Asian Journal of Pharmaceutical Sciences 18 (2023) 100857

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/AJPS

Review

Nanotechnologies meeting natural sources: Engineered lipoproteins for precise brain disease theranostics

Ruoning Wang[a,b,1,*], *Xinru Zhang*[a,b,1], *Kuanhan Feng*[a,b], *Wei Zeng*[a,b], *Jie Wu*[a,b], *Danni Sun*[a,b], *Ziyi Lu*[a,b], *Hao Feng*[a,b], *Liuqing Di*[a,b,*]

[a] *School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, China*
[b] *Jiangsu Provincial TCM Engineering Technology Research Center of High Efficient Drug Delivery System, Nanjing 210023, China*

ARTICLE INFO

Article history:
Received 8 June 2023
Revised 3 September 2023
Accepted 8 September 2023
Available online 19 October 2023

Keywords:
Biological modulation
Brain diseases
Blood-brain barrier
Lipoproteins
Theranostics

ABSTRACT

Biological nanotechnologies have provided considerable opportunities in the management of malignancies with delicate design and negligible toxicity, from preventive and diagnostic to therapeutic fields. Lipoproteins, because of their inherent blood-brain barrier permeability and lesion-homing capability, have been identified as promising strategies for high-performance theranostics of brain diseases. However, the application of natural lipoproteins remains limited owing to insufficient accumulation and complex purification processes, which can be critical for individual therapeutics and clinical translation. To address these issues, lipoprotein-inspired nano drug-delivery systems (nano-DDSs), which have been learned from nature, have been fabricated to achieve synergistic drug delivery involving site-specific accumulation and tractable preparation with versatile physicochemical functions. In this review, the barriers in brain disease treatment, advantages of state-of-the-art lipoprotein-inspired nano-DDSs, and bio-interactions of such nano-DDSs are highlighted. Furthermore, the characteristics and advanced applications of natural lipoproteins and tailor-made lipoprotein-inspired nano-DDSs are summarized. Specifically, the key designs and current applications of lipoprotein-inspired nano-DDSs in the field of brain disease therapy are intensively discussed. Finally, the current challenges and future perspectives in the field of lipoprotein-inspired nano-DDSs combined with other vehicles, such as exosomes, cell membranes, and bacteria, are discussed.

* Corresponding authors.
E-mail addresses: ruoningw@njucm.edu.cn (R. Wang), diliuqing@njucm.edu.cn (L. Di).
[1] These author contributed equally to this work.
Peer review under responsibility of Shenyang Pharmaceutical University.

https://doi.org/10.1016/j.ajps.2023.100857

1. Introduction

Brain disorders, such as neurodegeneration, cerebrovascular diseases, and malignant tumors, have been reported in more than 700 million people worldwide [1]. Systemically administered medications have long been the most important route of drug delivery to treat diseases. However, the effectiveness of these conventional strategies may be compromised because of the following obstacles. First, the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB) hamper the permeation of poisonous substances and hinder the entry of 98% of therapeutic agents into the brain [2]. Second, systemic administration of drugs always leads to "on brain but off lesions" distribution and increases the incidence of adverse effects. Undesired side effects, such as fatigue, muscle pain, and nausea, have been observed after the treatment of most central nervous system (CNS) diseases. Third, complex pathological changes can develop resistance to treatment by causing multiple tolerance mechanisms that weaken the drug molecule function or by activating multiple pathways that reduce the foci response to therapeutic agents. Finally, although certain promising candidates, such as nucleotide- and peptide-based novel drugs, have emerged, these require strict storage conditions, time-consuming preparation, and appropriate transporters before clinical application.

The emergence of nano drug-delivery systems (DDSs) represents a quantum leap toward ensuring safe and efficient drug delivery to lesions. By leveraging passive or active targeting capability, various drugs relying on engineered nano-DDSs can penetrate physiological barriers and be delivered to designated locations, leading to "on brain and on lesions" distribution with enhanced concentration and accumulation. Owing to their inherent ability for synergistic delivery, nanovehicles and drugs with complementary mechanisms or different functions can achieve synchronous spatiotemporal transport to regulate microenvironments and reduce resistance. Additionally, nano-DDSs provide protective zones to prevent the degradation of active components before they land [3]. Several nanomedicines, such as Doxil, Abraxane, Vyxeos, have been recently approved for clinical cancer treatment. However, poorly biocompatible materials usually cause unexpected immunological responses, oxidative damage, and cellular dysfunction after long-term administration. Furthermore, the treatment effects of available nanomaterials or medicines remain are not yet optimal, indicating that biological barriers cannot easily be overcome. Therefore, the cumulative concentration in lesions needs to be further strengthened.

Lipoproteins, which are natural nano-sized particles, display inherent BBB-penetrating, brain-tumor-homing, and inflammation-resisting properties compared with other native biomaterials such as cell membranes, exosomes, phages, and viruses. Overexpressed receptors, including low-density lipoprotein receptors (LDLRs) and scavenger-receptor-type B1 (SR-BI) receptors, exist in brain epithelial cells and diseased sites (such as brain tumor cells and inflammatory tissues) and confer preferential lipoproteins for crossing the BBB and accumulating in lesions via receptor-mediated binding. Additionally, apolipoproteins, the primary protein component of lipoproteins, have been proven to exhibit antioxidant and anti-inflammatory activities, which are beneficial for alleviating the inflammatory and degenerative pathology of neurodegenerative diseases. However, complicated purification, susceptibility to oxygen, and uncontrollable quality standards are the three major limitations hindering their widespread application and translation. Owing to properties such as tractable assembly, highly ordered, and easily controlled, lipoprotein-inspired nano-DDSs with diverse sizes and shapes have received considerable attention in addressing the aforementioned limitations. Common structured shapes, including nanotubes, spherical particles, nanodisks, and worm-like nanomicelles, are instrumental in improving the loading capability of both hydrophobic and hydrophilic drugs with extended systemic circulation times. Without changing the original characteristics, functional moieties can be engineered to further improve BBB penetration, enrich lesion accumulation, and respond to unique microenvironments such as pH, inflammation, and reactive oxygen species, which conform to disease characteristics and offer personalized theranostics [4]. Generally, functional molecules can be delivered via three methods, namely surface modification, covalent binding, and core capsulation.

Lipoprotein-inspired DDSs are becoming more important as they permit the generation of nanoparticles (NPs) with enhanced BBB penetration and lesion accumulation to enable and control the release of multiple agents for brain disease diagnosis and therapy. Thus, a review that summarizes the current research trends and highlights the directions for future development is necessary. In this review, obstacles in the treatment of brain disorders are identified. The structural features and characteristics of lipoproteins are described. Subsequently, the design and application of lipoprotein-inspired nano-DDSs in brain disease treatment over the past 5 years are presented. Finally, the directions for further development to strengthen curative effects, improve security, and promote clinical conversion are extensively discussed.

2. Obstacles in brain-targeting drug delivery

Conventional intravenous administration is the mainstream drug delivery modality for brain disease theranostics. However, four challenges can be encountered in this method: i) two natural barriers, ii) "off lesion distribution, iii) complex pathological changes, and iv) "off clinical translation, termed BDCT critical issues. Missing any points of BDCT will reduce the curative effects and further cause noticeable side effects. Over the past few decades, nano-systems, including liposomes, inorganic NPs, micelles, and dendrimers, have been widely used to address the limitations of conventional approaches, exhibiting enhanced BBB penetration and targeting of diseased lesions. Unfortunately, the therapeutic effects can be subject to rapid clearance, limited accumulation, off-target effects, and potential toxicity (Fig. 1).

