

REVIEW ARTICLE

Cancer, Drug Targeting and Targeted Therapies

Mumin Alper Erdogan

Izmir Katip Çelebi University,
Faculty of Medicine,
Department of Physiology,
Izmir, TURKEY

ABSTRACT

Cancer is one of the major causes of mortality worldwide and advanced techniques for therapy are urgently needed. Although there are many newly advanced research on new strategies, current treatments are still limited to surgery, radiotherapy, chemotherapy and immunotherapy. These new nanocarriers could be increased the concentration and therapeutic index of drugs in the tumor tissue, and further pharmacokinetic properties. The development of novel nanocarriers has allowed a major drive to improve drug delivery in cancer. The major aim of most nanocarrier applications has been to protect the drug from rapid degradation after systemic delivery and allowing it to reach tumor site at therapeutic concentrations, meanwhile avoiding drug delivery to normal sites as much as possible to reduce adverse effects. Nowadays, many researchers from various disciplines are working on developing new drug delivery systems in order to minimize the increasing problems of old drugs and translate them to clinical efficacy. Nanotechnology focuses on the formulation of therapeutic agents in liposomes and nanoparticles (nanoparticles, nanocapsules, micelles and dendrimers). These nanocarriers can provide targeted drug delivery to the diseased part of the body. Nanocarriers have really great potential in cancer treatment, diagnosis and imaging. Further studies in nanomedicine will improve therapeutic window of drugs with immensely reduced side effects leading to improved patient outcomes. It is foreseen that in the next 10 years, we will witness the widespread use of these treatment methods in combination with standard treatments not only for cancer but also for other diseases.

INTRODUCTION

Cancer ranks first among the causes of morbidity and mortality all over the world and is expected to continue to be the main cause of death in the coming years¹. Despite advanced and detailed research on new interventions, current treatments are still limited to surgery, radiotherapy, chemotherapy and immunotherapy².

Most treatment deficiencies are based on drug resistance, pharmacological or toxicity related problems. In contrast, the introduction of nanocarriers increased the concentration and therapeutic index of drugs in the tumor tissue, and further pharmacokinetic properties, increased circulation time, increased cellular uptake, increased dispersion volume, increased half-life, and so on increase the effectiveness of existing therapies and improve therapeutic window and associated clinical success².

Advances in nanotechnology are expected to enable the development of new therapeutics in cancer and the widespread application of new diagnostic methods. When all these developments are considered, biomaterials and nanotechnology can offer a new opportunity to improve survival in cancer patients.

Most of the commonly used drugs do not show their activities in the body by selective distribution in pathological organs, cells or tissues. Usually, these drugs prefer to be

distributed throughout the body. Moreover, drugs can be used to reach the area where they will affect; organs, cells and intracellular compartments. During this period, drugs can accumulate in normal organs and tissues that are not involved in the pathological process. This is necessary to achieve the therapeutic concentration of the drug in the required body compartments; In addition to the necessity of taking large amounts by the patient, it is the cause of many negative side effects. Drug targeting provides new solutions to all these problems. Drug targeting; and the ability to selectively and quantitatively collect in the target tissue or organ, irrespective of the chemical composition and mode of administration of the drug active ingredient. Thus, the concentration of the drug; it will be high in diseased areas and will be at minimum level to prevent negative side effects that may occur in other areas³.

The aim of drug targeting; selective transport, absorption and dispersion of the pharmacological agent to the site of action. With this selective targeting, unwanted side effects are reduced, the optimal therapeutic response is achieved, and substances with toxic effects at high doses can be used safely⁴.

