormonal drugs, antigrowth factor, and antivascular drugs. **Table 1** lists several breast cancer drugs from different classes that are in use or in clinical trials.

2.2 Drug resistance in breast tumors

Many tumor cells may be intrinsically resistant to specific anticancer agents, or over time cancer cells may become resistant to a given drug. Although response rate of metastatic breast cancer to first-line treatments is upwards of 70%, response rate may fall to < 20% if the disease progresses past first-line therapy. Resistance of cancer to chemotherapy may be caused by either host factors, such as poor tolerance to the drug's effects, as well as the body's metabolism, or alterations in the genetics of cancer cells. Several mechanisms have been suggested to explain cancer chemoresistance or poor response to anticancer agents. Membrane efflux transporters and tumor cell alterations are the two most common cellular mechanisms that either cause expulsion of anticancer agents from within the cell or prevent drug's action in cancer cells.

2.2.1 Membrane efflux transporters

Alteration in the drug export pumps (ATP-binding cassette membrane transporters) responsible for the removal of the therapeutic drugs [4] is one of the most studied cellular mechanisms of cancer drug resistance. The ATP-binding cassette (ABC) membrane transporter family, including P-glycoprotein (P-gp), multi-drug resistance protein (MRP) and breast cancer resistance protein (BCRP), plays a role in preventing the accumulation of toxic substances in normal tissues, such as the gastrointestinal tract and brain [5]. In tumors, ABC membrane transporters are associated with multi-drug resistance [6] and poor prognosis [7].

P-gp is the most widely studied ABC membrane transporter, and has been shown to be expressed in upwards of 40% of breast cancers [5]. P-gp can bind and expel a broad variety of hydrophobic drugs within the plasma membrane of the tumor cell, including some of the most common anticancer agents, such as doxorubicin, daunorubicin and paclitaxel [8]. Owing to the size and complexity of the P-gp binding regions, it is difficult to develop inhibitors to increase cytoplasmic availability of the drug.

The MRP transporter is another ATP-dependent organic ion transporter expressed in both normal breast tissue and

Table 1. Examples of different classes of drugs for treating breast cancer.

Drug type	Drug name	Targets (mechanisms)	Phase of development	Clinical outcomes	Ref.
Chemotherapeutic	Doxorubicin	Anthracycline; inhibits DNA synthesis	Approved	Highly effective antibiotic-based chemotherapeutic. Major adverse side effect is cardiotoxicity. Liposomal formulation available for clinical use	[91,92]
	Pacitaxel	Taxane; mitotic inhibitor	Approved	Better response rates, but not survival rates, compared with anthracyclines as a monotherapy. Effective in combination with anthracyclines	[91,93]
	Capecitabine	Alkylating agent; inhibits DNA synthesis	Approved	Significant tumor control rate after pretreatment in metastatic breast cancer patients. Increased risk of cardiotoxicity	[94,95]
	Cyclophosphamide	Alkylating agent; inhibits DNA synthesis	Approved	Significantly increases survival rate when used in combination with anthracyclines or taxanes	[96]
	Vinflunine	Alkylating agent; inhibits DNA synthesis	Phase II	Effective after treatment with anthracyclines and taxanes. Major side effect is leukopenia	[97]
Hormonal	Tamoxifen	Selective estrogen receptor modulator	Approved	Standard treatment for estrogen receptor-positive breast cancer for premenopausal women significantly increases survival. Increased risk of endometrial cancer	[98,99]
	Raloxifene	Selective estrogen receptor modulator	Approved	As effective as tamoxifen for treatment of estrogen receptor-positive breast cancer. Increases risk for venous thromboembolism and fatal stroke	[100,101]
	Fulvestrant	Estrogen receptor antagonist	Phase II	More effective than SERMs for estrogen receptor-positive breast cancer in postmenopausal woman	[102,103]
	Anastrozole	Aromatase inhibitor	Approved	Well-tolerated treatment after tamoxifen therapy. Side effects include gastrointestinal disturbance	[104,105]
	Letrozole	Aromatase inhibitor	Approved	Significant increase in endocrine therapy (delayed time to chemotherapy) compared with tamoxifen in metastatic breast cancer	[106]
Antigrowth factor	Bevacizumab	Inhibits VEGF receptor	Phase III	Low response rate in metastatic patients, with a high percentage of adverse side effects including hypertension	[107]
	Trastuzumab	Anti-HER2 Antibody	Approved	Significantly improves survival rate for HER2-positive breast cancer. Associated with cardiac dysfunction	[108,109]

Table 1. Examples of different classes of drugs for treating breast cancer (continued).

Drug type	Drug name	Targets (mechanisms)	Phase of development	Clinical outcomes	Ref.
Antivascular	Combretastatin	Antivascular	Phase II/III	Causes significant reduction in tumor blood flow. Can be tolerated in combination with paclitaxel and carboplatin. Side effects include significantly altered blood flow in normal tissue, such as brain, heart and kidneys	[87,110]
	Vadimezan	Tumor-vascular disrupting agent	Phase II	Causes significant reduction in tumor blood flow. Can be tolerated in combination with docetaxel	[111]

breast cancer cells [9]. Early expression of MRP1 in cancer patients has been shown to be correlated with poor prognosis [10]. Unlike P-gp, MRPs readily transport negatively charged drugs and possess the ability to transport a wide range of molecules, including weak organic bases such as anthracyclines [11]. MRP1 is widely expressed in human tumors, and has been found to increase expression in the presence of chemotherapy [10].

