REVIEW

Low density lipoprotein bionanoparticles: From cholesterol transport to delivery of anti-cancer drugs

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Abstract In this review article, we highlight the importance of low-density lipoprotein (LDL) and its implications in the field of drug delivery to cancer cells. LDL is naturally occurring bionanoparticles (BNPs) with a size of 18–25 nm. These BNPs specifically transport cholesterol to cells expressing the LDL receptors (LDLRs). Several tumors overexpress LDLRs, presumably to provide cholesterol for sustaining a high rate of membrane synthesis. LDL BNPs are biocompatible and biodegradable, favorably bind hydrophobic and amphiphilic drugs, are taken up by a receptor-mediated mechanism, have a half-life of 2–4 days, and can be rerouted. Drugs can be loaded onto LDL BNPs by surface loading, core loading, and apoprotein interaction. LDL may be used as a drug carrier for treatment of atherosclerosis, cancer, and in photodynamic therapies.

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1. Introduction

Nanoparticulate matter is a collection of particles with at least one dimension that is smaller than 1 μm but larger than atoms and molecules. The size of nanomaterials is similar to that of most biological molecules and structures (Buzea et al., 2007). Fig. 1 represents definition of nano and micro sizes and some biological nanomaterials.

The therapeutic or diagnostic agents of interest are encapsulated within nanoparticles using a polymeric matrix and are adsorbed or conjugated onto the nanoparticle surface (Misra et al., 2010). Nanoparticles may be targeted to specific sites via the receptors on target cells that elicit specific biochemical interactions (Misra et al., 2010). The universal structural topology of nanoparticles consists of a core compartment with terminal surface groups (Misra et al., 2010). Nanosized materials (5–100 nm) are used in various applications. The use of therapeutic nanoparticles as unique drug delivery systems will soon be a significant addition to current cancer therapeutics. This technology has enabled the manipulation of the biological and physicochemical properties of materials to facilitate more efficient drug targeting and delivery (Buzea et al., 2007).

Bionanotechnology, a subdivision of nanotechnology focuses on the development of novel nanoscale materials from biological building blocks (Lee and Wang, 2006). Natural

![Figure 1](image_url)  
Figure 1 Represents size of nanomaterials compared to biological components and definition of nano and micro sizes.
nanoparticles, bionanoparticles (BNPs), include viruses (Rae et al., 2005), lipoproteins (Skajaa et al., 2011) and nanoerythrosomes (Paygude, 2013). Viral nanoparticles are emptied viruses with a diameter of 30 nm that can carry drugs. They are naturally biocompatible, particularly with viruses that do not cause human diseases (Singh et al., 2007). Some viruses have a natural affinity for receptors on tumor cells, such as transferrin receptors (Singh et al., 2006).

Nanoerythrosomes have been proposed as encapsulation systems for macromolecular drugs (Paygude, 2013). They added benefits like greater retention time, bypasses macrophage uptake and systemic clearance (Paygude, 2013). The use of nanoerythrosomes looks promising for a safe and sure delivery of various drugs (Paygude, 2013).

Nucleic acids, ferritins, self-assembled protein cages, enzyme complexes and peptides have been extensively studied as starting materials for nanomaterial synthesis. These biogenic systems self-assemble primarily based on multiple non-covalent interactions to become highly organized nano-systems with a diverse array of shapes and sizes (Lee and Wang, 2006).

Lipoproteins are BNPs that transport cholesterol and other lipids in the blood; their size ranges from 8 to 1200 nm. Because they are endogenous carriers, lipoproteins are not recognized as foreign entities by the human immune system and are not absorbed by the reticuloendothelial system (RES). As a result, lipoprotein nanoplatforms may provide a solution to the biocompatibility issues associated with most synthetic nanostructures (Zheng et al., 2005).

The targeting of tumor cells by antineoplastic drugs is often characterized by low selectivity. As a result, different types of natural and synthetic delivery systems have been proposed as carriers for improving the selectivity of antitumor drugs (Polo et al., 2002; Singh et al., 2007). Nanoparticles can be targeted to cancer cells by attaching monoclonal antibodies or cell-surface receptor transporter ligands that bind specifically to molecules found on the surfaces of target cells (Zheng et al., 2005).

A number of receptors for hormones, growth factors, folate acid, vitamin B12, low density lipoprotein receptors (LDLRs) and others are overexpressed in cancer cells (Sega, 2008). The overexpression of the LDLRs in various tumor cells has been attributed to the large quantities of cholesterol and fatty acids required for supporting rapid proliferation. Therefore, the incorporation of drugs into low density lipoprotein (LDL) may be an efficient method of targeting tumor cells (Firestone, 1994; Lundberg, 1993). LDL is naturally occurring bionanoparticles (LDL BNPs) that has long been used as vehicles for the selective delivery of diagnostic and therapeutic agents to tumor cells (Zheng et al., 2005).

