Minireview Article

Aptamer Functionalized Liposomes for Cancer Treatment

Abstract

Cancer is a common disease affecting a large number of people. It is considered that 1 out of 3 people will be diagnosed with cancer in the course of their lives. Targeted therapy is gaining popularity since one can specifically target certain proteins, peptides, tissue environment that can stop the spread of cancer. One way to specifically target cancer is by the use of aptamer-liposomes. This review paper describes the use of aptamers as a new class of ligands with high specificity for various cancers. Liposomes are being used as the vehicles for the delivery of the anti-cancer drugs and for the attachment of aptamers to their surface. The main FDA approved (or in clinical trials) formulations of liposomes, aptamer, and aptamer-liposomes are being presented in this review. Aptamer-liposomes seem to be very attractive systems for the delivery of drugs for the treatment of various types of cancer.

Keywords

Aptamers, Liposomes, Nanodrugs, Nanoparticles

Main Text

Introduction

Cancer is a life-threating disease that continues to be very difficult to address. Even though there are a lot of drugs for the treatment of cancer, conventional chemotherapeutic drugs exhibit increase toxicity, rapid clearance, and non-specific distribution in the body.[1,2]

Nanomaterials seem promising carriers for the chemotherapeutic drugs. Their size varies from 1 nm to 100 nm and they have a high surface to volume ratio with a good drug release profile. They can take advantage of the leaky vasculature of tumors in order to successfully reach the target and deliver the drug. They also can reduce the drugs' toxicity and increase their solubility.

The most well-known nanomaterials used as nanodrugs are liposomes, polymeric nanoparticles, micelles, nanocrystal nanoparticles, inorganic nanoparticles.[3] There are FDA-approved nanodrugs available for clinical use from all these categories.[4] The first FDA approved nanoformulation, Doxil, was approved in 1995 for the treatment of

ovarian cancer. Currently, there are more than 50 FDA approved nanodrugs for clinical use. These nanodrugs do not necessarily show increased efficacy as compared to the conventional drugs, but they show reduced toxicity. A way to improve the efficacy of the treatment is to target the cancer site.

Size, shape, long-circulation, and surface characteristics of nanomaterials are all important properties. Size of the nanomaterials is very important since the size affects the uptake of the drug and the biodistribution. The size also affects the encapsulation efficiency. The size of the nanoparticles should not exceed 200 nm. Shape is also important. Most common nanomaterials are spherical, but rods, cylinders have been used with success. Surface modifications are extremely important. The surface should be hydrophilic so that they are not captured by the macrophages and they are able to circulate for a longer time. This is most commonly done by coating the nanomaterials with polyethylene glycol (PEG).[5]

Most of the FDA-approved drugs are liposomal formulations and some polymeric formulations. Liposomal formulations are some of the best and most studied nanoparticle based systems to target and effectively deliver drugs and genes to cancerous cells and not only. Liposomes have been discovered in 1961 by Bangham. Liposomes are models of biological membranes, made out of lipids. In an aqueous environment, there is the spontaneous tendency for the polar heads of the lipids to stay in contact with water and for the non-polar hydrophobic tails to avoid water. This leads to the formation of spherical vesicles that have a polar internal cavity in which polar drugs can be encapsulated and a non-polar, hydrophobic area which is called the lipid bilayer, in which hydrophobic drugs can be loaded. Liposomes can also be functionalized for long-circulation and/or targeting. Depending on the application, liposomes can be prepared from a variety of lipids and mixtures of lipids.

Liposomes are models of biological membranes and they can deliver the encapsulated drug by passive targeting or by active targeting. In passive targeting, the liposomes fuse with the biological membrane and deliver its payload. For active targeting, the liposomes have to be modified by attaching ligands to their surface. The ligands should be chosen so that they specifically recognize and bind to a specific molecular marker of disease. The targeting ligands increase treatment efficiency.[6]

There is a variety of targeting ligands that can be used such as antibodies, small peptides, and aptamers. Aptamers are a new class of targeting ligands. They proved to be great targeting ligands with very high affinity for their ligands as well as easy chemical modifications to enhance their stability in biological fluids.[7]

However, there are a number of limitations for the use of aptamers such as rapid excretion via renal filtration and low in vivo binding affinity for their targets. Liposomes can help with both these limitations by attaching the aptamers to the surface of long-circulation liposomes.