A more recently discovered ABC membrane transporter is the BCRP, which is widely expressed in several breast tumor lines and cancer cells. Cells overexpressing BCRP were found to be resistant to anthracyclines [12] and other folate-dependent drugs. It has been shown that folate deprivation results in a downregulation of BCRP expression [13]. Sulfated estrogen can be transported through the BCRP [14], and estrogen downregulates expression of BCRP by means of post-transcriptional inhibition [15].

2.2.2 *Tumor cell alterations*

Another mechanism that renders cancer resistant to anticancer agents, specifically taxanes, is the alteration of microtubules within the tumor cells. Although there is no clinical evidence of its effect, microtubule alterations have been studied and linked to anticancer drug resistance in cancer cell culture [16]. Paclitaxel causes mitotic arrest by binding to the β-subunit of tubulin in the microtubules. Paclitaxel-resistant cancer cell lines have been shown to express mutations in tubulin [17]. The expression of microtubule-associated proteins (MAPs) may increase or decrease sensitivity to chemotherapy. An increase in MAP4 expression has resulted in an increase in paclitaxel binding [18].

There are several other examples of alterations in the morphology of tumor cells that indicate an increase in resistance against anticancer drugs. Alterations in growth factor signaling pathways, for example, such as PI-3 kinase and HER2, are strongly correlated with resistance to selective estrogen receptor modulator (SERMs) [19]. Long-term estrogen deprivation via SERMs or aromatase inhibitors may initiate hypersensitivity to estradiol in cancer cells, thus decreasing the effectiveness of the anti-estrogen treatment [20]. Variations in drug resistance mechanisms found in a single tumor may also be the result of heterogeneity in the tumor cell population [21].

2.2.3 *Heterogeneity of tumor cells*

Tumors often show morphological and physiological intra-tumor heterogeneity, including the expression of cell surface markers and growth factor and hormonal receptors, metabolism, motility, and angiogenic, proliferative, immunogenic and metastatic potential [22]. This intra-tumor heterogeneity may be another reason for the low therapeutic efficacy in treating tumors, especially breast tumors [23,24]. The cause of tumor heterogeneity has been explained by two major theories: cancer stem cells and clonal evolution.

Cancer stem cell theory posits that a subgroup of cancer cells has the ability to self-renew and differentiate into phenotypically diverse progeny of non-tumorigenic cancer cells, which compose the bulk of the tumor and lose their ability to differentiate [25]. Recent gene expression microarray studies of intra-tumor heterogeneity have shown that cells responsible for cancer relapse are minor subpopulations of the original tumor [26]. Therefore, treatment may be achieved by targeting only cancer stem cells that are responsible for the differentiation and proliferation of most of the tumor population. However, it has been suggested that cancer stem cells have intrinsic features such as quiescence and DNA repair that make them more resistant to therapy [27].

According to the clonal evolution theory, a single mutated cell with genomic instability produces a population of tumor cells with different heritable traits. The microenvironment of the tumor cells and its alterations create selection pressures that act on this population. Some mutations can adapt better to the microenvironment than others [28]. When the tumor is treated with drugs, some population of cells may undergo genetic changes and mutations that resist the drug. This population has selective advantage over the rest of the

population and proliferates more extensively, which results in the tumor having a higher and higher population of resistant cells with time. Consistent with this theory, breast cancers consist of different subpopulations of cancer cells with varying phenotypic features, including gene expression, and immunogenic and metastatic potential [29,30].

Regardless of the cause, tumor heterogeneity complicates our understanding of tumor development, and presents significant challenges for the development of successful treatments. Moreover, high genetic heterogeneity of tumors causes much difficulty in identifying the cancer stem cells and/or the possible pre-existing clones that may be resistant to therapeutic intervention.

Even though many different anticancer drugs from different classes have been developed recently, the drug resistance developed in many tumor cells often reduces their treatment efficacy significantly. The traditional view of tumor as a tissue with a single type of cell is now being replaced by an approach that considers the tumor as a 'smart organism' with multiple functional components and different types of cell. Consistent with this more sophisticated view of the tumor, combinational therapies using different classes of drugs to treat tumor by different mechanisms have been shown to be successful in many applications. Therefore, multi-drug therapy represents an emerging approach for improving treatment efficacy and reducing adverse side effects. However, undesirable side effects are often associated with a large number of tissues that may be affected by these different drugs. In the next section, targeted drug delivery systems used in cancer treatments that may be able to increase the efficacy and reduce the side effects of many anticancer drugs are discussed.