With targeting, conventional, biotechnological and gene-based drugs can be selectively transported to specific areas of the body such as organs, tissues and cells. Liposomal formulation Doxil[®] (Doxorubicin) and nanoparticle formulation Abraxane[®] (Paclitaxel) as new drug delivery systems in the field of drug targeting have been approved by the American Food and Drug Administration (FDA). In addition, tumor targeting studies with specific monoclonal antibodies are currently being performed. Monoclonal antibodies such as Erbitux[®] (Cetuximab) used in the treatment of colorectal cancer, Panitumumab (Vectibix[®]) and Trastuzumab (Herceptin[®]) used in antiangiogenic therapy; Small molecule tyrosine kinase inhibitors such as Imatinib (Gleevec[®]), Erlotinib (Tarveca[®]), Sorafenib (Nexavar[®]), Sunitinib (Sutent[®]); They are FDA approved and used in the clinic⁵.

HISTORY OF DRUG TARGETING

Paul Ehrlich's ideas have been the cornerstone of innovations in chemotherapy and more generally in pharmacology. Ehrlich's concept of "receptor" forms the basis of his ideas.

Ehrlich suggested that drugs should be studied for their actions and affinities against the cells to which they are directed according to their chemical composition. Ehrlich's aim was to design chemical compounds with specific affinities to pathogenic organisms (such as antitoxin-toxin interaction)⁶.

While working on microorganisms, Paul Ehrlich searched for chemicals that could stain specific microorganisms to make them more visible on a microscope. In his research, he concluded that some chemicals can fight bacteria as well as dye. He called these chemicals "magic bullets." Ehrlich was able to develop his first magic bullet by proving the selective efficacy of the drug "Salvarsan" (Asphenamine) used in the treatment of syphilis⁴.

Paul Ehrlich's concept of "magic bullet" consists of two parts: First one is responsible for recognizing and linking the target, while the latter achieves the therapeutic effect on that target. Today, the concept of magic bullets consists of three interrelated parts: medicine; the targeting moiety, pharmaceutical carriers used to increase the number of drug molecules per targeting moiety. Pharmaceutical carriers; soluble polymers are microcapsules, microparticles, cells, ghost cells, liposomes, lipoproteins, and micelles. All of these can be targeted in one direction or another direction³.

DRUG TARGETING STRATEGIES

- a) Direct administration of the drug to the affected area (organ, tissue)

- b) Passive accumulation of the drug with permeable vascularity (Passive Targeting)
- c) Physical targeting due to abnormal pH or temperature at the target site, such as tumor or inflammation
- d) Use of vectors with high specific affinity for the affected region (Active Targeting)³

DIRECT APPLICATION OF DRUGS

In some cases, drug targeting can be achieved in a simple way. The drug is administered directly to the pathological site. Some successful examples of this approach are; direct application of hormonal drugs into the joints in the treatment of arthritis; and direct application of thrombolytic enzymes used in the treatment of myocardial infarction caused by thrombus between the coronary vessels³.

PASSIVE TARGETING

Passive targeting is the transport of medication to certain regions through natural physiological processes and factors in general. Passive targeting uses anatomical differences between normal and pathological tissues to transport drugs to the required site. Studies have shown that the permeability of blood vessel walls is increased in some cases (such as tumor cells). As the tumor exhibits loose vascularity, the drug delivery system spontaneously penetrates the interstitium through the blood vessel walls. This is called increased permeability and adhesion (EPR effect)^{4,7}.

The EPR effect was observed not only in tumor cells but also in areas of inflammation. Maeda et al. Have shown that excessive bradykinin release in the infection or inflamed areas produces the EPR effect. The only difference between infection-based and EPR effect in the tumor cell is the duration of drug retention time. When infection occurs in the normal cell, the retention time is less than that of the cancer cell, since the lymphatic drainage system is still functioning, so that the infection can dissipate within a few days. However, it may take weeks for macromolecular or lipid drugs to adhere to the cancerous cell. The EPR effect has been greatly utilized in transporting various therapeutics to the site of action. Many studies have suggested evidence supporting the mechanism of passive targeting. In the 1980s and 1990s, nanocarriers were designed based on several passive targeting mechanisms. For example, doxorubicin (DOXIL) designed in liposomal formulation was observed to be 6-fold more effective than free doxorubicin⁸.