LDL BNPs are recognized by and internalized in cells through specific membrane receptors that interact with the apoprotein B-100 (apoB-100) (Polo et al., 2002). The entry of nanoparticles into cells is mediated by several mechanisms, including clathrin-mediated transport, caveolae-dependent endocytosis, macropinocytotic uptake (Radu et al., 2010). Other mechanisms such as electrostatic forces, Van der Waals or steric interactions are involved in the entry of nanoparticles into cells (Radu et al., 2010).

The purpose of this review article is to highlight the use of LDL BNPs as a system of drug delivery to cancer cells and to discuss the opportunities and challenges faced by the use of LDL BNPs. The properties of LDL BNPs and the manner in which these properties affect the efficiency and specificity of BNPs as a drug delivery system are described. We will also discuss the advantages and disadvantages of LDL BNPs and the attempts to improve their therapeutic efficacy in cancer treatment.

2. Lipoproteins as natural drug delivery systems

Lipoproteins are spherical nanoparticles characterized by an insoluble core consisting of cholesteryl esters and triacylglycerol surrounded by a shell of amphipathic phospholipids and specialized proteins termed apolipoproteins (Wasan and Cassidy, 1998). Lipoproteins differ in their content of proteins and lipids and are classified into five main categories based on their density: chylomicrons, very low-density lipoproteins (VLDLs), intermediate density lipoproteins (IDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs) (Wasan and Cassidy, 1998). Lipoproteins are conventionally described by their density, which is reflected by an increased protein/lipid ratio, and are classified by their surface apolipoprotein content, which subsequently governs the ultimate fate of the particle (Chung and Wasan, 2004). Fig. 2 shows the general structural features of lipoproteins.

Lipid transport is regulated by apolipoproteins as well as lipoprotein receptors, lipolytic enzymes, and transfer proteins; all of these molecules act in concert to maintain cholesterol and triacylglycerol homeostasis (Chung and Wasan, 2004).

The hydrophobic nutrients, such as triacylglycerols and cholesteryl esters, are delivered from the liver and intestine to other tissues in the body for storage or catabolism and energy production by lipoproteins (Vickers and Remaley, 2013). Many of the recently discovered functions of HDL are, in fact, not strictly conferred by its ability to promote cholesterol flux, but by the other molecules it transports, including a diverse set of proteins, small RNAs, hormones, carotenoids, vitamins, and bioactive lipids (Vickers and Remaley, 2013). Based on the ability of HDL to interact with almost all cells and transport and deliver fat-soluble cargo, HDL has the remarkable capacity to affect a wide-variety of endocrine-like systems (Vickers and Remaley, 2013).

Lipoproteins are also involved in other biological processes, including coagulation, tissue repair, and immune reactions (Wasan and Cassidy, 1998). Lipoproteins also have a strong potential to serve as drug-delivery vehicles due to their small size, long residence time in the circulation and high-drug payload (Alanazi, 2003; Sabnis and Lacko, 2012). Consequently, lipoproteins and synthetic/reconstituted lipoprotein preparations have been evaluated with increasing interest toward clinical applications, particularly for cancer diagnostics/imaging and chemotherapy (Sabnis and Lacko, 2012).

Lipoproteins transport hydrophobic drugs, including halofantrine, amphotericin B, and cyclosporine. Furthermore, lipoproteins are the major transporters of vitamin E in circulation (Chung and Wasan, 2004). Also, antiarrhythmic, antidepressants, antimalarials, antifungals, and immunosuppressants, associate with plasma lipoproteins. The pharmacokinetic properties, tissue distribution, and pharmacological actions of these drugs are affected by their interactions with lipoproteins (Chung and Wasan, 2004).

Understanding the binding mechanisms of lipoproteins and hydrophobic drugs can predict their therapeutic effects and
toxicities. Moreover, understanding the mechanisms by which lipoproteins loaded with therapeutic agents are taken up by cells may provide novel methods for drug delivery and targeting (Chung and Wasan, 2004).

In the last few decades, LDL has been investigated for its ability to deliver drugs to cells expressing the receptors (Gu et al., 2011; Jiang et al., 2006). Because most tumor cells over-express LDLRs, these receptors have been utilized as a delivery method for a number of anticancer drugs (Glickson et al., 2009). The role of lipoproteins in lipid transport and drug delivery is illustrated in Fig. 3.