Liposomes are made out of phospholipids to which other materials can be added for stability, targeting, detection. Cholesterol is usually incorporated to change the membrane permeability. There are several ways to prepare liposomes. One of the most used methods to prepare liposomes is by the hydration of a thin lipid film made from evaporating the chloroform from the solution of lipids dissolved in chloroform. The thin-film is then hydrated with a solution of the drug to be delivered, if the drug is hydrophilic. If the drug is hydrophobic, it will be added to the lipids solution in chloroform before the formation of the thin lipid film.[8,9]

Other methods to prepare liposomes are reverse-phase evaporation[10], detergent-dialysis, sonication[11], dehydration-rehydration[12], and solvent-vaporization method[13]. Each method has its advantages and disadvantages. Most of these methods involve some common steps, such as the formation of the lipid film by evaporating lipids to dryness and the hydration of the lipid film with the aqueous solution of interest, which leads to the formation of multilamellar vesicles (MLV). The most reproducible method for liposomes synthesis is the thin lipid film method.[14,15]

Based on their size and number of bilayers, there are three classes of liposomes: small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), and multilamellar vesicles (MLV). Multilamellar vesicles are formed very easily by just hydrating a lipid film. In order to form unilamellar vesicles the solution of MLV's has to go through different mechanical processes that will disrupt the multilamellar vesicles. Vesicles with a diameter of less than 100 nm are considered small unilamellar vesicles, and vesicles with a diameter of more than 100 nm are considered large unilamellar vesicles. During the liposomes formation steps, their size, homogeneity, charge, lamellarity can be controlled.

There are several types of liposomes such as conventional liposomes, stealth liposomes, and targeted liposomes. The first generation of liposomes are called conventional liposomes. They are rapidly cleared after injection. The second-generation of liposomes are called long-circulating liposomes or stealth liposomes and they are able to evade interception by the immune system. These liposomes are covered with a polymer, polyethylene glycol (PEG).They have a long-circulation time in the bloodstream, favorable pharmacokinetic distribution and ability to find a target. The third generation of liposomes are long-circulation and targeted to a disease site. Targeting can be achieved by attaching small molecule ligands, peptides, antibodies, or aptamers.

There are several FDA approved liposomal formulations, most of which act through passive delivery. The first liposomal formulation approved for use in clinics was Doxil™. Doxil was approved in the US in 1995 for the treatment of ovarian cancer. Another approved liposomal formulation is DaunoXome for the delivery of daunorubicin in the treatment of advanced HIV-associated Kaposi's sarcoma. Other liposomal formulations for cancer therapy are: Depocyt, Myocet, Mepact, Onivyde, and Marqibo.[16]

There are FDA approved liposomal drugs designed to treat other diseases than cancer. Abelcet (1995), Amphotec (1996) and Ambisome (1997) are clinically approved for treatment of fungal infections.

DepoDur is a liposomal formulation approved in 2004 for administration before surgery as a pain management drug. Another liposomal formulation for pain management is Exparel, a liposomal local anesthetic drug approved in 2011.

There are also liposomal formulations for viral infections. Epaxal is one of these formulations and it's a vaccine for treatment of Hepatatis A.[16]

One of the few targeted liposomal formulations for breast cancer, MM-302 by Merrimack Pharmaceuticals announced positive Phase I trial results in 2015. MM-302 is based on a PEGylated liposome targeting ErbB2 (HER2) using an antibody fragment as a ligand to deliver doxorubicin.[17]

Aptamers possess a series of properties that make them very attractive ligands for tartgeting therapy. Aptamers are short single-stranded nucleic acid oligomers with a high affinity to specific targets. Aptamers are selected using a method called Systematic Evolution of Ligands by Exponential Enrichment (SELEX). Aptamers like antibodies display high specificity and affinity for their targets. However, unlike antibodies, they lack immunogenicity, can be easily synthesized and modified.[18, 19]

Aptamers possess other advantages as well such as a smaller size (3 nm) as compared to antibodies (~15 nm) that allows them to penetrate in tissues. Aptamers are also very stable at higher temperatures than antibodies.[20]

Aptamers are synthesized by SELEX.[21, 22, 23, 24] SELEX involves three steps. The first step involves incubation of the target with a selection of randomized sequences. The next step involves separation of the non-bound sequences from the bound sequences followed by the amplification of the bound sequences by PCR. These steps are repeated until the bound sequence provide the wanted affinity for the target. The process can be done *in vitro* or *in vivo.* However, if the aptamers are selected *in vitro*, there is the possibility that they will not be functional *in vivo*. The best approach would be to inject the library in an animal, harvest the tissue or organs and extract the bound sequence followed by amplification by PCR in order to create a new library. This will ensure that the aptamers are compatible with the *in vivo* applications.