3. Targeted therapy

Most conventional chemotherapeutic and hormonal agents now in use in the clinic to treat breast cancer are administrated into the systemic circulation by means of intravascular injection or oral administration. These anticancer agents are often distributed throughout the body, and may affect both cancerous and normal cells, causing systemic toxicity and adverse effects to healthy tissue. To reduce these adverse effects, the allowable dose of the drug to the patient is often significantly lower than the required dose for adequate tumor treatment. In addition, rapid elimination and accumulation of the drug by the reticuloendothelial system, such as the liver and the spleen, reduces further the effective drug dose to the cancerous tissue. Targeted therapy using nano/microparticulate pharmaceutical carriers has emerged as an important strategy for maintaining a sufficient therapeutic dose of anticancer drugs in the tumor tissue while reducing systemic toxicity and adverse side effects in non-targeted, healthy tissues. Some of these therapies have already been approved by the FDA for standard use in treating cancer patients. For example, Abraxane™ (Abraxis Bioscience Inc., NJ), an albumin-paclitaxel nanoparticle, has been approved for the treatment of metastatic breast cancer. The basic rationale behind this approach is that by modifying the pharmacokinetics of the therapeutic drugs using special properties of these carriers [31,32], the drugs can accumulate at the tumor tissue and execute their therapeutic effects efficiently, while reducing adverse systemic side effects. Targeting moieties, such as antibodies or ligands, may be linked to the drug carriers to enhance the affinity and specificity of the carriers to the targeted tissue [33,34]. In this section, various targeting strategies for drug delivery systems are discussed, and carriers and targeting moieties used in cancer treatments are presented.

3.1 Targeting strategies

Cancerous tissue often requires large amounts of oxygen for its survival and growth, requiring rapid vascularization, resulting in leaky and defective architecture, and impaired lymphatic drainage [35]. The leaky architecture of tumor vasculature allows certain sized particles to accumulate passively at the tumor site by the enhanced permeability and retention effect (EPR effect). Particles with a longer half-life in the circulation and smaller sizes will accumulate more abundantly at the tumor site. The size of gap junction between endothelial cells of the leaky tumor vasculature varies from 100 to 600 nm [36]. The size and surface characteristics of nanoparticles may be controlled to increase circulation times of the particles. Particles that are < 100 nm in diameter and possess a hydrophilic surface generally evade uptake by the reticuloendothelial system in circulation [37]. Plasma protein adsorption, aggregation and ligand desorption, which occur when particles make contact with blood components, may also be avoided by coating nanoparticles with a hydrophilic surface. Passive targeting can also be achieved by local administration of the therapeutic drug into tumor tissue. However, this approach is highly invasive, and some tumors are difficult to access. Nanoparticles that accumulate at the tumor site either slowly release their encapsulated drug into the extracellular space, or release the drug inside the cell after they are engulfed by the cell membrane via endocytosis. The drug release process can be controlled by utilizing the unique microenvironment in tumor tissue.

3.2 Targeting moieties

Targeting moieties can be attached to the nanoparticles to achieve better selective targeting and improve cell uptake of drug carriers. The interaction of a moiety with its target molecule can be lectin–carbohydrate, ligand–receptor, or antibody–antigen [38-40]. The choice of moiety must be specific either to the tumor-bearing organ, tumor tissue, cancer cells, or to intracellular organelles inside cancer cells. The level of expression of the target molecule must be significantly higher in the tumor tissue compared with that of the normal tissue to avoid systemic toxicity from the anticancer drug. By using these interactions, targeted drug delivery systems can selectively deliver the drug to the tumor tissue, tightly bind

to the cancer cells to avoid the systemic leaking, and improve cell uptake of drug carriers.

Starting with Kornfeld *et al.* in 1971 [41], several applications in using sugar–lectin interaction for receptor-mediated drug targeting have been reported. Cancer cells often express different glycans on their cell membranes compared with those expressed on normal cells. These glycans can be used as targets in a drug delivery scheme while utilizing lectins as a targeting moiety. Likewise, the glycans may also be used as a moiety to target lectins expressed on the surface of the cells. Galactosamine-targeted polyHPMA-doxorubicin (known as PK2) has been used successfully in targeting the liver of cancer patients by attaching the targeting moiety galactosamine to a polymer backbone [42]. However, targeting lectin- or glycan-conjugated drug vehicles to cancerous tissue is limited by the toxicity and cost of many lectins [43], as well as the organ specificity of the lectin–carbohydrate complex [44].

A great deal of effort has been put into identifying cell surface antigens and receptors that could serve as targeting moieties if expressed exclusively on tumor cells, or significantly overexpressed on the tumor cells compared with normal cells. Several antibodies have been shown to initiate specific signaling cascades, which can be used as targeting moieties for targeting tumors [45,46]. For example, Herceptin (Trastuzumab), a humanized monoclonal antibody that specifically binds to the HER-2 receptor, was approved by the FDA in 1998 as a targeted antibody therapy for HER-2-positive breast cancer. HER-2 is an ideal target for tumor cells because it is easily accessed by its ligands on the extracellular portion of the receptor. Several breast cancer treatment studies have been performed using the HER-2 receptor as a target. The anti-HER-2 antibody, its Fab′ fragment (slower clearance rate by body compared with the intact antibody) and the highly internalizable scFv fragment have all been used as targeting moieties. Drug carriers conjugated with HER-2-specific moieties have been shown to preferentially accumulate and bind to the breast cancer cells and release their encapsulated chemotherapeutic agents to breast cancer cells [47-49].