2.1. Lipoproteins as bionanoparticles (BNPs)

Lipoproteins are BNPs consisting of hydrophobic core (cholesterol esters and triacylglycerol), amphipathic shell (phospholipids and free cholesterol) and apolipoproteins (Skajaa et al., 2011). The particle size of lipoproteins ranges from 10 to 1200 nm, and the size of each type of lipoprotein is as follows: chylomicron, 75–1200 nm; VLDL, 30–80 nm; IDL, 25–35 nm; LDL, 18–25 nm; and HDL, 8–12 nm (Glickson et al., 2009).

3. LDL as a bionanoparticle

LDL is a spherical BNP with a particle size of 18–25 nm and is composed of a core and surface coat. The core is hydrophobic and consists of esterified cholesterol and triacylglycerol (Alazni et al., 2004). An LDL particle also contains an outer surface layer of phospholipids surrounded by a single apoB-100 protein (Prassl and Laggner, 2009). Free cholesterol intercalates between the phospholipid fatty acid chains, providing a degree of rigidity to the LDL particle (Nikanjam et al., 2007a). LDL contains approximately 50% free and esterified cholesterol, 25% proteins, 20% phospholipids, and 5% triacylglycerol. Over 95% of the LDL apo-protein is apoB-100 (Rajman et al., 1999). apoB-100 is exposed at the surface, allowing for receptor recognition with nine amino acids at residues 3359–3367 serving as the binding domain for the LDLR (Segrest et al., 2001). Fig. 4 displays schematic model of an LDL nano-particle.

The most important role of LDL is the delivery of cholesterol to extrahepatic tissues for utilization in a number of processes, including steroid production and membrane synthesis (Fielding and Fielding, 1996).

LDL particles have multiple distinct subspecies that differ in particle size and particle number (Berneis and Krauss, 2002). Particle number and particle size can be efficiently measured by nuclear magnetic resonance spectroscopy. Analytical studies have shown that LDL particles exhibit a large heterogeneity of size, density, and composition due to differences in their relative contents of cholesterol esters, triacylglycerol, and apoB-100 (El Harchaoui et al., 2007). This heterogeneity has been identified through the use of density gradients, rate zonal and analytical ultracentrifugation, and non-denaturing gradient gel electrophoresis (Krauss, 1994). Distinct LDL sub-populations vary in isoelectric point, electrical charge, hydrodynamic properties, and immunoreactivity (Chapman et al., 1988).

Measurements of LDL subfraction diameters using staining electron microscopy have established that the mean particle diameter decreases with increasing density. The structure of LDL particles of different densities varies with respect to both the size of the core and the width of the surface shell (Rajman et al., 2009).
et al., 1999). LDL subfraction patterns were divided into four main groups: LDL I to LDL IV (Austin et al., 1988). In an alternative classification based on particle diameter, two major subclasses of LDL in each profile are: subclass A, with a particle diameter of 25.5 nm or greater, and subclass B, with a particle diameter less than 25.5 nm (Austin et al., 1988). Individuals with the pattern B LDL subclass had higher triacylglycerol and cholesterol compared to those with pattern A LDL (Maki et al., 2000). In kinetic turnover studies, where the quantity of radioactive products with labeled LDL was measured in the urine, two LDL pools were found: a rapidly cleared pool A, likely consisting of larger LDL particles, and a slowly cleared pool B, likely consisting of smaller LDL particles (Rajman et al., 1999).

3.1. The LDL receptors

The LDLR is a single-chain transmembrane glycoprotein responsible for the binding and endocytosis of LDL and is the founding member of the LDLR superfamily (Xu et al., 2013). Each member of the LDLR family undergoes receptor-mediated endocytosis, is expressed in a number of different tissues and has a wide range of different ligands that are not specific to the LDL particle. The LDLR consists of five distinct domains, including a ligand binding domain, epidermal growth factor (EGF) precursor-homology domain, O-linked sugar domain, membrane-spanning domain, and cytoplasmic tail (Brown and Goldstein, 1986).

The ligand binding domain consists of 292 amino acids with 40 amino acids repeating 7 times (with little variation). This domain has many cysteine residues, which cluster with negatively charged amino acids, to form binding sites for apoB-100. EGF precursor homology region (containing approximately 400 amino acids) is like the EGF precursor. O-linked sugar chains bond to the 58 amino acids found in the third domain. The hydrophobic domain consists of 22 hydrophobic amino acids that span the cell membrane. The cytoplasmic domain or the cytoplasmic tail (containing 50 amino acids) is

Figure 3  Role of lipoproteins in transport of lipids and delivery of hydrophobic drug delivery. Lipoprotein-mediated lipid and drug transport in mammals involves several different lipoproteins. The hydrophobic nutrients, such as triacylglycerols and cholesteryl esters, are delivered from the liver and intestine to other tissues in the body for storage or catabolism and energy production by lipoproteins. As well the drugs can be delivered by similar mechanism.