PHYSICAL TARGETING

This targeting mechanism is physical targeting based on local temperature increase in different tissues and organs and / or formation of pathological events with acidosis. The difference between normal and tumor tissue pH is also noteworthy. For example; The pH-sensitive release nanoparticles will be able to accumulate in these tissues at a high rate and release more here because of the pH^{9,10}.

ACTIVE TARGETING

The specific interactions between the active targeting drug delivery system and the target cells are briefly defined as ligand-receptor interactions. The principle of Active Targeting is based on the use of targeted ligands capable of specifically binding to receptor structures directed to the target structure, such as antibodies and peptides. Examples of targeted ligands from drug delivery systems used in active targeting to tumor cells are folate, transferrin, galactosamine. The success of active targeting is ensured by the correct selection of targeting means that exhibit high affinity for cell

surface receptors and exhibit chemical modifications to produce appropriate conjugation. Active targeting can be achieved by identifying pathological cells of various molecules condensed in the pathological region by targeting ligand-receptor, antigen-antibody interactions or targeting aptamers. Aptamers are DNA or RNA oligonucleotide sequences that selectively bind with high affinity to the target utilized in the active targeting of therapeutics. Targeted therapeutic agent; with the aid of a carrier capable of binding with the cell or tissue-specific ligand, preferring a high accumulation of drugs in the pathological structure. Thus, as well as being capable of combining with different targeting ligands, variable nanosystems can provide important opportunities to overcome physiological barriers and effective cellular uptake of the drug. Various nanosystems can reach higher concentrations in cellular uptake than normal drugs^{7,8,11}.

ADVANTAGES OF DRUG TARGETING

Advantages of drug targeting; transport of the active substance to pathological regions or special cells in the body by reducing the majority of the side effects and the dose, reaching the active substances to the unreachable regions and targets (eg intracellular regions, viruses, bacteria, parasites), depending on the pharmacological receptor, dosage and decrease in frequency. In this way; drug administration protocols will be simplified, reducing the amount of drug required to achieve therapeutic effect, as well as reducing treatment costs³.

Selective transport of the drug in the body has two vital benefits: It provides the optimal interaction of the drug at the desired rate of activity at the site or sites of action. A second benefit, of equal importance, is to reduce the dose of the active substance and limit it to the distribution of the active substance only to the target organ. Thus, any side effects or side effects that can occur can be substantially minimized, and site-specific drug delivery can significantly improve the therapeutic index of the drug. Targeted drugs will benefit some uncontrolled intracellular infections, central nervous system diseases, immune system diseases, cancer and cardiovascular diseases⁴.

DRUG DELIVERY TO SPECIFIC AREAS

In drug targeting systems; The development of effective drug depends on understanding the appropriate mechanisms, which will increase selectivity. These mechanisms; biochemical, physiological and immunological. Therefore, researches on developing drug targeting systems are intertwined and require multidisciplinary studies. Drug molecules have effects on specific receptors in specific regions or on specific tissues of the body. The interaction of the specific receptor with the drug molecule may increase the pharmacological response and in some cases will be promising in terms of clinical benefit. When the drug is given by normal route (oral or injection), the drug will be able to spread throughout the body. In the meantime, the relationship will not only be in the desired region, it may be affected in other regions, undesirable reactions may occur, as well as undesirable side effects. The observed benefit from the drug can only be achieved when the drug molecules are targeted to the desired site⁴.

DRUG DELIVERY SYSTEMS

Transportation of the drug to the structure where it will act is one of the main problems in the pharmaceutical and biotechnological industries. Therefore, drug delivery systems have always been an area of interest to researchers. Recent advances in biotechnology and the research of other sciences related to these fields help to discover and rationally design many new drugs. However, most drugs are limited by poor solubility, high toxicity, high dose, accumulation of drug due to poor solubility,

nonspecific transport, in vivo degregation and short half-lives. Today, many researchers from various disciplines are involved in the development of specific new drug delivery systems in order to minimize the increasing problems of drugs and to turn new developments into clinical efficacy. Targeted drug delivery is defined as the specific release of bioactive agent to a specific structure at a certain rate. Targeted drug delivery systems deliver drugs more effectively and relatively more practically to today's drugs, increase patient compliance, extend drug half-life, and reduce healthcare costs. Therefore, the development of techniques capable of selectively transporting drugs to pathological cells, tissues or organs is now one of the most important areas of drug research.