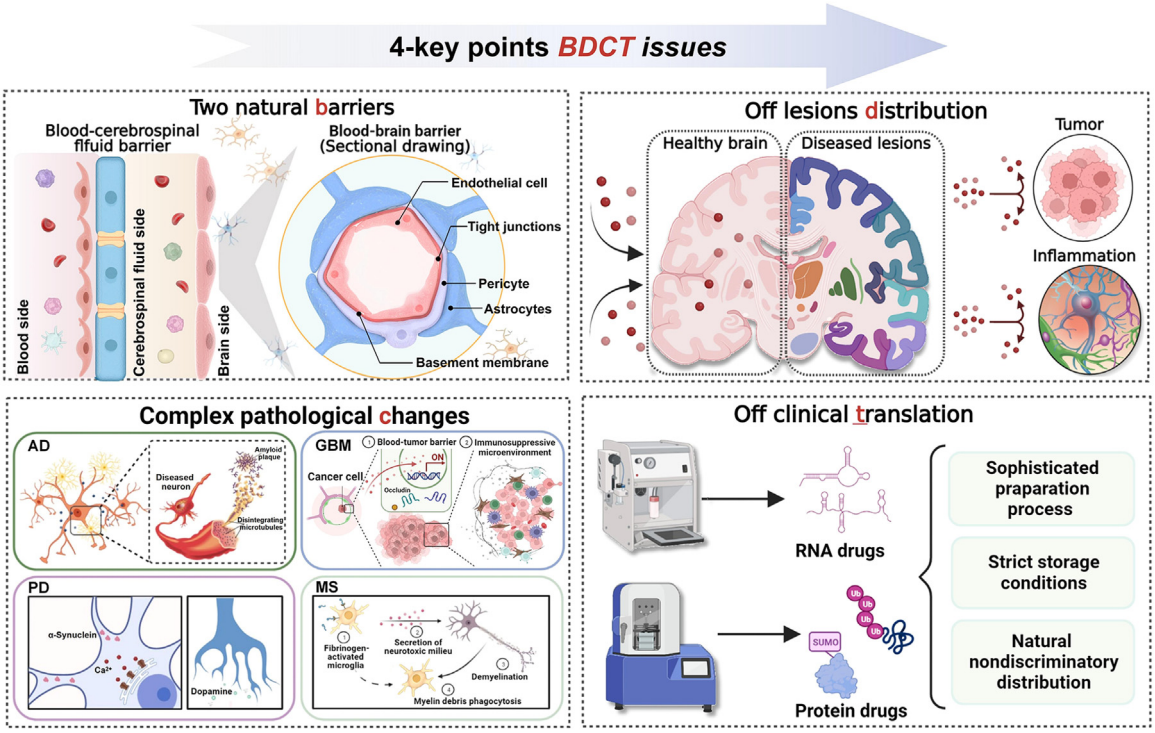

Fig. 1 – Schematic diagram of the proposed BDCT key issues of brain drug delivery: two natural barriers, off lesions distribution, complex pathological changes, and off clinical translation. The above-mentioned issues are commonly decreased the efficiency brain diseases treatment.

2.1. *4-key-point BDCT issues*

"Two natural barriers", the BBB and BCSFB, represent the first obstacles that limit delivery efficiency when therapeutic agents enter the blood circulation and are ready to enter the brain. The BBB is generally considered as a crucial limitation for therapeutic conveyance to the brain, resulting in insufficient drug accumulation and reduced therapeutic effects. The BBB is a special neuroglial membrane comprising endothelial cells, a basement membrane, and a tight junction, which separates blood from the brain parenchyma. The BBB is responsible for brain safety and contributes to invalid drug delivery. Except for necessary nutrients and gasses, most macromolecules, such as immune cells and therapeutic agents, can be intercepted by the BBB. To improve permeability, drugs with unique physicochemical properties, including low molecular weight (<400 Da) and high lipophilicity, should be designed. However, these drug particles still exhibit an undesired ability to cross the BBB because exogenous substances are probably expelled from brain endothelial cells by efflux transporters. Except for the BBB, the BCSFB hinders the efficacy of the systemic administration of therapeutic paradigms. Structurally, the BCSFB consists of choroid plexus epithelial and capillary cells, which share a smaller area than the BBB. No tight junctions exist in the capillary cells, and they hardly offer resistance to diverse components. However, these cells are surrounded by a single-molecular layer of polarized epithelial cells that are linked together by tight junctions. These junctions form a barrier that restricts the entry and movement of exogenous substances and prevents the free diffusion of cerebrospinal fluid (CSF) [5]. Therefore, it plays as significant in secreting and maintaining CSF stabilization. Although drugs rarely exhibit significant difficulties when entering or crossing the BCSFB, detectable drug concentrations in the brain remain dismal. As the BBB and BCSFB have different structures and properties, drugs entering the BCSFB are not equal to those entering the brain or lesions. Moreover, even if drugs are injected directly into the CSF, they cannot easily reach the deep brain parenchyma [6].

The concentration of drugs accumulated in lesions is another critical factor affecting treatment efficacy. "Off lesion distribution" infers drug non-discriminated distribution in the entire brain. It significantly decreases the clinical translation rate of most prosing agents. Off-lesion distribution causes drugs to indiscriminately attack normal and pathogenic tissues, thereby inducing serious toxic and adverse effects. This indistinguishable destruction of tissues and cells is attributed to the poor affinity between therapeutic agents and diseased sites. Therefore, increasing the accumulation and specificity of drugs in lesions, rather than allowing them to cross natural barriers, can be more practical for improving treatment efficiency. However, the targets between different brain disorders may exhibit differences; thus, explicit knowledge of the pathogenic and pathological characteristics of diseases is required for high-precision targeting design. For example, overexpressed receptors, such as transferrin receptors (TfRs), SR-B1, and glucose transporters (GLUTs), are recognized as promising targets for developing brain tumor drugs with improved precise

distribution [7]. Nevertheless, curbing the progression of inflammation and repairing peripheral nerve injury are two significant therapeutic hallmarks of neurodegenerative disorders (NDs), including Alzheimer's disease (AD) and Parkinson's disease (PD).

In addition to improving drug concentration in lesions, "complex pathological changes" should be considered during the drug design process to achieve good therapeutic effects. For NDs, including AD and PD, four issues should be considered: i) pathological aggregation of the microtubule-associated protein tau, ii) abnormal levels of iron and calcium in the brain, as well as the degeneration and loss in neurons, have been demonstrated several neurodegenerative processes, iii) neuroinflammation caused by toxic stimulation, infection, traumatic injury, or autoimmunity can promote the malignant process of NDs, and iv) mutations in cell death pathway-related proteins, such as apoptosis, necroptosis, and autophagy, have a bearing on the ND development. Recent preclinical studies have reported that basement membrane thickening and increased CNS exposure to P-gp substrates caused by AD may lead to low penetrability and accumulation of therapeutic agents in the brain and neurocytes [8]. For brain tumors, including primary brain tumors and brain metastases, surgical resection, radiotherapy, chemotherapy, or their combination are still mainstream treatment approaches in clinical research. However, a complete resection of all malignancies is extremely challenging because of their infiltrative properties and the non resectability of normal tissues. By releasing cellular signaling molecules, tumors reverse their microenvironment to hypoxia, low pH, and high H_2O_2, thus promoting tumor angiogenesis, immunosuppression, and heterogeneity, which provide significant support for improving tumor proliferation, differentiation, and invasion. Additionally, the formation of the blood-brain tumor barrier (BBTB) limits therapeutic outcomes. The BBTB exhibits better easy-penetrating properties than the BBB. However, it still hinders drug delivery at effective concentration because it is similar to the BBB in function and is characterized by heterogeneous permeability. Furthermore, the local damage to the BBTB associated with high-grade tumor does not effectively enhance therapeutic agents into lesions [9].

The research and development of drugs should not stop in the laboratory but should enter clinical trials and become marketable. "Off clinical translation" is primarily attributed to narrow therapeutic windows, single therapeutic targets, and potential systematic toxicity. When interacting with enzymes or other substances in the blood, the physicochemical properties of drugs may cause unexpected alterations and uncontrollable side effects. Recently, gene therapies, such as those using plasmid DNAs, antisense oligonucleotides, small interfering RNA (siRNA), and microRNAs (miRNAs) have been widely discussed owing to their satisfactory and enduring therapeutic effects. Gene therapy can treat diseases at the following three levels: (i) choosing a healthy copy of the gene to replace the disease-induced gene, (ii) disturbing or inactivating the disease-induced gene, and (iii) introducing a new disease-regulating gene or mimics into the body to assist in disease treatment. However, such biological products are more susceptible to the human environment than conventional chemicals, compromising their clinical development and application. Almost all RNA-interfering (RNAi) drugs are unstable in the serum, and some RNAi degradation sequences can induce unnecessary immune responses and cause damage to normal tissues. Other biological medicines also have limitations, such as rapid clearance, premature degradation, and short half-lives [10]. For example, the tissue-type plasminogen activator, a thrombolytic agent classified as glycoprotein, has been reported to share high affinity with fibrin, a thrombus component, and exhibits favorable thrombolytic effect without disturbing the normal coagulation balance. However, its clinical application has two major limitations: a half-life of a few minutes and degradation products that can lead to neurovascular toxicity. Therefore, developing reasonable DDSs to deliver fragile drugs with high levels of security is necessary.