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important in attaching the clathrin molecules and creating the endocytosis vesicles (Brown and Goldstein, 1986). Fig. 5 represents the structure of LDLR. The LDLR consists of five distinct domains, including a ligand binding domain, epidermal growth factor (EGF) precursor-homology domain, O-linked sugar domain, membrane-spanning domain, and cytoplasmic tail. Ligand binding domain has negative charge allowing the binding of positively charged residues of apoB-100.

Figure 4 Schematic model of LDL nano-particles. Spherical LDL BNP with a particle size of 18–25 nm and is composed of hydrophobic core consists of esterified cholesterol and triacylglycerol. An LDL particle also contains an outer surface layer of phospholipids surrounded by a single apoB-100 protein. Free cholesterol intercalates between the phospholipid fatty acid chains.

Figure 5 The LDL receptor structure. The LDLRs consist of five distinct domains, including a ligand binding domain, epidermal growth factor (EGF) precursor-homology domain, O-linked sugar domain, membrane-spanning domain, and cytoplasmic tail. Ligand binding domain has negative charge allowing the binding of positively charged residues of apoB-100.

Uptake of LDL particles occurs through both receptor- and non-receptor-mediated pathways. On average, 30–40% of the total plasma pool of LDL is cleared from the body each day, and of this portion of the circulating LDL pool, approximately two thirds are removed by receptor-mediated uptake (Wasan and Cassidy, 1998). Through electrostatic interactions between the highly cationic receptor binding sequences on apoB-100 and the complementary anionic sequences on the cell surface, the LDL particle binds to the LDLR embedded in clathrin coated pits on the cell surface (Brown and Goldstein, 1986).

The receptor-ligand complex internalizes into vesicles and is transformed into an endosome. Inside the endosome, the receptors dissociate from the lipoprotein and are either metabolized or recycled back to the coated pits at the cell surface. Subsequently, LDL is delivered to lysosomes where it is degraded (Wasan and Cassidy, 1998). The turnover time for cell surface LDLRs is approximately 24 h (Brown and Goldstein, 1986). Cholesteryl esters are hydrolyzed to free cholesterol, the free cholesterol, now available to the cell, can be used in the production of membranes, re-esterified and stored, or removed from the cell and excreted in the bile (Wasan and Cassidy, 1998). The empty LDLR is recycled to the plasma membrane to mediate another round of LDL binding and internalization (Xu et al., 2013). apoB-100 is broken down into its amino acid components.

This pathway could be useful for drug delivery, and may then be directed toward tumors expressing LDLR. Any drugs incorporated into the LDL particle accumulate within the targeted cell, providing a very effective mechanism for drug delivery (Chu et al., 2013). Accordingly, LDL-based hydrophobic/amphiphilic drug delivery systems (DDS) can be internalized and degraded by regular cell metabolism, and these properties can be used in targeted therapies for cancer (Chu et al., 2013). Fig. 6, shows cellular uptake of LDL loaded with drug.

3.2. Role of apolipoprotein B-100 in LDLR binding

apoB-100 is located on the short arm of chromosome 2 consisting of 4560 amino acids and has a molecular weight exceeding 500,000 Daltons. This apoprotein is secreted from hepatocytes as a protein compound in VLDL particles. VLDL particles undergo intravascular processing during which all other types of apoproteins are removed such that apoB-100 remains the sole apoprotein in LDL particles. Each LDL particle contains one apoB-100 molecule (Veniant et al., 1999).

The complete primary structure of apoB-100 has been identified, and its secondary and tertiary structures have been proposed. The LDL receptor-binding domain is present at residues 3359–3367. This domain is formed by positively charged residues that interact with the negatively charged regions of the LDL receptor (Rall et al., 1981). The function of apoB-100 is to maintain the integrity of LDL particles and to control the plasma levels of LDL by binding to its receptors (Brown and Goldstein, 1986). The apoB-100 molecule is modeled as a belt that surrounds the LDL particle. Therefore, the diameter of the LDL particle and apoB-100 has implications for the binding affinity of apoB-100 to its receptors (Miserez and Keller, 1995).