Folate-conjugated drug carriers are another popular vehicle for targeting cancer cells by means of surface moieties. The folate receptor is expressed in very low numbers or is absent in most normal tissues. However, the cell surface receptor for folate is significantly overexpressed on the surface of various cancers, including ovarian, brain, kidney, breast and lung malignancies [50,51]. Drug delivery systems using folate as targeting moiety have shown > 1000-fold higher affinity to the targeting receptors and can efficiently deliver the encapsulated drug to the tumor cells [50].

Synthetic peptides or aptamers can be designed to have similar, sometimes even higher, specificity and affinities to the targeted receptors. One of the most popular peptide ligands is based on the RGD sequence, which is a cell binding site in the extracellular matrix protein, fibronectin and integrins. This peptide has been successfully attached to liposomes to selectively target antivascular agent combretastatin to breast tumor in a mouse model, resulting in significant tumor control (**Figure 1**) and reduced side effects [52]. Aptamers are single-stranded RNA or DNA oligonucleotides that are designed to bind to a variety of proteins. Aptamer-functionalized liposomes have been used to target the vascular endothelial growth factor receptors [53]. However, their high cost, instability *in vivo* [54] and high immunogenicity [55] have limited their application.

Although all of the above-mentioned targeting moieties offer the possibility of improved delivery of agent to the target cells *in vitro*, some of these systems may not be effective *in vivo*. This is largely because target molecules expressed/overexpressed on the surface of the particular cells are not necessarily in direct contact with the bloodstream. Therefore, cell-specific targeting systems designed using these targeting moieties have to pass through the endothelial cell layer to reach the targeted cells. One way to circumvent this problem is using specific target moieties on the surface of endothelial cells. However, the expression levels of many adhesion molecules, for example, in the vasculature are not significantly different in tumor tissue compared with normal tissue. A new approach may be to target upregulated moieties in tumor vasculature as an alternative to using existing antigens/receptors on the surface of cancer cells [52,56]. For example, it has been shown that localized radiation therapy upregulates adhesion molecules, such as E-selectin and ICAM-1, on the surface of endothelial cells in mammary tumors (**Figure 2**), which can then be used to selectively deliver antivascular drugs to the tumor site [52,56]. A similar approach has also been used to selectively target angiogenic compounds to adhesion molecules that are upregulated after a myocardial infarction [57-59]. An advantage of this approach is the accessibility of the tumor vasculature for larger drug vehicles, such as liposomes, which may not accumulate passively in the tumor using the EPR effect.

There are many biologically active therapeutic agents, including macromolecular drugs, which must be delivered inside the targeted tumor cells in order to exert their therapeutic action. For example, gene therapy agents act in cell nuclei, pro-apoptotic drugs target mitochondria, and lysosomal enzymes must reach the lysosomal compartment to be effective [60]. If released outside the cells, most of these agents will become deactivated or degrade before reaching their target organelles within the cells, even if they manage eventually to enter the cells. Fortunately, some of these targeting moieties not only provide the specific recognizing and binding abilities, but also help the drug carriers enter the cancer cells to release the encapsulated drug. This internalization of the drug carriers by the targeting moieties usually occurs by means of receptor-mediated endocytosis.

3.3 Multi-drug targeting systems

As discussed above, therapeutic limitations and undesirable side effects of many current drugs are usually due to a

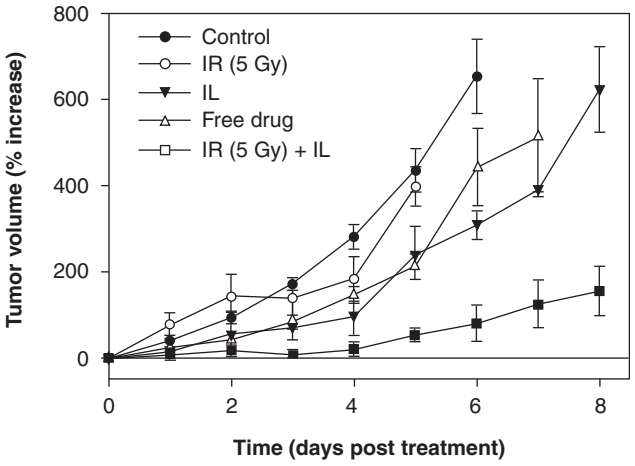

Figure 1. Targeted delivery of combretastatin to irradiated melanoma tumor using RGD-conjugated immunoliposomes significantly reduced the rate of tumor volume growth starting from day 3 post-treatment and prolonged the lifespan of the tumor-bearing mice.

Reproduced with permission from [52].

IL: Immunoliposome treated; IR: Irradiated; Systemic: Combretastatin injected intravenously (mean ± s.e.m., n = 4 – 6 animals per group).

multitude of mechanisms. Therefore, a targeted delivery system designed to deliver multi-drugs to specific locations within the tumor and its surrounding tissue could result in maximal therapeutic efficacy and minimal adverse side effects. For example, one can design a delivery system to deliver two intracellular drugs and one extracellular drug to a tumor tissue by two different drug carriers. One drug carrier can be designed to bind and enter the cancerous cells by receptor-mediated endocytosis (e.g., folate receptor) and then release the encapsulated intracellular drugs into the cells, whereas the other drug carrier can be designed to accumulate passively and release the encapsulated drug in the interstitial space (such a proposed scheme is illustrated in **Figure 3**). This targeted multi-drug delivery strategy can not only preferentially deliver several different drugs in high doses to the locations where they work best in treating tumor, but also limit the adverse systemic side effects. To the authors' knowledge, this innovative drug delivery scheme has not been described in the literature before.