2.2. *Non-biomimetic nano-delivery system*

Over the past 20 years, nanotechnology has been explored for the treatment of various diseases with ideal targeting capabilities, poor side effects, and higher patient compliance. Although several carriers have been used for brain delivery, some problems still need to be addressed.

Liposomes were the first nanocarriers approved by the FDA and have achieved success in clinical therapy. Liposomes are vesicles comprising phospholipids and cholesterol. They exhibit several characteristics, such as low toxicity and high loading efficiency, and their properties (size, shape, and composition) can be easily modified to render them suitable for drugs. Many studies have demonstrated that drug concentrations in lesions, including camptothecin, doxorubicin (DOX), and paclitaxel (PTX), can be significantly enhanced by loaded liposomes, which exhibit good therapeutic effects. However, the stability of liposomes is often undesirable. When exposed to complex biological environments, they are easily affected by proteins and undergo unexpected transformations, causing drug leakage and toxic effects on surrounding tissues. Although cationic liposomes have received considerable attention as carriers for delivering sensitive compounds, such as nucleic acids, cytotoxicity is still a serious risk that limits their application. Results have shown that cationic liposomes can reduce cell mitosis, form vacuoles in the cytoplasm, and destroys some significant cell proteins, such as protein kinase C [11]. Moreover, pure liposome-DDSs cannot easily cross the BBB, indicating that the drug concentration at the lesion site is not optimal. Other limitations of liposomes include fast systemic elimination and the inability to consistently control drug release. Some of these limitations have been addressed by coating the surface with hydrophilic polymers (*e.g.*, PEG). Nevertheless, these non-biocompatible tailored-NPs usually cause adverse immunological responses. Approximately 15% of patients experience allergic reactions, including skin reactions and peripheral neuropathy, during AD treatment [12]. Additionally, oxidative damage in the biological milieus and cytoplasmic vacuolation with long-term administration pose potential threats to treatment.

Inorganic nano-DDSs primarily consist of gold, silica, iron oxide, and quantum dots and became well-known in the 1990s. Owing to their small sizes and superior ability to accumulate in lesions, inorganic NPs are typically used for diagnosing, treating, and assessing prognostic outcomes in combination with imaging. Compared with liposomes, inorganic NPs share desirable inherent properties in photothermal therapy under photostimulated thermogenesis to promote the decomposition of pathological complexes such as amyloid-beta (Aβ) deposition in AD. Furthermore, their ability to provide on-demand drug release under external stimuli can meet clinical demands. Studies have not sufficiently demonstrated that inorganic NPs are suitable for drug delivery. However, their non-biodegradability, toxicity, and irreproducible and susceptible results in tests remain in the very early stages of clinical trials. After long-term systemic administration, inorganic nano-DDSs are barely eliminated from the body, leading to sustained accumulation in the reticuloendothelial system and potentially triggering inflammatory reactions, including fibrosis or cancer [13].

Amphiphilic copolymers can self-assemble into nanoscale particles, such as polymeric micelles and NPs. Over the past decade, studies have fabricated micelles to realize the loading and controlled release of insoluble drugs, such as PTX and DOX, to treat gliomas and other tumors. Many studies have demonstrated that micelle-based nanocarriers offer numerous attractive properties, including effective drug delivery, high drug-loading ability, and sufficient stability. For example, neprilysin and mRNA can be loaded into PEG micelles to achieve co-delivery with good performance in synergistic AD therapy. However, inherent toxicity and insufficient targeting ability remain the two primary challenges preventing the development of micelles for the treatment of brain diseases. Additionally, the micellar system easily dissociates instantaneously at the critical micelle concentration, leading to drug leakage and affecting the drug delivery efficiency [14].

Dendrimers have been used to treat various disorders for approximately 10 years. Owing to their large loading space and good water solubility, diverse types of dendrimers, such as polyamidoamine and polypropylene mine, have been used as drug vehicles and several of them have entered clinical trials. Recently, polyamidoamine dendrimers have been wrapped in a maltose histidine shell. They appeared effective in both BBB permeability and synapse protection and alleviated AD brain damage. Moreover, after being modified on the surface with the TAT peptide or lactoferrin (a common modifier enhancing the permeability of the BBB), polyamidoamine dendrimers exhibit increased therapeutic efficacy. Although the outcomes appear promising in laboratory models, the application of dendrimer-DDSs to brain treatment has limitations because of accumulated toxicity and the complexity of manufacturing. In the case of polyamidoamine and polypropylene imine, numerous dendritic macromolecules are densely covered with a large number of terminal amino groups on their three-dimensional spherical surfaces. As the branching number increases, the terminal amino groups increase and their cationic properties become stronger. Excess cations can cause cell membrane rupture and apoptosis leading to severe cytotoxicity. In addition, the potential danger of degradation limits their applications in organisms. Taxotere®, a docetaxel anti-tumor product launched in 2010, was used to treat solid tumors by destroying the mitosis of tumor cells. However, severe myelotoxicity (*e.g.*, neutropenia, leukopenia, and anemia), hepatotoxicity, and allergic reactions caused by surfactants severely restrict its application in organisms [15].

3. Lipoprotein

Thus far, the application of conventional nano-DDSs has been limited by their cytotoxicity because of the lack of biocompatibility and degradability. Therefore, a delivery system with good performance in terms of systemic security, targeting ability, biological barrier penetration, and effective drug loading is required. As endogenous substances, lipoproteins show great potential for drug delivery and have gained considerable attention. In this section, we provide a comprehensive introduction to the physicochemical properties and physiological functions of lipoproteins (Fig. 2) and focus on their advantages as nano-delivery platforms.

3.1. *Properties and functions of lipoproteins*

Lipoproteins can be classified into chylomicrons (CMs), very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Although lipoproteins possess structures similar to that of a core comprising triglycerides and cholesterol esters and are covered by at least one layer of phospholipids, they share diverse characteristics in size, physicochemical properties, and physiological functions, as they differ in lipid composition and major apolipoproteins. The characteristics of lipoproteins are presented in Table 1.

CMs have the smallest density and largest volume of lipoproteins with lengths within the range of 75–1200 nm in human plasma. Structurally, the core of CMs comprises nonpolar substances (triglycerides and cholesterol) and is surrounded by a single molecular layer of phospholipids, which are inlaid with apolipoprotein (ApoB-48) and free cholesterol. After absorption by intestinal epithelial cells, dietary fats are esterified to form cholesterol ester (CE) and then combined with apolipoproteins from the rough endoplasmic reticulum to form nascent chylomicrons. Subsequently, they move into the lymphatic system and further drift toward the blood, evolving into triglycerides catalyzed by lipoprotein lipase. The residual parts, generally including a high amount of CE and diverse apolipoproteins, are eliminated by the liver. The process lasts only a few minutes [16]. Therefore, they are responsible for transmitting exogenous triacylglycerols and cholesterol into the body. Some drugs with high lipophilic ability (*e.g.*, fat-soluble vitamins) can form prodrugs by associating with TG and synthesizing CMs, which are finally absorbed by the intestinal lymphatic system.