A central role in this process appears to be exerted by the arginine residue at position 3500 of the apoB-100 protein,
which stabilizes two clusters of basic amino acids at residues 3147–3157 and 3359–3367 that ensure the binding of apoB-100 to the LDL receptor (Ma¨ rz et al., 1993). apoB-100 can be purified and used to reconstitute/synthesize apoB-100 containing lipoparticles as hydrophobic/amphiphilic compound delivery vehicles. The reconstituted apoB-100 lipoparticle may be an ideal carrier for transporting hydrophobic and amphiphilic compounds. Consequently, synthetic nanoparticles with a small fragment of apoB-100 or LDL-dextran mixture were developed (Chu et al., 2013).

3.3. LDL and cell growth

Lipid metabolism contributes to the transformed phenotype of cancer cells. In particular, aberrant regulation of cholesterol homeostasis has been associated with multiple types of cancer (Smith and Land, 2012). Because of their diverse biological roles, lipids contribute to several aspects of tumor growth, energy production, and redox homeostasis (Santos and Schulze, 2012). Cholesterol is an essential component of the cell membrane. The primary pathway of cholesterol production is de novo synthesis; however, another mechanism of obtaining cholesterol is the cellular uptake of circulating LDL. Several pieces of evidence have demonstrated that the LDLRs have a role in cell growth and tumorigenesis (Notarnicola et al., 2008). An increase in levels of cholesterol was observed in tumors relative to that in normal tissue. Moreover, low serum cholesterol levels have been associated with the presence of tumors in cancer patients, suggesting that cholesterol may accumulate in tumor tissue (Smith and Land, 2012).

3.4. The LDLRs in cancer cells

Lipoproteins are a major mechanism by which cholesterol is transported and delivered to at least some types of cancer cells, and some cell types may endogenously generate cholesterol. Not surprisingly, LDL plays a role in cholesterol delivery to some types of cancers and up-regulation of the LDL receptor in malignancy has been reported (Damiano et al., 2013).

It has been demonstrated that LDLRs are overexpressed in various human cancer cell lines. Glioblastoma multiforme (GBM) is a highly aggressive tumor that accounts for approximately 85% of primary brain tumors in adults. A study on seven GBM cell lines showed that these cells have high LDLR expression (Chu et al., 2013). However, studies on the distribution of LDLRs in normal rat and monkey brain tissue suggest that normal brain tissue, particularly the gray matter of the cortex, has relatively low LDLRs (Chu et al., 2013).

Several studies have demonstrated that the LDLRs have a role in cell growth and tumorigenesis (Notarnicola et al., 2008). Compared to normal cells, the LDLRs are overexpressed in many malignancies, including acute myelogenous leukemia (three to 100-fold compared to normal cells), adrenal adenoma (eightfold), and colon (sixfold), pancreatic, lung, brain, and prostate cancers (Firestone, 1994). Among the laboratory models of human cancer, B16 melanomas and HepG2 hepatomas are rich in LDLRs (de Smidt et al., 1990; Rensen et al., 1997). Overexpression of LDLRs occurs in several cancer cell lines due to the cell’s increased need for lipids in the synthesis of new membranes (Graziani et al., 2002). A large body of evidence has demonstrated that some types of cancer cells have high LDL requirements due to both elevated LDLRs’ levels on rapidly growing tumor cells and a depletion of LDL in the blood of cancer patients (Bildstein et al., 2011). Tumor cells generally have high cholesterol requirements because they are rapidly dividing and because cholesterol is required for new membrane synthesis.

The LDLRs are overexpressed in several cancer cell lines due to the rapid growth of neoplastic cells and their corresponding need for structural lipids (Bildstein et al., 2011). As a result, LDLR activity may be higher in cancer cells than in normal cells. In fact, increased LDLR expression has been
observed in several malignant diseases, including colon cancers, prostate tumors, adrenal tumors, gynecological cancers, lung tumors, leukemias, and brain tumors (Nikanjam et al., 2007a,b). Growth factors may be important in the regulation of LDLR gene expression (Samadi-Baboli et al., 1993).

Using immunocytochemistry to study the localization of the LDLRs, relatively few LDLRs were found in the neurons and glial cells of monkey and rat brains. Additionally, LDLR binding was evaluated using homogenates from human intracranial tumors and the surrounding normal tissue and shown to be highly variable between tumor tissue and normal brain tissue. The boronated protoporphyrin that associates with LDL is endocytosed into the human glioblastoma cell line (Lenka Maletinska et al., 2000), suggesting that LDL may be used as a drug delivery system for tumors expressing the LDLRs (Rensen et al., 2001).