3.4 Drug carriers

The main route of administration for a nanoparticle-based targeted delivery system remains systemic intravenous delivery into the bloodstream. Therefore, the interaction of the drug carriers with the components of plasma, the specific and non-specific binding to blood cells and vascular endothelium, and the final biodistribution in various organs are of great interest. Particle size and surface characteristics of the drug carriers are two critical properties that affect circulation time in the blood and the efficiency of reaching the tumor tissue by means of the EPR effect. These characteristics are also very important for the internalization of drugs into the cancer cells via either pinocytosis or receptor-mediated endocytosis. The design of the drug carriers also controls drug release profiles. In this section, some of the commonly used drug carriers for targeted drug delivery systems and some of the drug carriers now being tested in clinical trials are discussed (**Table 2**).

3.4.1 *Polymers*

Both natural and chemically synthesized polymers are used extensively for developing nanoparticles for targeted drug delivery. Recently, a nanoparticle formulation of paclitaxel has been applied successfully clinically for the treatment of metastatic breast cancer [61]. The carrier of this formulation is non-toxic, non-hemolytic and non-immunogenic. If designed appropriately, polymeric nanoparticles may serve as a drug carrier that may specifically deliver the drug to tumor tissue or cells.

Polymer-based targeting nanoparticles must be rendered cell/tissue-specific in order to increase further their nanoparticle specificity and efficiency in internalizing to the targeted cells. To achieve this goal, targeting moieties may be attached to the surface of the nanoparticles, together with protective polymer, such as PEG [62]. Polymeric nanoparticles can also be designed to control the release profile of the drug by adding properties such as positive charge or pH-sensitivity. Cationic polymers have been used for efficient intracellular delivery of DNA to the nucleus [63]. Positively charged nanoparticles are believed to interact easily with negatively charged cells and improve transfection efficiency [64]. The pH-sensitivity function can also assist nanoparticles to release encapsulated drugs into the cytoplasm rather than into the endosome where the drug may degrade [65].

3.4.2 *Liposomes*

Liposomes are one of the most popular and well-investigated drug carriers. Liposomal drug delivery vehicles have been studied intensively in the past few decades because the biodistribution of therapeutic agents can be tailored to the needs of the targeted tissue. Drug carriers such as DOXIL, which is an FDA-approved liposomal formulation of doxorubicin [66,67], can effectively control the pharmacokinetics and biodistribution of the drug, in order to avoid or target specific tissues, thus achieving therapeutic index enhancement via both toxicity reduction and/or efficacy enhancement [68]. Drugs can be loaded either into the internal, trapped aqueous space (hydrophilic drugs), or between the lipid bilayer membranes (hydrophobic drugs). The grafting of PEG onto the liposome surface results in stealth liposomes, which have been shown to prolong circulation lifetime (10 – 50 h versus minutes for non-PEGylated liposomes) [69]. These long-circulating liposomes are passively accumulated in the tumor tissue by the EPR effect [35], eventually releasing the encapsulated drug in the interstitial space. Immunoliposomes are long-circulating liposomes that are conjugated with targeting ligands on their