Advances in the field of nanotechnology, especially nanoparticles (NP) with many applications in the clinical area has had a significant impact on the pharmaceutical industry. Nanotechnology focuses on the formulation of therapeutic agents in liposomes and nanoparticles (nanoparticles, nanocapsules, micelles and dendrimers). These formulations provide targeted drug delivery to the diseased structure. Since nanoparticles have the potential to be used in the diagnosis and treatment of many diseases, it may be thought that they will be more involved in drug delivery system technology in the near future. Various branches of science, especially in the field of health nanotechnology applications are becoming widespread and the replacement of traditional drugs with new drugs is accelerating. In this process; nanotechnology and biotechnology are leading the development of numerous drugs produced by the pharmaceutical industries. Various active substance release systems and targeting systems have been developed to minimize disintegration and loss of active substance, to prevent harmful side effects, and to increase bioavailability and rates of action. Some of these systems are liposomes, nanoparticles, active substance polymer conjugates and polymeric micelles^{7,8}.

LIPOSOMES

Liposomes were discovered in the 1960s, and doxorubicin (Doxil) in the liposomal formulation was FDA approved for treatment as an anticancer agent in the 1990s. Liposomes are biocompatible, non-immunologically reversible vesicular structures that contain phospholipid bilayers, ranging in size from nanometers to several micrometers. Liposomes have aroused great interest as they play an important role in the formulation of drugs in enhancing versatility and therapeutic effect. Various problems such as poor solubility, short half-life, poor bioavailability, strong side effects of various drugs have been largely overcome by liposomes. The improved safety and efficacy thus achieved have been achieved for a wide range of classes of drugs including antitumoral agents, antivirals, antimicrobials, vaccines, gene therapeutics. Currently, liposomes are used by pharmaceutical scientists to reduce the toxicity and side effects of drugs. Liposomes which can increase vascular permeability in tumor tissues are used in various diseases such as cancer^{9,12,13}.

Liposomes are nanoparticles that form hydrophilic heads and the distribution of phospholipids with hydrophobic anionic / cationic long chain tails which form the closed membrane structures by themselves. Hydrophilic agents or hydrophobic agents such as drugs or siRNA can be included in the internal compartment and hydrophobic membrane, respectively. Today, many liposomal anti-cancer drugs have been used successfully in clinical or advanced clinical research. Doxorubicin, which has improved pharmacokinetics and tissue distribution with polyethylene glycol (PEG), has received FDA approval for the treatment of Kaposi's sarcoma in 1996¹⁴. Other approved liposomal formulations; Non-PEG Doxorubicin (Myocet), Liposomal Daunorubicin, Liposomal Amphotericin B and Liposomal Cisplatin etc.¹⁵. Anti-sense oligonucleotides are also popular and are transported within these structures¹⁶. Liposomes containing anti-Bcl-2 were tested in vivo in rodents after their in vitro success and no toxicity was

observed after 6 weeks of i.v treatment¹⁷. Anti-Raf treatment LErafAON has also shown significant success in Phase I studies in advanced solid tumors as a treatment method that increases the efficacy of chemotherapy and radiotherapy¹⁸.

NANOPARTICLES

Nanoparticles; These are matrix systems which are prepared with natural or synthetic polymers, varying in size from 10-1000 nm, which are called nanospheres or nanocapsules according to the preparation method, where the active substance is dissolved, trapped and / or adsorbed or bound to the surface. Nanocapsules are vesicular systems, the drug is trapped in a cavity and surrounded by a polymer membrane. Nanospheres are matrix systems where the drug is physically and uniformly dispersed¹⁹.