VLDL is synthesized in the liver via a complicated multistep process. Functionally, VLDL plays a critical role in the distribution of triglycerides to peripheral tissues by interacting with lipoprotein lipase [17]. Although many

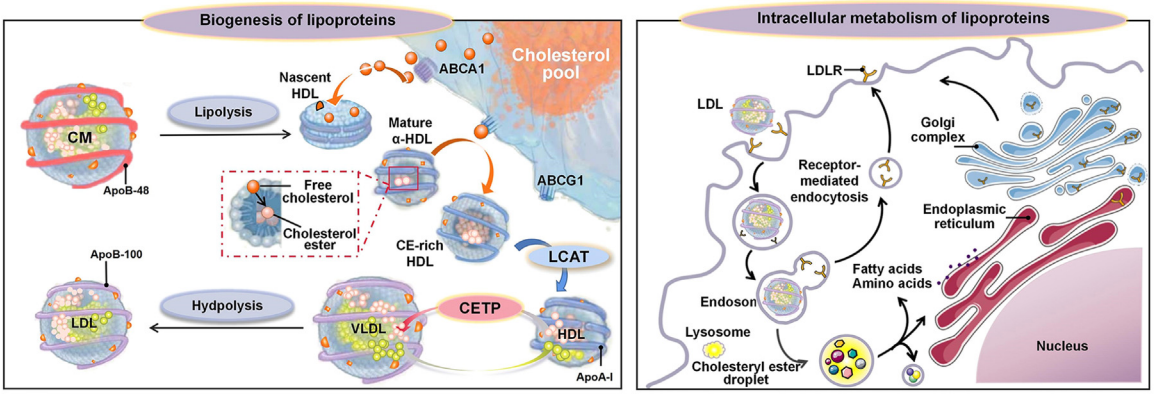

Fig. 2 – Schematic depiction of the different lipoproteins, indicating their biogenesis of various lipoproteins and their intracellular metabolism. The left panel illustrates formation of CM, HDL, VLDL, and LDL, as well as their relationship. The right panel illustrates the intracellular metabolism process of final LDL and their metabolism products, including fitty acids and amino acids.

Table 1 – Characteristics of 4 kinds of lipoproteins within the human body.

Lipoprotein	Size (nm)	Density (g/ml)	Major apolipoprotein Subpopulation	Number of amino acid residues	Function
Chylomicron	75–1200	<1.006	ApoB-48	2152	Important carrier for lipid transport and metabolism.
VLDL	30–80	0.95–1.006	ApoB-100	4536	Transport of endogenous triglycerides synthesized in the liver.
			ApoE	299	Ligand for LDL-R and LRP, participate in the conversion and metabolism of lipoproteins.
LDL	18–25	1.006–1.063	ApoB-100	4536	Transport of endogenous triglycerides synthesized in the liver.
HDL	5–12	1.06–1.210	ApoA-I	243	LCAT activator.
			ApoA-II	77	Structural and functional apolipoprotein.
			ApoE	299	Ligand for LDL-R and LRP.

preclinical studies have shown that the delivery stability of VLDL-like nanocarriers is reliable, the same nanocarriers were not widely used because of the contingency and irreproducibility of experimental results. Currently, VLDL is not suitable for drug delivery.

As a hydrolysate of VLDL, LDL (18–25 nm in diameter) is the most abundant spherical lipoprotein in human plasma. Through amphipathic α-helix protein–lipid interactions, ApoB-100 overlaps with the surface of LDL and can be specifically recognized by LDL receptors during systemic circulation, eventually completing cholesterol delivery. LDL receptors are single-chain transmembrane glycoproteins that are widely distributed in various organs and are present in many cell membranes. During the metabolism of LDL, its receptor specifically recognizes ApoB-100 and then guides LDL to bind to its specific region. After binding, a clathrin-coated pit is formed and freed from the cell membrane to generate vesicles. At a low pH, the vesicle promotes the separation of LDL from the receptor. Subsequently, the receptor is turned back to the cell membrane, whereas the LDL remnant particles arrive at the lysosome, where the remnant can be broken down into fatty acids, amino acids, and cholesterol to provide nutrients to cells. In addition, LDLR is overexpressed in some malignancies, such as rectal, brain, and metastatic prostate cancers, providing natural targeting for designing nano-DDSs [18].

As one of the most important members of the lipoprotein family, HDL shares the smallest particle size, and its structure changes with the content of its internal components. Owing to the differences in the cholesterol ester content in the core, each mature HDL particle exhibits an exceptional size and shape, ranging from 7 to 13 nm. Relying on a variety of apolipoproteins embedded in the phospholipid layer, HDL particles can be recognized by diverse receptors and catalyzed by enzymes. As the central component of HDL, ApoA-I, a 28-kDa protein, can combine with ATP-binding cassette receptor A1 (ABCA1) to absorb phospholipids and free cholesterol to ultimately generate discoidal nascent HDL. Depending on the differences in composition and shape, two forms of HDL particles are formed during maturation: discoidal and spherical HDLs. In human blood, HDLs exist predominantly in spherical formations, with amphipathic helices of ApoA-I located on the surface. Functionally, HDLs collect cholesterol from peripheral tissues and transport it to the liver for elimination. Therefore, HDLs are considered important factors in circumventing cardiovascular diseases by inhibiting the formation and rupture of atherosclerotic plaques. Thus, HDLs exhibit a high binding affinity for some

receptors overexpressed in lesions and natural anti-oxidation and anti-inflammatory properties, rendering them suitable for drug delivery with promising potential for precise targeting and biosafety [19].

3.2. *Lipoprotein-inspired nano-delivery system*

Recent advances in lipoproteins have opened a novel course of diagnostic and therapeutic agents coated with biological vehicles to achieve precise cancer treatment. These biomimetic nanomedicines often have a "core–shell structure" that imitates the biocompatibility of the source lipoprotein and provides natural barrier penetration, lesion-homing ability, and anti-inflammatory effects.

3.2.1. *Natural lipoprotein nano-delivery system*

Lipoproteins are widely distributed in biological fluids, including blood and cerebrospinal fluid. In early studies, natural lipoproteins have been distinguished from other biological components owing to their unique sizes and shapes or by using immunocapture assays. To improve purity and reduce time consumption, lipoproteins have been isolated from other components via several methods, such as ultracentrifugation, size-exclusion chromatography, and immune-based assays. After separation, lipoproteins can be used as effective carriers of drugs, photosensitizers, and genes. The aforementioned substances can be loaded into lipoproteins in three ways: i) hydrophobic contents can directly enter the core of lipoproteins by replacing the original nucleoplasm, ii) cargoes can covalently be coupled to apolipoproteins through amino acid residues, and iii) cargoes can be inserted into the amphiphilic phospholipid layer via van der Waals forces. Natural lipoprotein-like nanocarriers can retain the characteristics of lipoprotein particles to the greatest extent possible and exhibit high biocompatibility. In our previous study, three types of lipoprotein-based nano-systems were re-assembled using whole endogenous components, effectively preserving the advantages of natural lipoproteins and enhancing hydrophobic drug (PTX) delivery [20]. However, such receptor-mediated mechanisms have a major limitation, that is, receptors exist in both lesions and healthy tissues, causing undesirable accumulation. To address this limitation, we designed a novel native HDL-like NP and endowed this special system with rerouting capacity. By linking with $\alpha v\beta 3$-integrin specific cyclic-RGDyk peptide, NPs were enabled to keep away from the original receptors (SR-BI) and then specifically bind with others [21]. To achieve high targeting and penetration and facilitate the application of synergetic chemo-phototherapy in diseases, we constructed a dual-loaded HDL-induced nanomedicine (pHDL/PTX-ICG) for both treatment and imaging. Upon near-infrared irradiation, this nanosystem exhibited satisfactory drug release, and precise chemo-phototherapy could be realized by relying on NIR fluorescent probe guidance with negligible toxicity. LDLs share properties similar to those of HDLs in terms of structure and composition. Some research groups have developed a new type of nanocarrier using LDL to encapsulate PTX micelles and siRNAs. Results showed that such NPs are directly taken up by cancer cells, thereby promoting the release of drugs and genes, increasing the concentration of drugs in cells, reducing drug efflux, and prolonging circulation time [22]. However, these disadvantages cannot be prevented because the purification of natural lipoproteins is complex and costly. First, many other unrelated biological substances, such as extracellular vehicles (EVs), which are similar in size and density to lipoproteins, are present in the blood, blocking the efficiency of their separation. Second, given the complexity of purification, a highly reliable production technology is required. Unfortunately, most manufacturers lack these requirements. Third, endogenous lipoproteins and apolipoproteins cannot easily be obtained in significant quantities from serum, indicating that their supply is limited and costly. Finally, for short shelf life and easy oxidation, the application of natural lipoproteins is highly restricted.