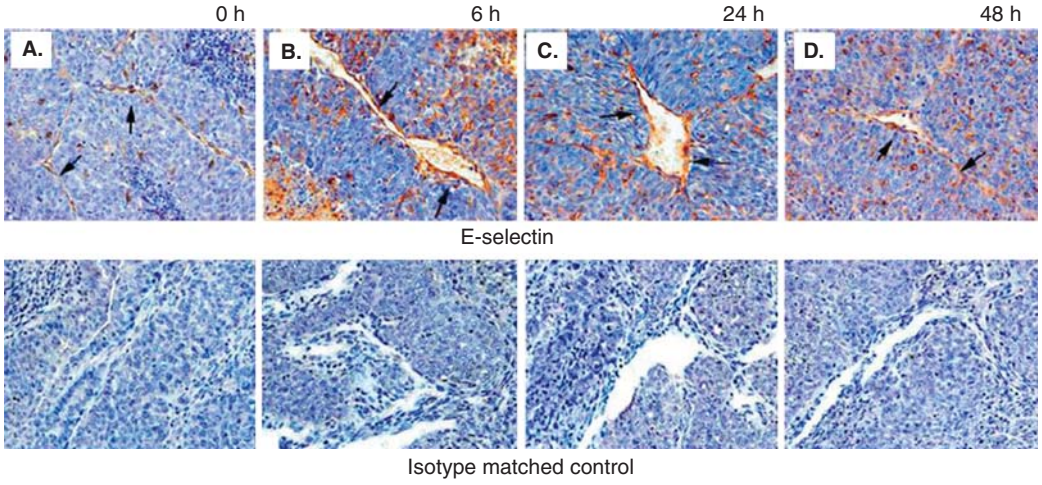

Figure 2. Localized radiation therapy upregulated adhesion molecules on the surface of the irradiated endothelial cells in tumor tissue in mammary tumors. Immunohistochemistry shows that, compared to baseline (**A**), expression of E-selectin significantly increased in irradiated tumors 6 and 24 h post-irradiation (**B** and **C,** respectively), before returning to a near basal level at 48 h post-irradiation (**D,** arrows). Brown color staining indicates upregulation of E-selectin in capillaries with arrows pointing to positive endothelial cells in large vessels. Isotype matched controls are shown for each time point to demonstrate the specificity of the immunolabeling. This upregulation of E-selectin was in turn used to target drugs to mammary tumors.
Reproduced with permission from [56].

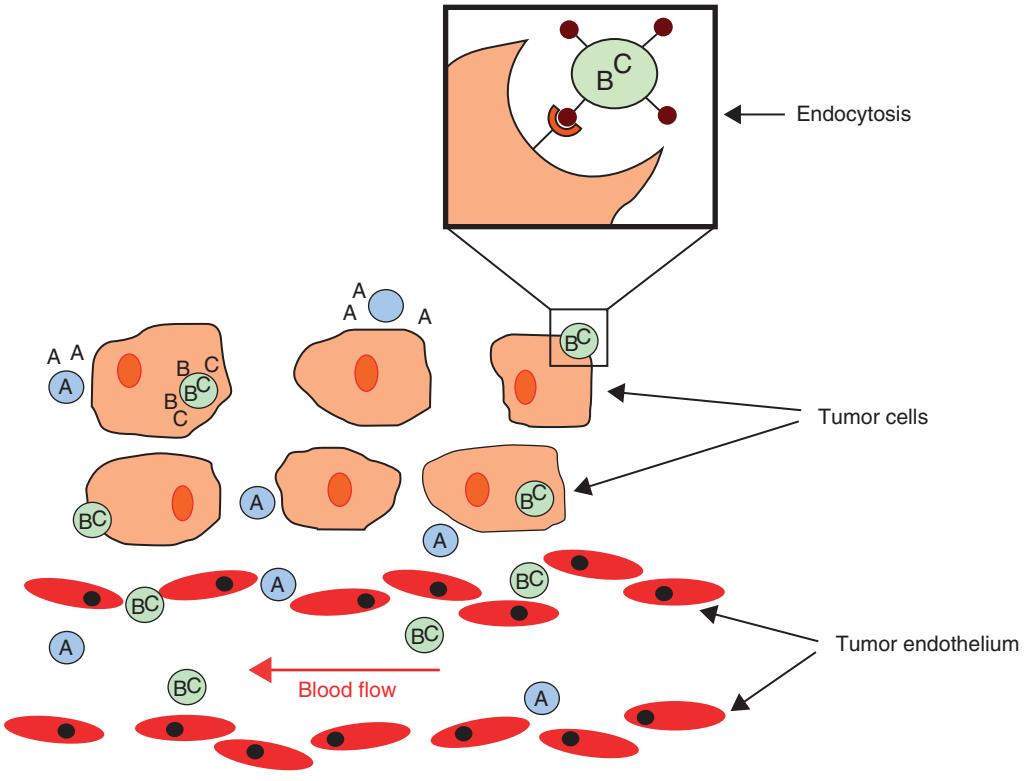

Figure 3. A schematic of the proposed strategy for targeted multi-drug delivery. Drug carrier 1 (blue circles) is loaded with one extracellular drug A, and drug carrier 2 (green circle) is loaded with two intracellular drugs (B and C). Drug carrier 1 can be designed to accumulate passively and release the encapsulated drug A in the interstitial space. Drug carrier 2 can be designed to bind and enter the cancerous cells and then release the encapsulated intracellular drugs B and C into the interior of the cells.

Table 2. Examples of nanoparticle-drug conjugates for breast cancer drug delivery.

Drug carriers	Structure	Drug being delivered	Clinical trials	Cancer types	Ref.
Polymers	Nanoparticle albumin-bound paclitaxel (Abraxane)	Paclitaxel	Phase III trial	Breast cancer	[61]
	Conjugate of paclitaxel and poly-L-glutamate (CT-2103)	Paclitaxel	Phase II trial	Breast cancer	[112]
	Poly-L-glutamate camptothecin (CT-2106)	Camptothecin	Phase I trial	Solid tumor (including breast cancer)	[113]
	Polymer-doxorubicin (PK1)	Doxorubicin	Phase III trial	Breast cancer	[114]
	SN-38-incorporating polymeric micelles (NK012)	SN-38	Phase I trial	Breast cancer	[115]
	Cremophor-free, polymeric micelle formulation of paclitaxel (Genexol-PM)	Paclitaxel	Phase II trial	Breast cancer	[116]
Liposomes	PEGylated liposomal doxorubicin (CAELYX/Doxil)	Doxorubicin	Phase III trial	Breast cancer	[117]
	Non-PEGylated liposomal doxorubicin (Myocet (M))	Doxorubicin	Phase I trial	Breast cancer	[118]
	Liposomal paclitaxel formulation (Genexol®-PM)	Paclitaxel	Phase I trial	Solid tumor (including breast cancer)	[119]
	Liposomal cisplatin (Lipoplatin)	Cisplatin	Phases I, II and III	Solid tumor (including breast cancer)	[120]

surface to increase ligand binding efficacy [70]. Even though immunoliposomes accumulate in tumor tissue by the EPR effect [71], compared with long-circulating liposomes, they quickly bind to and internalize in the tumor cell by means of ligand–receptor interaction after accumulation in the tumor tissue, thus delivering more encapsulated drug to the target tissue [72].