Obtained by using natural or synthetic polymers; The advantages of nanoparticles used to target proteins, peptides and genes as well as drugs are related to two main properties. The first of these properties is that the nanoparticles have small particle sizes. Thus, they are taken into the cells through small capillaries and allow the active substance to accumulate in the target area. The second is the use of biodegradable materials in the preparation of nanoparticles. Biodegradable materials provide controlled release of the active substance in the target tissue over periods of days or even weeks. In addition, nanoparticles; they increase the stability of drugs / proteins or peptides, they can be easily sterilized, their active substance loading capacity is high and thus the intracellular distribution of the active substance is increased. By this way, the release and bioavailability of the drug administered in the form of nanoparticles can be increased in oral administration¹⁹.

In the preparation phase, the polymers can be isolated from their natural sources (such as Chitosan produced from Chitin) or synthesized for the intended structure (poly-lactic-co-glycolic acid / PLGA). PLGA, Chitosan, human serum albumin, alginate and hyaluronic acid polymeric structures are frequently used in preclinical studies. Chitosan nanoparticles are a prominent candidate in siRNA transport due to positive electrical charges. Electrostatic interactions between negatively charged siRNA and positively charged chitosan form a safe circulating carrier for siRNA. Src and fgr siRNAs achieved significant tumor reduction in the orthotopic ovarian ca model with chitosan coated nanoparticles²⁰. Albumin-coated paclitaxel (Abraxane) is the first polymeric formulation approved by the FDA for the treatment of metastatic breast cancer²¹. The lung was also approved for CA. The gp60 receptor, albumin 60 kDa glycoprotein, binds to albumin and is introduced into the tumor by triggering caveolin-1 mediated transport²².

MICELLES

Micelles are considered to be spherical particles composed of corona (term describing the hydrophobic ends of amphiphilic copolymers in a micelle structure) which are stabilized by the core and hydrophilic polymer chains formed morphologically from hydrophobic blocks. Micelles as drug delivery systems; solubilize the active substances with low solubility and thus increase their bioavailability. They can remain in the body for a sufficiently long period of time to allow the active substance to be collected in the required region. The size of nanometers allows them to accumulate in areas with weak vascularization. Micelles can be targeted by binding to specific ligands. They can be produced in large quantities, easily and reproducibly. They are able to protect the active ingredient from inactivation in the biological environment, thus avoiding undesirable side effects⁷.

Polymeric micelles are formed from self-formed amphiphilic copolymers in size of 10-100 nm. They consist of a hydrophobic core and a hydrophilic corona layer. Micelles

increase the bioavailability of hydrophobic drugs and can protect the drugs from inactivation of the surrounding tissue²³. Polymeric micelles can be used for both active and passive targeting. Genexol-PM is a polymeric micelle loaded with paclitaxel which is examined for the treatment of breast, lung and pancreas. Pluronic and NK911 are phase I phase doxorubicin loaded micelle formulations²⁴. Polymeric micelles that are modified with ligands such as folate (to the Folate receptor) and mAb C225 (which binds to the EGF receptor) are also present for active targeting. In the Nude mouse xenograft model, the doxorubicin loaded PLGA-b-PEG polymeric micelle formulation was found to increase tumor uptake and provide significant tumor regression²⁵.

DENDRIMERS

Dendrimers are repetitive, branched, spherical large molecules. It is characterized in that various molecules can be inserted between the branches of the nested structures, the numerous end groups, which may also be reactive. A dendrimer consists of a core, branching units around the core, and surface groups, also called branched functional groups. Diversity of dendrimers is provided by functional groups. Branching units allow the dendrimers to grow in a repetitive manner. The co-surface groups of dendrimers are well suited for certain drug delivery applications, with excellent encapsulation properties and largely controllable chemistries. Depending on the surface groups, the drug can be loaded into the molecular dendrimer. Dendrimers can function as drug delivery systems either by drug encapsulation within the dendritic structure or by the interaction of electrostatic or dendrimer with covalent bonds to the functional group at the end of the drug²⁶.