3.5. Sources of LDL

LDL was isolated from plasma using ultracentrifugation. Sodium bromide (1.0 mL; 1.025 g/mL) containing 0.01% (w/w) NaCl, 0.3 mmol/L Na₂EDTA, and 1 mmol/L benzamide hydrochloride was gently added to 2 mL of plasma, and the mixture was separated by ultracentrifugation at 400,000g (at the bottom of tube) at 4 °C for 5 h. The bottom fraction (2.0 mL) was then transferred to another tube, and the density was increased to 1.063 using NaBr (1.151 g/mL). After another centrifugation at 400,000g at 4 °C for 5 h, the top fraction was decanted, and the container was filled with nitrogen and stored in the dark at 4 °C until analysis. The LDL fractions were protected from the sun and artificial light, which can cause oxidation, and analyzed within 24 h of preparation (Aoki et al., 2012; Kader and Pater, 2002). LDL can also be purchased from commercial suppliers (Huntosova et al., 2012).

3.6. Advantages of LDL BNPs as drug carriers

Using LDL as a drug carrier may circumvent many of the issues encountered with synthetic carriers, and LDL also has important advantages compared to other nano-delivery systems (Huntosova et al., 2012). The following list provides the characteristics of LDL particles that may be exploited if the particle is used as a drug vehicle.

(1) LDL BNPs are natural carriers and are therefore biocompatible. LDL particles are non-immunogenic because LDL escapes recognition by the mononuclear phagocytic system. This feature may provide a solution to the biocompatibility problem associated with most synthetic nanodevices.

(2) LDL BNPs are biodegradable. LDL particles are internalized into cells and digested in lysosomes by acid hydrolysis and enzymatic degradation, releasing free cholesterol, fatty acids, and amino acids. All degradation products are recycled by the cell.

(3) The small LDL particle size of 18–25 nm is within the ideal range of 10–100 nm for nanoparticle drug carriers. Therefore, they are neither quickly eliminated by the kidney nor captured by RES (Davis et al., 2008).

(4) Hydrophobic drugs can be loaded in the hydrophobic core while amphiphilic drugs can be loaded in the amphipathic shell of LDL.

(5) Drugs are protected from hydrolytic and enzymatic degradation in the plasma when sequestered in the hydrophobic core of LDL nanoparticles.

(6) LDL has a large core capacity. Therefore, the lipid fraction can have substantial quantities of lipophilic drugs loaded inside it. apoB-100 can also be covalently conjugated with a ligand or with diagnostic and therapeutic agents.

(7) LDL BNPs may prolong the circulation half-life (2–4 days) of drugs because they are not cleared by the RES. Substantially unmodified LDL is also not rapidly cleared from the bloodstream by the RES.

(8) LDL BNPs can be targeted to tumor cells because the LDLR is highly expressed in most tumor cells. Cancer cells require large amounts of cholesterol for the synthesis of new membranes. Consequently, many cancer cells have higher LDLRs than normal cell. Neoplastic cells readily internalize and degrade LDL using the high-affinity receptor pathway.

(9) LDL nanoparticles may be rerouted to receptors other than the LDLR. Alkylation of the lysine side chains of apoB-100 eliminates LDLRs-binding activity. Alkylation of 20% of the lysine side chains completely abolishes LDLR binding. Therefore, LDL nanoparticles can be redirected to alternative targets. The alkylated lysine ε-amo side chains have appropriate receptor-targeting ligands. The addition of folic acid ligands to the amino groups redirects the particle to folate receptors (Glickson et al., 2009).

3.7. Disadvantages of LDL BNPs as a drug carrier

LDL particles are isolated from human blood, and as a result, there is concern about the introduction of pathogens that produce infectious diseases. Commercial sources of LDL that provide pathogen-free blood proteins are available. Although LDL has proven to be a useful vehicle for delivery of lipophilic drugs and diagnostic agents to tumors, its application is largely limited to LDLRs-related diseases. Specifically, it has a limited use in cancer therapy because many tumors do not overexpress the LDLRs, whereas some normal tissues express. Native LDL, though, is less than ideal as a targeting agent since it is difficult to isolate in large quantities and is variable in composition and size (Nikanjam et al., 2007b).

A key limitation to this application is the existence of receptors on normal cells, such as those in the RES that lead to high background binding. This issue can be resolved to some extent by judiciously selecting the targeting particle dimensions, by attaching polyethylene glycol groups to the particle surface to minimize non-specific binding, or by choosing appropriate receptors, but the problem cannot yet be entirely eliminated and remains a confounding issue in the use of this delivery system (Glickson et al., 2009).