3.2.2. *Artificial lipoprotein nano-delivery system*

Producing synthetic lipoproteins, which allows for stringent control of the compounds and properties of NPs, is an alternative to lipoprotein separation from biological fluids. Synthetic lipoproteins can exert more excellent properties, such as higher stability, better availability, and superior yields, and are more easily obtained than wild lipoproteins. Additionally, synthetic lipoproteins can be separated from solutions via dialysis or density gradient-based ultracentrifugation. Because of the diversities in size and contents of various lipoproteins, several differences in production, including technology, equipment, cost, and time, can be observed. For example, owing to the larger size of ApoB, synthetic LDL requires more processing than synthetic HDL during manufacturing. Moreover, the sizes and shapes of synthetic lipoproteins can be adjusted using different kinds of lipids and setting various stoichiometric ratios between lipids and proteins. For example, higher lipid-to-protein proportions and long-saturated lipids can be used to prepare larger discoidal lipoproteins, whereas smaller ones are synthesized. Furthermore, synthetic lipoproteins may not exhibit significant differences from natural products, and the uniformity of their shapes, sizes, safety, and properties has been demonstrated in several studies. Recently, several studies have focused on the development of sHDL. Our group developed special biopeptide-lipid hybrid particles (PPL/ICG), which were formed by using a PLGA polymeric core together with indocyanine green (ICG) and a lipoprotein shell-decorated ApoA-I biomimetic peptide. Compared with conventional lipoprotein NPs, PPL/ICG has the following advantages: (1) sufficient stability in plasma, (2) satisfactory systemic circulation time, (3) high level of biosafety, and (4) favorable therapeutic capability [23]. Gong et al. constructed an s-HDL-like NP containing (DTX-sHDL). As a drug carrier, sHDL exhibits favorable loading capability and natural biocompatibility. Furthermore, the data demonstrated that special nanomedicines can realize efficient treatments with reduced toxicity.

Reconstituted lipoproteins are another common artificial lipoprotein. Essentially, no significant difference exists between reconstituted and synthetic lipoproteins. As a common drug delivery tool, rHDL-NPs can also be used to transport numerous therapeutic agents, such as chemotherapy drugs, siRNAs, photosensitizers, and imaging agents. We engineered a dual-loaded rHDL nano-DDS

Table 2 – Summary of the characters between conventional and lipoprotein-inspired nano-DDSs in brain disease treatment.

Nano-DDSs	Typical representatives	Advantages	Disadvantages
Liposome	i. Liposome PEGylated liposome ii. Cationic liposome iii. Liposome conjugated with targeting molecules iv. Liposome conjugated with antibodies	i. Low toxicity ii. High loading efficiency iii. Easy modification iv. Approved by FDA with promising clinical transformation	i. Poor biocompatibility ii. Drug leakage iii. Insufficient BBB penetration iv. Short biological half-life v. Adverse immunological responses
Inorganic nano-DDSs	i. Gold nanoparticle ii. Silica nanoparticle iii. Iron oxide nanoparticle iv. Quantum dots	i. Small particle size ii. Superior ability to gather in lesions iii. On-demand release drugs under suitable external stimulation	i. Poor biodegradability with obvious toxicity ii. Irreproducible trial results iii. Insufficient BBB penetration iv. Easy to induce inflammatory reactions
Amphiphilic copolymers	Micellar	i. Self-assemble with small size ii. High loading efficiency and controlled release of insoluble drugs iii. High stability	i. Limited BBB-crossing and tumor-homing ability ii. Potential toxicity
Dendrimers	i. Polyamidoamine ii. Polypropylenemine iii. Polypropylene imine	i. Larger loading space ii. Better water solubility iii. High stability	i. Severe myelotoxicity and allergic reactions ii. Complex manufactures iii. Cationic properties inducing cytoxicity
Lipoproteinnano-DDSs	i. Natural lipoprotein nano-DDSs (native HDL and LDL) ii. Lipoprotein-inspired nano-DDSs (D4F, ApoE, and ApoA-I)	i. Biocompatibility and biodegradability ii. Natural BBB penetration and lesion accumulation iii. Natural neuroprotective and anti-inflammatory ability iv. Prolonged circulation time v. Multiple drug loading capacity with high plasticity	i. Complicated purification and cost-consuming ii. Easily oxidation

using vascular endothelial growth factor-specific siRNA (siVEGF) and PTX. This NP formulation exhibited noticeable pathological cytotoxicity and targeting in lesions and promoted the inhibition of tumor growth without remarkable side effects. In addition to good tumor treatment capability, some studies have shown that statin-loaded rHDL shows great potential for both targeting lesions and preventing the degradation of atherosclerotic plaques. In addition to rHDL, rLDL has gradually attracted considerable interest. Compared with natural LDL, rLDL has a smaller particle size and unique transport pathway. Moreover, it is more effective in promoting cell-selective absorption of drugs. rLDL can be absorbed by tumor cells through a receptor-mediated mechanism, thereby selectively providing drugs for tumor therapy. An amphipathic hybrid peptide has been used to replace natural apoB-100 to prepare biomimetic LDL nanocarriers encapsulated with PTX. Results showed that the NPs could enhance the anti-tumor effect of the drug, reduce side effects, and prevent lysosomal decomposition [24].

These results suggest that lipoproteins should be considered when constructing precise nano-DDSs for brain disease treatment. First, lipoproteins share inherent biocompatibility and biodegradability. Following cellular uptake, lipoproteins eventually decompose into reclaimable biological components, ensuring complete biosafety and preventing adverse immune responses. Second, lipoproteins cannot easily be detected using mononuclear phagocytic systems (MPS) and thus rarely cause adverse toxicity responses. Third, because the particles are usually smaller than 30 nm, HDL and LDL exhibit a high ability to protect themselves from being easily captured by MPS. This may be vital in prolonging its circulation time. Furthermore, their small sizes enable them to permeate solid tumors. Fourth, lipoprotein particles possess high loading capacity and good plasticity. The large hydrophobic core inside the particles contains numerous therapeutic agents. Moreover, their unique core-shell structure renders them suitable for direct drug loading. In addition, the components of lipoproteins can be selected based on demand. Finally, lipoproteins show great potential for targeting lesions. For example, owing to the overexpression of LDL receptors in many lesions, LDL exhibits unique binding ability to tumors, and HDL, especially ApoE-HDL, exhibits high binding annuity to the pathological marker Aβ of AD, providing some valid remedy for these diseases. The characteristics of conventional and lipoprotein-inspired nano-DDSs for brain disease treatment are presented in Table 2.

4. Lipoprotein-induced nanocarriers for brain diseases

Brain diseases, mainly including neurodegenerative disorders and tumors, are the leading cause of elder-related deaths worldwide. Several attempts have been made to address these distressing diseases. However, therapeutic effects

Table 3 – The application of lipoprotein-based DDSs in the brain diseases.