The main advantage of dendrimers is that many anti-cancer agents can be conjugated to the central core or functional terminal groups²⁷. In addition, depolymerization of dendrimers makes it possible to control the modified release profiles of the payload²⁸. For example; The biocompatibility and release properties of polyamidoamine (PAMAM) dendrimers can be increased by PEGylation and acetylation, modification with anionic or neutral molecules²⁹. Again doxorubicin was conjugated with PEGylated PAMAM dendrimers via acid-sensitive linkages, and doxorubicin was released under acidic conditions. In the ovarian ca xenograft model generated by the SKOV3 cell line, the highest PEGylation dendrimers showed maximum tumor accumulation. In another study, the PHSCN peptide was used. This peptide interacts with the $\alpha 5$ subunit of integrin to block its activity. This peptide-modified polylysine dendrimers showed a significant decrease in the number of invasive human breast cancer cells³⁰. The same dendrimers significantly inhibited lung colony formation in tumor mice.

ACTIVE SUBSTANCE-POLYMER CONJUGATES

The use of large biological molecules in the peptide-protein structure as a drug active ingredient is increasing day by day. Most of the peptide-protein substances are disintegrated in the stomach when taken orally or the plasma half-life is very short when injected. In order to solve the transport and pharmacokinetic problems of these substances in the body, researchers have focused on polymers. These polymers can be listed as PCL (polycaprolactone), PE (polyethylene), PEG (polyethyleneglycol), PEO (polyethylene oxide), PLA (polylactic acid), PLGA (poly (lactic-co-glycolic acid). Nowadays, polyethylene glycol (PEG) is the most widely used polymer in the formulation of peptide-protein drugs³¹⁻³³.

ADVANTAGES OF PEG-ACTIVE SUBSTANCE CONJUGATES

1. PEG masks the protein surface by steric inhibition and protects it against degrading agents.

2. It increases the molecular size of the polypeptide and consequently decreases renal ultrafiltration.
3. Contact of antibody or antigen processing cells is also inhibited by PEG chains.
4. Protein immunogenicity is reduced or eliminated.
5. PEG carries its physicochemical properties to the peptide or nonpeptide molecule to which it binds, thus changing the biodistribution and solubility properties of that substance.
6. Enzymes and bioactive substances are dissolved in organic solvents or aqueous solutions.
7. It prolongs the excretion of PEG-protein conjugate in vivo and the circulation time in the blood.
8. It stabilizes the physiological properties of protein and bioactive substances.
9. The pharmacokinetic properties of various active substances are improved.
10. It increases the accumulation in tumor tissues.

MONOCLONAL ANTIBODIES

Monoclonal antibodies (MAb) are obtained from cells called "hybridoma" that constantly produce antibodies by fusing mammalian cells capable of producing antibodies with infinitely divisible tumor cells. These cells are called eden monoclonal cells için because they are derived from a single type of hybrid cell. Monoclonal antibodies can be used in the diagnosis, purification and analysis of biological materials, as well as in the diagnosis and treatment of cancer and some autoimmune diseases, and in the prevention of tissue rejection in organ transplants. Monoclonal antibodies were first obtained from hybridomas formed by B cells and myeloma cancer cells of mice immunized with antigen in 1975 by Köhler and Milstein. The advantage of monoclonal antibodies is that they take less time to develop than a drug of chemical origin, thus making the cost less expensive. In addition, it has less toxic effects compared to other drugs that make them attractive³⁴.

GENE SILENCING WITH SIRNA

The discovery of RNA interference (micro RNA and siRNA mediated gene silencing) is considered one of the most important advances in biology in the last 10 years³⁵⁻³⁷. siRNA is widely used today as a powerful tool in gene research and in silencing post-transcriptional gene expression. Furthermore, potential siRNA applications are of great interest in the clinical use of this technology in the treatment of cancer and other diseases. The specifically designed siRNA can bind specifically to the target gene (mRNA) sequence and induce degradation of mRNA translation³⁷.