Aptamers were discovered in 1990s by by Tuerk and Gold. Since their discovery, there is only one FDA approved aptamer formulation, Macugen/Pegaptanib sodium, for different disease conditions such as age-related macular degeneration (AMD), diabetic macular edema and proliferative diabetic retinopathy.[25] AS1411 is an aptamer in Phase II clinical trials. It is a 26-base guanine-rich oligonucleotide (GRO) with an unmodified (phosphodiester) DNA backbone. AS1411, previously named ARGO100, forms a G- quadruplex structure and is the first aptamer in clinical trials for the treatment of human cancer.[26] A second therapeutic aptamer also in Phase II clinical trials for cancer

is NOX-A12 (Olaptesed Pegol, NOXXON), an antagonist of the chemokine CXCL12 (or SDF1). Thus far, NOX-A12 has only completed clinical trials for hematologic malignancies, although a new two-part trial (NCT03168139) is currently recruiting patients with colorectal or pancreatic cancer.[27] There is a total of 11 aptamers in different stages of clinical trials: Pegaptanib sodium/Macugen, E10030, ARC1905, AS1411 (AGRO100), NOX-A12, NOX-E36, NOX-H94, ARC1779, NU172, REG1 system, BX499 (formerly known as ARC19499).[28] The therapeutic aptamers used in oncology are AS1411 for the treatment of advanced renal cell carcinoma and NOX-A12 that fights fight tumor proliferation and metastasis.

Attachment of aptamers to liposomes can be done in two different ways: by non-covalent attachment or covalent attachment. Aptamers are negatively charged and they can be attached to cationic liposomes via electrostatic interactions. However, the covalent coupling of aptamers to liposomes is preferred since the covalent bond is stronger, creating a more stable delivery system. There are also two ways in which aptamers can be covalently linked to the liposomes. One way is by pre-conjugating the aptamers to the liposomal structure before liposomes' formation step. The drawback of this formulation is that a fraction of the aptamers will be located inside the liposomes prohibiting the specific targeting to the cancer site. Another way is by post-conjugation of the aptamers to the surface of pre-formed long-circulating liposomes. The conjugation can be done via thiol bonding or to activated carboxyl groups.[29] Jingjinh Li produced the AS1411 aptamer liposomes by EDC/Sulfo-NHS activation of pegylated liposomes containing carboxyl groups, followed by addition of the AS1411 aptamer for 2 hours at 37° C. The mixture was then washed, centrifuged, and the precipitate dispersed in PBS for future use.[30] These AS1411-liposomes possess affinity for over-expressed nucleolins on the surface of breast cancer cells (MCF-7 cells) as demonstrated by Catuogno et al.[31]

Mucin 1 (MUC1) is another target, a glycoprotein over-expressed on the surface of several cancer cells. 5TR1 and S2.1 are the aptamers that target MUC1. The researchers studied the effect of treatment with 5TR1 Doxorubicin loaded liposomes in C26 tumor bearing mice. The authors of the paper demonstrate significant reduction in the tumor growth and enhanced survival upon treatment with the 5TR1 aptamer.[32]

Sgc8 is another aptamer successfully conjugated to the surface of liposomes that targets protein tyrosine kinase 7 (PTK7) for the treatment of human acute lymphoblastic leukemia cells. Kang et al. demonstrated in vitro high specificity and efficiency for the leukemia cells.[33]

Baek et al. developed a doxorubicin loaded liposomes conjugated with anti-PSMA (prostate-specific membrane antigen) A9 aptamer (xPSMA9). The study was done in vitro and showed enhanced binding to prostate cancer cells (PSMA positive) along with the reduction in tumor size in prostate epithelial cells (LNCaP) as compared to non-targeted liposomes.[34] Another aptamer specific for prostate cancer is SZT01. Stuart et al. encapsulated N,N,N0,N0 -tetrakis(2-pyridylmethyl)-ethylenediamine (TPEN) in the

aptamer targeted liposomes. TPEN induces oxidative stress leading to cell death. The researchers were able to demonstrate reduced tumor growth in a prostate cancer xenograft model.[35]

Other studies show the specific delivery of doxorubicin by TSA14-liposomes. TSA14 is an aptamer specific for breast tumor cells. Moosavian et al. demonstrated in vitro improved cellular uptake and cytotoxicity as compared to the non-targeted liposomes. They also describe how to prepare aptamer-liposomes by incubating TSA14-NH_2 aptamer with DSPE-PEG(2000)-carboxylic acid for 2 hours at room temperature.[36]

Another aptamer-liposome formulation in cancer studies is the A6-liposomes prepared from pre-formed liposomes bearing a PEG-MAL group available for coupling the aptamer. A6 aptamer is specific for HER2 receptors in breast cancer cells. Powell et al. used A6 targeted liposomes to deliver siRNA to breast cancer cells.[37] Another aptamer for HER2 is HB5.[38]

Conclusions

Aptamers attached to various nanoparticles seem very promising for the treatment and diagnosis of various diseases.[39] Since Macugen, other aptamers failed to get approved by the FDA. The main problem is the lack of improvement over the existing treatment. Aptamers are heavily studied these days to improve their applications in targeted delivery and a number of them are entering clinical trials. This makes aptamers-liposomes very attractive delivery systems to study for treatment and diagnosis of many different diseases.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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