Modification	DDSs	Pathology	Experiments	Ref
ApoE3	rHDL	AD	*In vivo* SAMP8 and SAMR1	[32]
ApoE3 α-M	rHDL	AD	*In vitro* bEnd.3 cells *In vivo* Balb/c nude mice, APP/PS1 transgene mice, SAMP8, and SAMR1	[33]
GM1 αNAP	rHDL	AD	*In vivo* SD rats and ICR mice	[34]
ApoE4	LSPR	AD	Detection limit of the biosensor Real time measurement of aggregation	[36]
Serotonin modulator ApoE Nerve growth factor	Liposome	AD	*In vivo* Male Wistar rats	[37]
ApoE3 ApoE4	Nanoparticle	AD	*In vitro* Wild-type (K1) and HSPG-deficient (M1) CHO cells *In vivo* Wild-type C57BL/6 mice	[38]
mApoE Phosphatidic acid	Liposome	AD	*In vivo* APP/PS1 Tg male mice	[39]
mApoE Phosphatidic acid	Liposome	AD	*In vivo* Neonatal rats	[40]
ApoB siRNA	rLDL	Lewy body disease	*In vitro* N2A	[41]
ApoE3 Porphyrins	Nanoparticle	Gliomas	*In vitro* U87-MG, U87-MG-GFP, HepG2, and ldlA7 *In vivo* Nude mice	[42]
ApoE siRNA	Chimeric polymersomes	GBM	*In vitro* U-87 MG-Luc cells HA1800	[43]
ApoE SDF1-mimic peptide	SLNP	Gliomas	*In vivo* NOD/SCID mice bearing GICs-derived glioma	[44]
ApoE Activating transcription factor-5 siRNA	rHDL	GBM	*In vitro* C6 cells	[45]
Paclitaxel-alpha linolenic acid	sLDL	GBM	*In vitro* HepG2 *In vivo* U-87 MG	[46]
mApoE Chlorotoxin DOX	Liposome	GBM	*In vitro* hCMEC/D3	[47]
R4F Curcumin DiR-BOA	HDL-mimicking peptide- phospholipid scaffold	MS	*In vivo* C57BL/6 mice	[48]
ApoE JQ1	Nanoparticle	MB	*In vitro* HD-MB03 and DAOY cells *In vivo* NSG mice	[49]
ApoA1 anti-CD15 LDE225	Lipoprotein-like nanoparticle	MB	*In vitro* DAOY human MB cells and PZp53 cells *In vivo* Jax 008,831 and Math-Cre-ER-Ptch flox/flox mice	[50]
K16ApoE Tripeptidyl peptidase 1	Lipoprotein-like nanoparticle	Lysosomal storage disorder	*In vivo* $Tpp1^{-/-}$ mice	[51]
K16ApoE Dabrafenib	Lipoprotein-like nanoparticle	Brain metastatic melanoma	*In vitro* MDCK cells, MDCK II, RBE4, hCMEC/D3, and SC-1800 *In vivo* NOD/SCID mice	[52]

remain daunting owing to incompetent biological barrier penetration and insecurity of vehicles, as discussed in the previous section. Moreover, although the requirement of crossing the BBB is a critical commonality in brain disorders, drug development requires numerous emphases because the properties, peculiarities, and pathological mechanisms of each disease are unique. For example, in chronic and degenerative diseases (*e.g.*, AD and PD diseases), most pathological changes are focused on long-term neuronal apoptosis and functional degeneration. Therefore, the disease progression speed should be slowed and memory damage should be rescued. Preventing recurrence is a key issue in glioma treatment. Additionally, traumatic events, such as brain injury, generally require rapid anti-inflammatory interventions.

Various modified lipoprotein NPs possessing properties such as effective BBB permeability, excellent therapeutic outcomes, and little-to-no-toxicity have been exploited. Successful brain-targeting lipoprotein-like DDSs are presented in Table 3. In this section, recent advances in

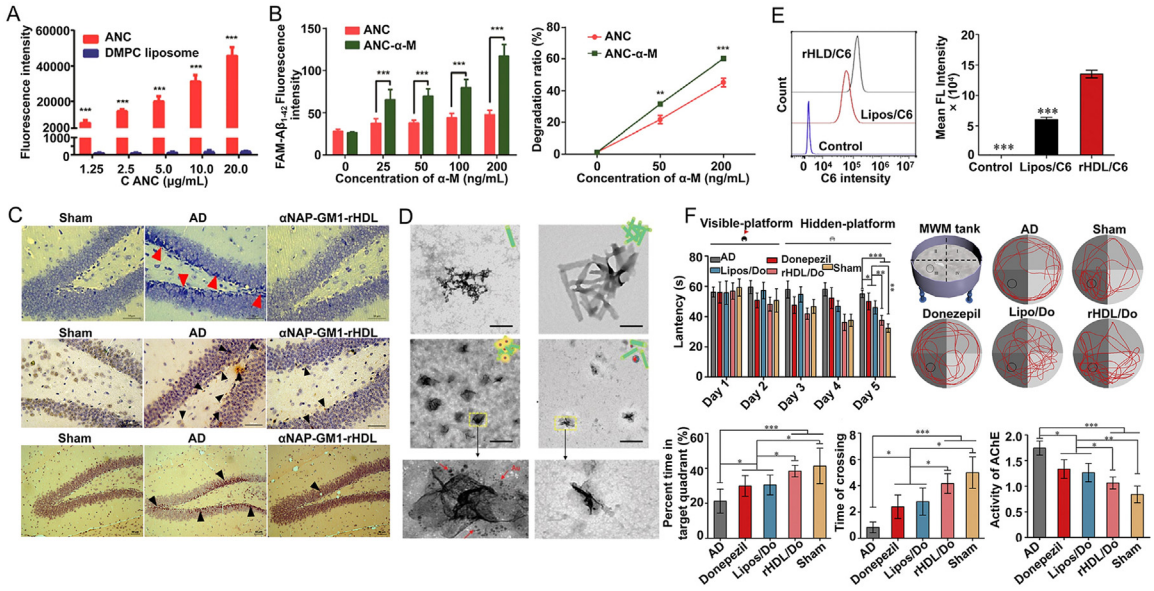

Fig. 3 – The therapeutic results of various kinds of lipoprotein-based DDSs in the AD. (A) Quantitative analysis of cellular uptake. Reproduced from [32] Copyright 2014 American Chemical Society. (B) Concentration of $A\beta_{1-42}$ in the cell lysates. Reproduced from [33] Copyright 2016 American Chemical Society. (C) Neuroprotective effects of RNAP-GM1-rHDL. (D) Morphological evaluation of the *in vivo* neuroprotective effects. Reproduced from [35] Copyright 2015 American Chemical Society. (E) FACS analysis of uptake efficiency. (F) Nanoformulations rescued memory deficits in AD mice. Reproduced from [37] Copyright 2019 Elsevier Ltd.

lipoprotein nanocarrier-facilitated brain treatment are discussed.

4.1. *Alzheimer's disease*

AD is a malignant and heterogeneous neurodegenerative disorder reported in more than 50 million patients worldwide. The core pathological changes in this disease are $A\beta$ deposition and the formation of neurofibrillary tangles, thus causing a series of pathogenic cascades and eventually developing into a loss in neurological function. With the development of related pathological research, many studies have demonstrated that the overall clearance impairment of $A\beta$ is the primary form of the advanced stage of this disease rather than $A\beta$ production. Therefore, improvement in $A\beta$ clearance has been widely considered the most crucial therapy for delaying the onset and slowing the progression of AD. HDL, which has the smallest particle size, is a suitable nanostructure for drug loading and delivery. ApoE, a subpopulation of apolipoproteins constituted in HDLs, exhibits superb binding ability with $A\beta$ to form compounds (ApoE3 > ApoE2), improving its degradation and efflux via a receptor-mediated pathway crossing the BBB [25]. Furthermore, lipidated ApoE has a higher-binding ability than isolated ApoE. As previously mentioned, ApoE-HDL may be essential in $A\beta$ degradation, and its derivatives, especially ApoE3-rHDL and its modifiers, might serve as novel and promising nanocarriers for AD treatment.