These double-stranded short RNAs (dsRNA) are cleaved into 21-base siRNA fragments via the DICER protein. The target mRNA binds with antisense chain and forms with RNA-Induced Silencing Complex (RISC). The RNA in the complex breaks down the target mRNA, the endonuclease (Argonaute 2), causing degradation and causing protein expression to stop. For therapeutic applications, synthetic siRNAs are used to target oncogenes, cancer cell proliferation, survival, invasion, angiogenesis, metastasis and genes that provide resistance to chemotherapy and radiotherapy, as well as genes that cause other pathologies^{38,39}.

RESTRICTIONS ON SYSTEMIC USE OF SIRNAS

The main limitation of siRNAs after systemic administration is rapid degradation by circulating nucleases with a half-life of 15 min. and renal clearance^{40,42}. Therefore, early studies have entered clinical trials, particularly in relation to local applications (intravitreal and intranasal routes)^{41,42}. Various chemical modifications have been used to improve the stability of siRNAs: phosphorothioate, boranophosphate backbone and sugar modifications such as 2-OMe, 2-fluoro, and 2-O-methoxyethyl (2-MOE)⁴¹. Another problem is the electrostatic recoil caused by the negation of the negatively charged cell membrane by the negatively charged cell membrane and therefore the need for special carriers is also important. In addition, albeit specific, these siRNA sequences can induce undesirable side effects by triggering the immune response³⁸. In summary, the development of reliable, stable, effective and tumor-specific delivery systems is of great importance in the translation of siRNA-based applications into the clinic. Nanotransmitters give great promise in this sense and have the potential to reduce siRNA-related toxicities and prevent off-target effects in normal tissues⁴³.

NANOCARRIERS FOR SYSTEMIC SIRNA ADMINISTRATION

Nanocarriers with particle sizes ranging from 1 to 1000 nm can overcome the problems associated with the systemic application of siRNAs⁴⁴. These carriers have been shown to successfully carry loads such as chemotherapeutic agents, oligonucleotides, drugs, peptides and imaging agents in in vivo systems. It is desirable that an ideal nanocarrier be reliable, non-toxic, biocompatible, biodegradable, non-immunogenic and capable of rapidly bypassing renal or hepatic clearance. It is also expected to be able to deliver the target siRNA to the desired tumor tissue and release this cargo to the target cytoplasm with maximal efficacy. The various nanoparticles of siRNA carriers are composed of natural or synthetic biodegradable nanomaterials. These are liposomes, micelles, polymers (chitosan, PLGA, PLA, etc.), carbon nanotubes, quantum dots, gold nanoshell or iron oxide magnetic carriers. Nanoparticles below 200nm passively accumulate in tumor tissue⁴⁴. This is due to increased permeability and EPR effect (Abnormal tumor vascularity, endothelial patency and fenestrations). Nanoparticles larger than 100 nm are taken up by the Reticulo-endothelial system in the liver, spleen, lung and bone marrow, and the circulation times of small-sized nanoparticles are thus longer⁴⁴. Very small nanoparticles and polymers with a MW of less than 40 kDa are removed by renal excretion. Physical properties of nanoparticles, electric charge, shape and so on. other properties determine the fate of the particle. For example; Negatively charged particles are cleared faster than positive charges and tend to be absorbed by phagocytic cells. Furthermore, due to negatively charged siRNA, they cannot achieve optimal loading effectivity⁴².

LIPOSOMAL NANOCARRIERS FOR SIRNA

Liposomal formulations are one of the most popular delivery systems and are used as effective systemic drug carriers because of their high degree of biocompatibility. Liposomes provide various advantages as siRNA delivery systems. They protect their load from degradation, preferably accumulate in tumor tissue (passive targeting), carry high concentrations of charge, target siRNAs specifically to tumor cells and microenvironment with high affinity-indicating ligands (Active Targeting), reliable in human and animal depending on lipid content and an effective systemic carrier system. Liposomes can be coated with PEG (Polyethylene Glycol) so that they can have longer circulating half-lives, preventing their detection and elimination by RES cells^{45,46}. PEGylation may serve as a linkage for the attachment of target ligands for specific targeting of liposomes and allows them to interact better with cell surface receptors in the targeted cells. For this purpose, targeting ligands such as peptides, monoclonal

antibodies (immunoliposomes), aptamers and chemical compounds can be used for targeted transport by binding with PEG.