3.8. Methods of loading drugs into LDL particles

The hydrophobic lipid core (cholesterol esters and triacylglycerol) and amphipathic phospholipid shell of LDL
nanoparticles allow substantial quantities of lipophilic drugs and amphipathic drugs to be loaded inside the LDL particle. The amino acid residues of apoB-100 can be covalently conjugated with diagnostic ligands or therapeutic agents. There are three ways to incorporate diagnostic and therapeutic agents into LDL particles: protein loading, core loading, and surface loading (Zheng et al., 2005). Fig. 7 represents the methods of loading drugs into LDL particles.

3.8.1. Surface loading
In this method, the therapeutic agent is non-covalently bound to the surface of the phospholipid shell. The probe is typically conjugated to one or two long hydrophobic fatty acid chains and to a cholesterol group. Very high product yields have been achieved with fluorescent or paramagnetic probes (Glickson et al., 2009). Surface loading is used for either diagnostic or therapeutic agents (Li et al., 2004) and paramagnetic Gd-chelated MRI probes (Corbin et al., 2006). The method is easy to implement but prone to high leakage rates because transfer of the surface probe to the outer phospholipid layer of the cellular plasma membrane is thermodynamically favorable. Thus, delivery of substantial amounts of probe to cells may occur by pathways not involved in receptor delivery (Glickson et al., 2009).

3.8.2. Core loading
In this method, either diagnostic or therapeutic agents are reconstituted into the non-polar cholesterol ester core of LDL nanoparticles. Core binding was used for the agents for photodynamic therapy (PDT) (Zheng et al., 2005). The lipid core of LDL can be extracted with a nonpolar solvent, such as heptane, while keeping the phospholipid and protein “shell” intact. As a result, the particle spontaneously reassembles with recovery of over 50% of the LDLR binding activity. Anti-cancer drugs have been either directly loaded onto LDL or the core lipids of LDL were replaced with drugs (Nikanjam et al., 2007b). LDL contains about 1500 molecules of cholesterol esters per LDL particle. Therefore, the development of anticancer cholesterol conjugate compounds mimicking the native cholesterol esters can be loaded into LDL by core loading (Radwan and Alanazi, 2013). This presents a potentially effective approach for targeted drug delivery to cancer cells via the elevated LDL receptors (Alanazi et al., 2003).

3.8.3. Apoprotein loading
Protein loading was performed by covalent conjugation of diagnostic or therapeutic agents to apoB-100. A number of investigators have attached chelating groups to lysine side chains (Glickson et al., 2009). Modification of apoB-100 in LDL particles results in their uptake by liver sinusoidal endothelial cells. Furthermore, attachment of 18F-containing ligands to the lysine-ε-amino groups has also been utilized for imaging (Pietzsch et al., 2004). Radio-iodination of tyrosine side chains for SPECT detection has been attempted but leads to a change in the protein’s transport properties (Sobal et al., 2004). Protein labeling has the advantage of producing stable products; however, a disadvantage is that covalent binding may modify the delivery characteristics of LDL because the receptor-binding site includes reactive lysines (Glickson et al., 2009).

4. LDL as a drug carrier

4.1. LDL as a drug carrier for atherosclerosis treatment
An excess of oxidized LDL in the aortic vasculature leads to LDL being consumed by macrophages, transforming them into foam cells (Ross, 1993). Over time, additional macrophages are attracted, the vasculature becomes congested, and the excess cholesterol forms plaques. Oxidized LDL may bind these

Figure 7 Methods of loading drugs into LDL nanoparticles. The lipid and phospholipid fractions of LDL nanoparticles allow substantial quantities of lipophilic drugs and amphipathic drugs to be loaded inside the LDL particles. The amino acid residues of apoB-100 can be covalently conjugated with ligands or diagnostic and therapeutic agents.
plagues more tightly than native LDL, facilitating an undesirable accumulation of the lipoprotein (Wang et al., 2001). This spontaneous accumulation of LDL at a target site makes LDL an ideal carrier for a therapeutic drug (Alanazi, 2003; Tauchi et al., 2000). In fact, dexamethasone, which prevents macrophage transformation into foam cells, was loaded into human LDL as palmitoyl dexamethasone, and after intravenous injection, the level of dexamethasone-loaded LDL was increased in the aorta and persisted for 7 days (Tauchi et al., 2001).

4.2. LDL as a drug carrier for anticancer drugs

A variety of in vitro and in vivo studies have indicated that tumor cells from the colon, kidney, lung, and brain are characterized by an enhanced expression of LDLRs. In many cases, the incorporation of drugs into the large LDL lipid vesicle does not impair their recognition by the LDLRs. Indeed, death of cultured tumor cells has been induced using various drug-LDL complexes (Polo et al., 2002). Lipoproteins may also be used as endogenous carriers to target tumor cells, which have high lipoprotein receptor activities (Jiang et al., 2006).