3.4.3 *Nanotubes*

Nanotubes have large internal volumes and external surfaces that allow more drugs/biomolecules to be loaded onto the nanotubes [73]. Drugs/biomolecules can be loaded inside the nanotube, or attached to the side of the nanotube wall, or trail behind the nanotube. Nanotubes have a very high optical absorbance in the near-infrared (NIR) region. This property allows them to be imaged using infrared fluorescence microscopy. Moreover, with continuous NIR irradiation, these nanotubes may be heated up, which could result in thermal destruction of the cells if the nanotubes are delivered into the cells [74]. The surface of the nanotube may be chemically modified to be hydrophilic and functionalized, and linked to a wide variety of molecules such as antibodies, peptides, imaging agents, or therapeutic agents [53,75,76] to facilitate the targeted delivery of anticancer agents to the cancer cells and enhance the endocytosis process. However, nanotubes are non-biodegradable, have a large surface area for protein opsonization, have a strong tendency to aggregate and may be toxic to humans.

3.4.4 *Quantum dots*

Nanoparticle size quantum dots (QDs) have recently been established as a model for molecular, cellular and *in vivo* imaging [77-79]. Quantum dots with a protective hydrophobic bilayer can be dispersed in aqueous solution and remain stable for long periods of time [80]. Quantum dots can be delivered into cells passively by means of endocytosis [81], or actively by receptor-induced internalization [82]. Targeting moieties such as antibodies, oligonucleotides, or small molecule ligands can be linked to water-soluble QDs [83]. Quantum dots have also been studied as a delivery vehicle for cancer imaging and therapy by conjugating a QD with an anticancer drug, such as doxorubicin [84]. However, owing to their chemical composition of toxic heavy metal atoms (e.g., Cd, Hg, Ph, As) [85,86], using QDs in humans may raise some safety issues. Compared with other drug vehicles, QDs have the advantage of fluorescence, and do not have to be modified to serve as imaging agents. However, QDs are solid particles, and drugs cannot be loaded inside, they must be attached to the outside.

4. Conclusion

Despite advances in treatment, breast cancer remains a significant cause of mortality for American women. Although anticancer drugs such as chemotherapeutics and hormonal therapies greatly improve prognosis of breast tumor development, overcoming drug resistance mechanisms in tumor cells, such as ABC membrane transporters, may require large systemic doses of drugs, which is associated with significant toxicity (cardiotoxicity, endometrial cancer, vascular events, infertility) to healthy tissue [1]. Significant changes in blood flow to normal tissue have also been reported with the systemic application of newer antivascular drugs [87]. Multi-drug therapies using different anticancer drugs to

overcome drug resistance induced by tumor heterogeneity may also result in systemic toxicity issues.

Targeted drug delivery systems provide a new approach to increase the therapeutic efficiency and reduce the adverse side effects of anticancer drugs in breast cancer treatments. Several targeted drug delivery systems are in clinical trials and a few are already on the market [66]. By modifying the pharmacokinetics and pharmacodynamics of their incorporated drugs, drug carriers not only reduce drug toxicity to the non-targeted tissue, but also increase bioavailability of therapeutic drugs that normally have a short circulation time in plasma to the site of the tumor [1]. Nanoparticle drug carriers with long circulation times can passively accumulate in tumor tissue by the EPR effect. However, their drug release process is still dependent on the surrounding environment, and the release of intracellular drugs may still need to pass through barriers such as cell membranes. Targeting moieties can be attached to the surface of nanoparticle drug carriers to assist the internalization of the drug into the targeted cell by receptor-mediated endocytosis and other mechanisms. However, effective therapeutic responses are still dependent on pathophysiological responses to the drug and its carriers and the selectivity of the drug carriers to tumor tissue. Furthermore, treatment efficacy of targeted therapies is highly dependent on the selection of the right breast cancer subtypes. Conventional diagnostic plus recently developed gene expression microarray [26] technologies can be used to classify breast cancer patients into the different subtypes, and the treatment strategy can be customized to each individual patient to achieve maximal treatment efficacy. This treatment strategy can also be applied to other tumor and tissue types. However, the unique barriers presented in different tissues have to be considered accordingly. For example, the strategy for intravenous delivery of therapeutic agents to brain tumors has to include methods, for example ultrasound, that allow the drug carrier to cross the blood–brain barrier efficiently [88].

A better understanding of tumor biology and physiological responses to nanoparticles, as well as advanced nanoparticle design, are needed to improve the therapeutic outcomes of breast cancer using a nanoparticle-based targeted drug delivery system. With greater understanding of the intercellular and intracellular signaling pathways in tumors, more and more therapeutic targets and targeting moieties will be developed, which is essential for progress in this emerging field. This will further facilitate the development of the treatment of breast cancer using a nanoparticle-based targeted drug delivery system.