In the past five years, ApoE-rHDL has been widely used as a safe and effective vehicle for AD treatment. Song et al. developed biomimetic ApoE3-rHDL that effectively permeates the BBB and enhances $A\beta$ degradation *in vitro* and *in vivo* (Fig. 3A) [26]. To achieve good therapeutic outcomes, α-Mangostin, a polyphenolic compound that inhibits the formation of $A\beta$oligomers and fibrils and improves $A\beta$cellular decomposition, was loaded into a carrier to form a novel nanoformulation (ANC-α-M). Compared with a blank team, ANC-α-M NPs exhibited the highest delivery efficiency and accumulation in lesions and produced admirable treatment results (Fig. 3B). However, all $A\beta$ cannot easily be caught relying only on ApoE, and single-drug loaded NPs cannot easily meet the urgent requirement for therapeutic efficacy. Therefore, the affinity of nanocarriers and $A\beta$ should be enhanced and their neuroprotective functions should be improved. Recently, Gao et al. investigated the therapeutic effect of a comprehensive biomimetic nanostructure on AD-induced damage [27]. In this study, lipid membranes were prepared using two common substances, dimyristoylphosphatidylcholine and monosialotetrahexosyl ganglioside (GM1), which can maintain the original structure and properties of lipoprotein [28]. Most importantly, the effectiveness of GM1 to bind with $A\beta$ has been widely investigated. Furthermore, a dual-domain peptide consisting of neuroprotective peptide NAPVSIPQ and lipid-associating peptide 4F alleviated neuronal inflammatory damages with insignificant side effects (Fig. 3C). Results showed that only two-week low-dose administration can effectively alleviate $A\beta$ deposition, inhibit neuronal change, and rescue recognition dysfunction (Fig. 3D). The aforementioned data prove that ApoE-induced biomimetic nanocarriers are a promising platform for AD treatment.

Monitoring the progression of the disease and producing therapeutic effects are other development directions for relieving AD. Lipoprotein-induced nano-DDSs have received

attention for early diagnosis because they can encapsulate metal particles and fluorescent hydrophobic probes for photothermal therapy and imaging. Martins et al. described ApoE3-AuNP-rHDL utilization, which efficiently targets Aβ_{1-42} and decreases Aβ aggregation, as a potential therapy for AD. In this nano-DDS, curcumin embedded in a lipid membrane contributed to achieving real-time aggregation-promoted fluorescence detection, and the gold core facilitated the disintegration of Aβ by rising the local temperature following NIR irradiation. The fluorescence intensity increases with the accumulation of Aβ, providing opportunities for early disease monitoring. The detection ability in the early stage and the light-driven decomposition signifies that this novel nanosystem can become a potential candidate for AD treatment [29].

Disequilibrium between Aβ anabolism and catabolism is certainly the problem that triggers events in AD pathology. However, an increasing number of clues suggested that emphasizing only Aβ disposal and neglecting their abnormal crosstalk with dysfunctional microglia may also impair therapeutic outcomes. To respond to these issues, Yang, et al. designed pHDL/Cur-siBACE1 relying on HDL-inspired nano-DDSs to recover microglial dysfunction and downregulating Aβ deposition. Compared with that of liposomes, the K_D values of pHDL/Cur-siRNA to Aβ_{1-42} monomer was 2.59-fold lower and to Aβ_{1-42} oligomer was 1.35-fold lower, indicating that the advanced nanoscavengers exhibited preferred Aβ binding and clearance capability [30].

In additional to Aβ accumulation, acetylcholine deficiency is a characteristic of AD requiring considerable attention. The cholinergic hypothesis explains the mechanism underlying a series of neurological injuries caused by AD, including memory loss and cognitive dysfunction. Donepezil has been used as a therapeutic agent for AD treatment. Unfortunately, high-dose administration is often required to address poor BBB permeability and achieve therapeutic goals, inducing severe systemic toxicity (such as diarrhea and anorexia) and hindering its application. Yang et al. designed bionic nano-DDSs using apolipoprotein A-I-reconstituted HDL, and donepezil was encapsulated [31]. This nanomedicine (rHDL/Do) can achieve both Aβ-targeting clearance and acetylcholinesterase (AChE) inhibition without any noticeable side effects. rHDL/Do can achieve efficient cargo loading, exhibit increased BBB permeability, and produce favorable therapeutic results (Fig. 3E and 3F). Furthermore, after daily administration, rHDL/Do exhibited a remarkable ability to inhibit AChE activity and reduce cholinergic dysfunction owing to the controlled release of donepezil. Therefore, these nano-DDSs produce beneficial effects in therapeutic applications.

4.2. *Parkinson's disease*

As the second most common neurodegenerative disease, PD also poses a significant threat to human health. The clinical presentation of patients consists of two primary aspects: motor symptoms, including static tremors and bradykinesia, and neurological symptoms, such as depression, cognitive disorders, and hallucinations. The primary pathological features of PD are the death of dopaminergic neurons and the presence of abnormal α-synuclein inclusions in the substantia nigra. Furthermore, studies have shown that α-syn can interact with lipoproteins, and ApoE appears to be significantly increased in comparison with the normal status in the CSF of patients with PD. Although studies have attempted to curb progression by exploiting many novel agents, conventional administrations are still largely inefficient because of penetration inadequacy. Recently, the construction of nano-DDSs has received considerable attention to address these issues. Suzuki et al. designed a new type of NPs and evaluated their effects in curbing PD. The NPs comprised a gold core and a phospholipid layer, which was inserted into Apo-B100. Data analysis results indicated that the NPs exhibited noticeable aggregation in AD and PD lesions. Their discovery may lead to further developments in the diagnosis and treatment of neurodegenerative diseases [32].

Studies have shown that antisense oligonucleotides can be used to reduce the synthesis of proteins associated with nervous system diseases during the treatment of neurodegenerative diseases. However, the ingress of siRNA into the CNS occurs primarily through intrathecal and intracranial injections, thereby requiring the development of simpler and easier methods. ApoB, the LDL receptor, can be used as a substituted peptide for nucleotides involved in BBB transport. The 11 amino acid sequences of ApoB are combined with nine-amino acid arginine linker. Studies have shown that siRNAs can be transferred to neurons and glial cells through the BBB. To verify the therapeutic value of this DDS, Ghosh, et al. implanted α-syn targeted siRNA into PD transgenic mice. Results showed that ApoB delivered siα-syn can protect the degeneration of neurons in the cerebral cortex, limbic system, and striatum nigra system and reduce neuroinflammation.

4.3. *Brain tumor*

Glioblastoma (GBM), which is the most common high-grade glioma, arises in glial tissues and accounts for approximately half of the morbidity. Comparison with other solid tumors, reduced drug permeability with GBM or other brain tumor may attribute to natural protection of brain to other external substances (or recognized as "poisons"). It seems that ATP-binding cassette transporters located in the BBB is of crucial importance to in this protective mechanism, to limit transport of toxic or therapeutic agents. Unfortunately, many chemotherapeutic agents lack BBB penetration and do not reach the required concentration in lesions, limiting the efficiency of current standard treatments, including surgery, radiation, chemotherapy, and their combination. Moreover, all tumor cells and tissues possess relatively independent characteristics owing to heterogeneity. This implies that each site has different sensitivities to drugs, leading to drug resistance and increasing treatment difficulty. The emergence of nanotechnology has received considerable attention in therapeutic and diagnostic settings. Instead of passive targeting through the enhanced permeation and retention effect (EPR effect), modified NPs represent potential candidates for ideal anticancer therapy with enhanced tumor accumulation. NP-functionalized cell-penetrating peptides or cation proteins can realize good cargo transport and