LDL may be used as carriers to conjugate water-insoluble anti-cancer drugs to lead to a higher accumulation of the drugs at specific locations (Jiang et al., 2006). This property may allow the particle to deliver its loaded drugs and avoid drug degradation (Lou et al., 2005). LDL has a high affinity for, and may accumulate in, tumor cells. LDL has also been used as a carrier to deliver anti-tumor drugs into hepatoma cells to treat hepatocellular carcinoma (Jiang et al., 2006).

Masquelier et al. (2000) investigated the possibility of using LDL as a drug carrier to increase the selectivity of anti-tumor drugs in cancer chemotherapy and found that the sequestering of anti-tumor agents with LDL does not influence the characteristics of the drugs (Jiang et al., 2006). Doxorubicin (DOX) has been coupled to human LDL to form an LDL-DOX complex. When the complex was injected into mice, more LDL-DOX accumulated in the liver than free DOX. In contrast, less LDL-DOX accumulated in the heart than free DOX (Chu et al., 2001). The synthetic lipoprotein coined “nano-LDL” targets cancer cells, specifically glioblastoma multiforme, is composed of a peptide fragment anchored to the particle via a lipid and binds the LDLRs (Nikanjam et al., 2007b). The nano-LDL particle was able to incorporate the paclitaxel prodrug at 0.33 mg/mL; the resulting nanoparticle was highly toxic to multiple glioblastoma multiforme cell lines expressing variable amounts of the LDLRs (Hackett et al., 2013). Most neurons do not express high levels of the LDLRs, which may make this vehicle highly selective for glioblastoma multiforme cells in the brain (Hackett et al., 2013).

4.3. LDL as a drug carrier for anticancer photodynamic therapy

Photodynamic therapy (PDT), the systemic administration of photosensitizers (PTS) followed by a local photoactivation of PTS, is a promising method for the treatment of solid tumors (Wilson and Patterson, 2008). Some porphyrins and other red light-absorbing photosensitizing agents, such as chlorins and phthalocyanines, accumulate in large amounts and are retained for prolonged periods of time by a variety of malignant lesions (Polo et al., 2002). Once photoactivated by irradiation with selected light wavelengths, the tumor-localized photosensitizers generate highly cytotoxic reactive oxygen species, inducing irreversible damage in the neoplastic tissue (Polo et al., 2002). PDT is currently approved for the treatment of bladder, esophageal, and lung tumors for both curative and palliative purposes. Several lines of evidence indicate that the efficiency and selectivity of tumor targeting by porphyrins and their analogs is enhanced by increasing the hydrophobicity of the molecule (Polo et al., 2002).

Suitable formulations of PDT agents in liposomal vesicles or LDL particles have been developed to allow for the systemic administration of poorly water-soluble porphyrin derivatives. Pharmacokinetic studies with tumor-bearing mice demonstrated that various liposome-associated porphyrinoids are efficiently transferred to serum lipoproteins, particularly to LDL, upon intravenous injection and consequently lead to a high tumor uptake (Polo et al., 2002). However, many PTS used in PDT are hydrophobic or amphiphilic and cannot simply be injected intravenously. In general, moderately hydrophobic PTS are preferentially transported in the bloodstream by albumins, whereas highly hydrophobic PTS interact mainly with lipoproteins, especially with LDL (Sharman et al., 2004).

As a result, LDL may play a key role in the targeted delivery of hydrophobic and amphiphilic PTS to tumor cells in PDT (Jin et al., 2011). The targeted delivery of drugs in complex with LDL into tumor cells is possible due to the enhanced expression of specific LDLRs in many types of transformed cancer cells compared to non-transformed cells (Huntosova et al., 2012).

5. Conclusions

LDL is a BNP that specifically transports cholesterol to cells expressing LDLRs. Because the LDLRs exhibit binding specificity, they can target drugs to site-specific cells. The properties of LDLs, such as small size, amphiphilic surface chemistry, and receptor-mediated uptake make them ideal candidates for therapeutics, imaging agents, and drug delivery vehicles.

The size distribution of these LDLs firmly within the nanoscale, advances within nanotechnology will continue to enable the development of these biomimics and to expand their potential into the therapeutic realm. LDL-BNPs can be directed specifically to tumor cells. As drug delivery system LDLs are biocompatible and biodegradable, have a longer half-life, and can be rerouted. Drugs can be loaded into LDL BNPs by surface loading, core loading, and apoprotein interactions.

Conflict of interest

The authors declare that there is no conflict of interest.

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