CATIONIC-LIPID-BASED LIPOSOMES

Cationic liposomes are one of the most widely used non-viral delivery systems of siRNA / shRNA or antisense oligos. Cationic lipids such as DOTAP and DOTMA can form complex or lipoplexes with negatively charged DNA or siRNAs and have high in vitro transfection effectivity^{45,47}. However, due to their very stable structure after entering into the cell in vivo, it is very weak in releasing siRNA and has limited success in gene downregulation^{46,48}. The high toxicity of cationic lipids clinically prevents them from being one of the most important candidates as siRNA carriers. The use of cationic liposomes in in vivo mouse models generates dose-dependent toxicity, pulmonary inflammation, hepatotoxicity, and systemic interferon type I response due to TLR4 activation^{46,48,49}. They also activate the complement system and cause rapid clearance by RES macrophages. Their toxicity is associated with ROS induction and increased intracellular calcium levels⁴⁸. DOTAP-based liposomes accumulate near the vessel and are preferably retained by the liver / spleen, causing a decrease in systemic anti-tumor treatment efficacy⁴⁷. In summary, although cationic liposomes offer certain advantages as siRNA carriers, their potential toxicity should be examined in detail before clinical trials, and lipid selection and proper formulation will reduce these toxicities.

TUMOR TARGETED NANOPARTICLES

Targeted delivery systems enhance the therapeutic window of drugs by increasing transport to the target tissue and reducing side effects. This concept has been demonstrated using tumor cell-specific antibodies. Tumor-targeted nanoparticles accumulate 10-100 times more in tumor tissue compared to passive targeting⁴⁷. Generally, high-affinity ligands are attached to the outer surface to increase the efficiency of siRNA transfer. Functional peptides, lipophilic molecules, PEG and aptamers are used in tumor targeting. Folate Receptor alpha (FR), transferrin receptor, AlphaVBeta3/5 integrin receptors and prostate specific membrane antigen (PSMA) are used for the most common targeting⁵⁰⁻⁵⁷. FR-alpha is a highly selective tumor marker and overexpresses in 90% of ovarian and nasopharyngeal cancers and some breast cancer. $\alpha V\beta 3$ and $\alpha V\beta 5$ are specifically expressed in angiogenic tumor epithelium and in some metastatic cancers. For example; Folate or RGD conjugated DOPC-liposomes showed better and longer-term silencing in vivo and exhibited better levels of antitumor activity than normal DOPC-based liposomes in two different ovarian cancer models. Similar targeting strategies can be implemented by coating liposomes with these specific antibodies. These strategies have been successfully applied in receptor specific transport of chemotherapeutic agents, radiopharmaceuticals, imaging contrast agents, peptides and siRNAs⁵⁸⁻⁶⁰. As a result, siRNA-laden tumor-targeted nanotransmitters can increase therapeutic efficacy and reduce toxicity associated with their burden

CONCLUSION AND FUTURE PROSPECTS

Nanocarriers have great potential in cancer treatment, diagnosis and imaging. The application of siRNA-based therapies appears to be the best candidate and is currently being tested in human trials in the clinic. Pharmacokinetics, pharmacodynamic parameters, most importantly toxicity and safety profiles of various siRNA carrier systems should be well defined and future studies will develop more effective and reliable carrier systems. Neutral lipid-based nanoliposomes seem to be very promising for effective and reliable transport of siRNAs, especially in the last decade. In order to further enhance the tumor efficacy of these carriers, tumor-targeting versions thereof

need to be developed. In conclusion, siRNA-based therapies in Phase I-III clinical trials show great promise in targeting oncogenes and signaling pathways that trigger cell proliferation, cell cycle, invasion/metastasis and resistance mechanisms in various cancers. It is foreseen that in the next 10 years, we will witness the widespread use of these treatment methods in combination with standard treatments not only for cancer but also for other diseases.

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