5. Expert opinion

Although several nanoparticle-based targeted drug delivery systems have been developed and have been shown to lead to promising therapeutic response, surprisingly most are still at the early stages of the long journey to clinical application. This may in part be because biological barriers to drug carriers, the release profiles of the enclosed therapeutic drugs *in vivo*, and the interactions of the released drugs with their surrounding environments still need to be better understood to achieve optimal drug transport into tumors. In addition, the heterogeneity of the breast cancer also reduces the efficacy of the cancer treatment. In general, further progress in the emerging field of targeted drug delivery may also be contingent on the discovery of new biomarkers that are present and/or upregulated in tissue and can be targeted for selective drug delivery [59].

In general, traditional therapeutic strategies try to eliminate tumor cells using cytotoxic drugs and/or radiation. More new treatment strategies are focused at present on either using targeted delivery systems to deliver high doses of cytotoxic drugs to the tumor without adverse side effects, or using antiangiogenic or antihormonal therapies to inhibit tumor growth. Although anticancer drug development has expanded significantly with the increase of molecular and biological knowledge, efficacy of these treatments remains mostly unchanged. This may be partially owing to the heterogeneity of tumors. Therefore, characterizing the heterogeneity of different types and subtypes of cancer through different stages of tumor development as well as determining changes in tumor heterogeneity in response to different treatment strategies should be an important area of study in this field. The treatment strategies should also be modified to address the heterogeneity in tumors. Multi-drug therapies using different classes of drugs to target different mechanisms may provide a more effective method for eliminating cancer cells and reducing tumor size compared with traditional single-drug targeting therapies.

Increased hydrostatic pressure gradients in tumor vasculature could be partially responsible for poor delivery and distribution of systemically administrated therapeutic drugs and/or drug carrying nanoparticles to tumors. This not only reduces the therapeutic efficacy, but also may induce unexpected side effects. Even though receptor-mediated endocytosis may help targeted delivery systems to release the encapsulated therapeutic drugs in the cells, overdosing may cause the excess drugs to leak back into the circulation. In these cases, optimization of dosing schedule and dose of therapeutic drug is critical for the prevention of unexpected side effects due to drug leakage. Therefore, owing to the heterogeneity of the breast cancer cell population, the dosing of the drug as well as delivery efficiency must be tailor-designed for each patient/tumor. This will present even more challenges to the drug delivery strategies.

For reasons discussed above, targeted multi-drug therapy may enhance therapeutic efficacy and improve long-term patient prognosis. A targeted multi-drug therapy may consist of different encapsulated drugs, multiple delivery approaches, or both. Using multiple therapeutic drugs, especially from different classes, to treat tumors can improve the treatment efficacy with a lower dose of each drug, which in turn reduces the adverse side effects. In fact, a combination of a

chemotherapeutic drug and a membrane transport inhibitor has been shown to increase the efficacy of cancer treatment [89]. In addition, ligand–receptor-mediated targeted delivery of nanoparticle–drug complex may overcome drug resistance associated with membrane efflux pumps by effectively transporting drugs into the targeted cells at a rate faster than they are being expelled by, for example, ABC membrane transporters. Therefore, ligand–receptor-mediated targeted delivery systems may be utilized to deliver a combination of cytotoxic drugs and other agents that block the action of drug resistance mechanisms (e.g., inhibitor of ABC membrane transport proteins) to the cancer cells.

Nevertheless, targeted tumor cells may develop other drug resistance mechanisms that need to be overcome to address multi-drug resistance mechanisms. Therefore, other combinational therapies may utilize targeted drug delivery to increase further the efficacy of anticancer agents. For example, tumor growth and development can be delayed or even stopped by blocking intercellular communications. Bevacizumab, an antiangiogenic compound, can block intercellular communications between tumor cells by inactivating VEGF pathways within the interstitial spaces. However, to be effective, this type of compound should be delivered to the interstitial space, not inside the cells. A combination of a therapeutic compound that blocks intercellular communication (e.g., anti-VEGF antibody) with a chemotherapeutic drug may also improve the therapeutic efficiency. In fact, a combination of chemotherapeutic and antiangiogenic agents has been shown to increase the survival rate in metastasis cancer patients [90].

Owing to the different mechanisms through which these drugs exert their therapeutic effects, encapsulation and targeting schemes should be tailored to the specific functions of each drug. For example, whereas antiangiogenic compounds may be most effective when delivered to the interstitial space instead of inside the cells, chemotherapeutic drugs work best when delivered intracellularly. The heterogeneity of the breast cancer cell population requires targeting several different cell types expressing various surface markers to reduce efficiently tumor size and eliminate cancer cells. An effective targeting multi-drug strategy may include several different targeting moieties. Taken together, an ideal multi-drug therapy includes targeted delivery of multiple agents from different drug classes to multiple targets in the tumor using diverse targeting approaches. This will maximize the therapeutic efficacy, overcome drug resistance, and reduce side effects.

Declaration of interest

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