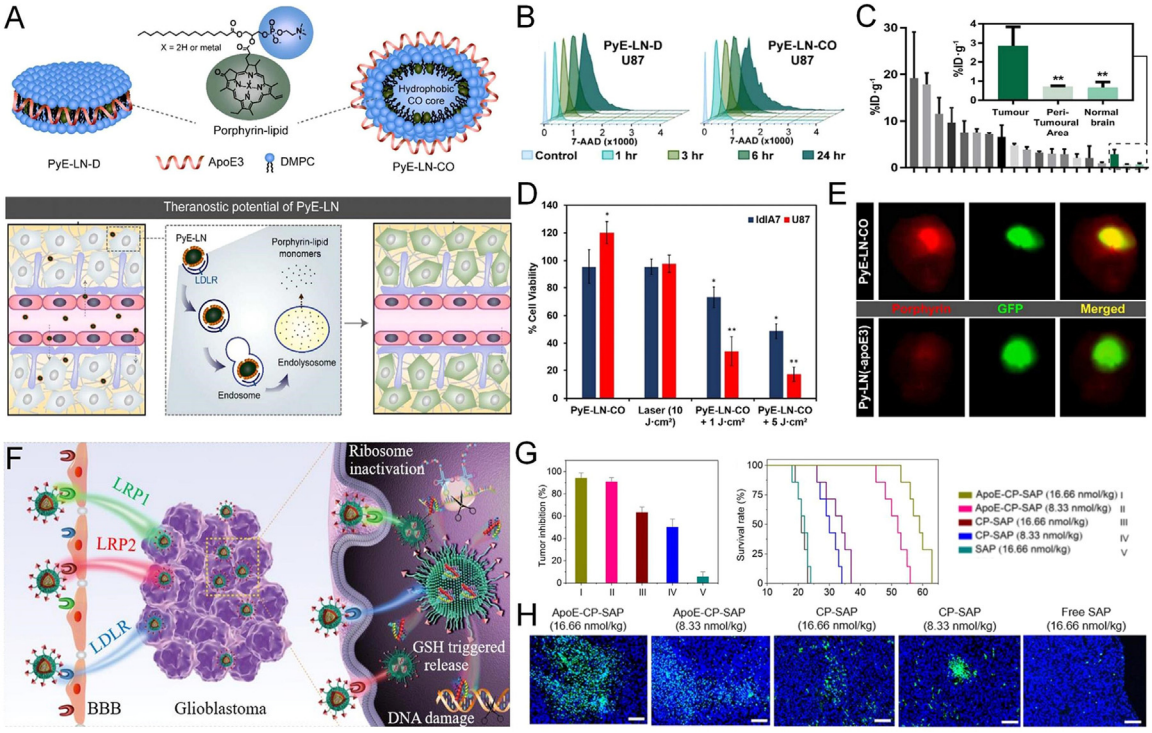

Fig. 4 – The therapeutic results of various kinds of lipoprotein-based DDSs in the GBM. (A) The preparation of PyE-LN-D. (B) The uptake analysis to tumor cells. (C) Porphyrin biodistribution in tumor-bearing mice. (D) pyE-LN PDT sensitization detection. (E) GFP signal co-localization in brains. Reproduced from [40] Copyright 2017 Elsevier Ltd. (F) Presentation of ApoE-CP for GBM targeted therapy. (G) Mean tumor inhibition rate and survival rate of tumor-bearing mice. (H) TUNEL assays of the GBM tissues. Reproduced from [41] Copyright 2019 American Chemical Society.

high cellular uptake while maintaining the integrity of cell membranes via absorption-mediated transcytosis However, nonselective cell internalization is a formidable obstacle to clinical applications. Transporter-mediated transcytosis (TMT) is another drug delivery approach that uses transporter-modified NPs. Owing to its high substrate selectivity, this process can lead to improved therapeutic outcomes and reduced side effects. The application of TMT has challenges sometimes because of the limitations of drug features, especially the efficiency of delivery into brain lesions. Notably, clinical conversion has several limitations [33].

Relying on receptors that are selectively combined with ligands, receptor-mediated endocytosis exhibits high specificity for drug delivery. Several receptors, including TfR, LDLR, and insulin receptors, are often overexpressed in lesions. This may open up new avenues for disease therapy. A study on brain disease reported that LDLR expression in malignant sites is higher than that in healthy tissues. Leveraging these traits, NPs can be endowed with favorable BBB permeability and specific targeting ability. For example, ApoE3 exhibits excellent LDLR binding activity. This has provided new insights into GBM treatment. Recently, several agents and adjuvants, such as resveratrol, curcumin, and DNA-loaded ApoE3-HDL, have been transported into GBM cells using LDLR overexpression [34]. Although their results were satisfactory *in vitro*, improvement was required both in deepening exploitation *in vivo* and searching the targeting specificity of ApoE3 NPs. Rajora et al. developed a multifunctional size-controlled NP (pyE-LN). The NP can be classified into discoid and spherical forms, depending on whether cholesterol-oleate (CO) is encapsulated or not. To improve the stability of the supramolecular structure and acquire unique intrinsic multimodal imaging and phototherapy capabilities, porphyrins were added into the shell (Fig. 4A). Compared with discoidal NPs, CO-loaded pyE-LNs exhibited a long blood clearance half-life and efficient glioblastoma cell internalization (Fig. 4B and 4C). In addition, they found that U87 cells expressing LDLR required approximately four times as many pyE-LNs as ldlA7 cells that did not express LDLR. Furthermore, the aggregation of pyE-LN in the lesion was 4-fold higher than that in healthy tissues, demonstrating its targeting capability. The viability of GBM cells was reduced by 83% following pyE-LN PDT sensitization. (Fig. 4D and 4E). Their results showed that pyE-LN is an ideal vehicle for GBM therapy. Similar effects were observed in other experiments. Jiang et al. investigated the therapeutic effects of ApoE-CP-sapor in (SAP) NPs against bEnd.3 and GBM tumor non-stem cells (U-87 MG) (Fig. 4F). Chimeric polymersomes are favorable carrier systems for drug delivery and have the potential to treat GBM. ApoE-CP serves as a delivery system for SAP, enabling high selectivity targeting and potent anti-tumor effects with increasing accumulation

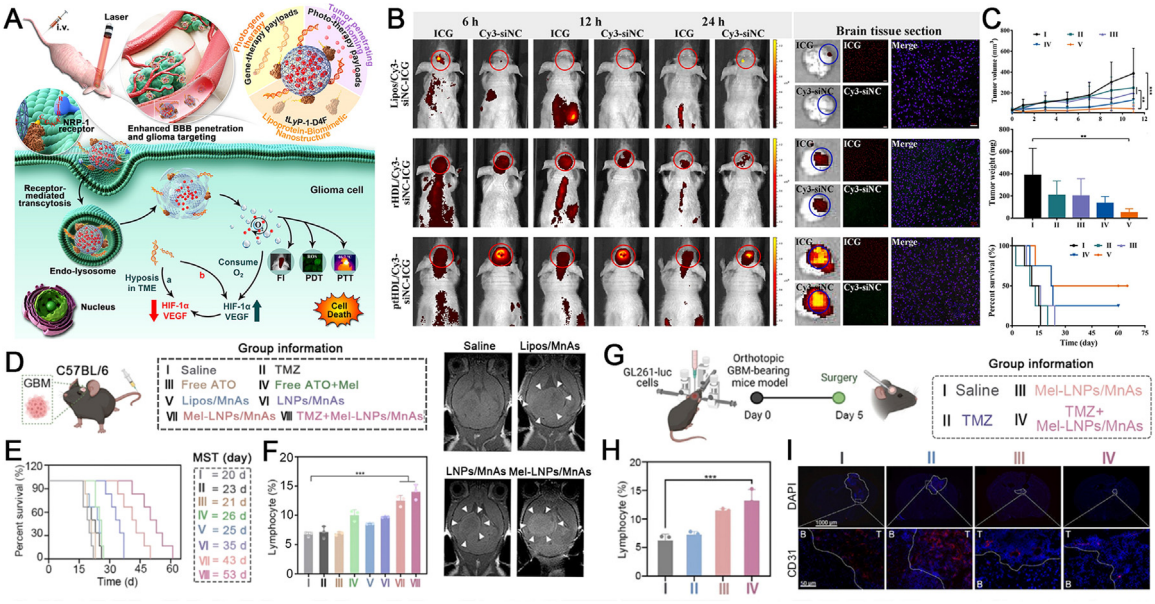

Fig. 5 – The therapeutic results of ptHDL/siHIF-ICG in the GBM. (A) The design of ptHDL/siHIF-ICG. (B) *In vivo* biodistribution of nanoparticles. (C) Evaluation of anti-tumor effect of ptHDL/siHIF-ICG Reproduced from [42] Copyright 2022 American Chemical Society. (D, E) Experiment design and survival analysis. (F) CTL activation represent adaptive immune response. (G) The design of experiment. (H) CTL activation represent adaptive immune response. (I) Coronal cryosections from brain with GBM. Reproduced from [43] Copyright 2023 American Chemical Society.

and penetration in lesions. A study has reported a complete inhibition of tumor growth using SAP-loaded ApoE-CP without any observable adverse effects (Fig. 4G and 4H) [35].

Phototherapy is considered an effective anti-tumor approach owing to its non-invasive properties, high penetration, and reduced toxicity. However, hypoxia, the most evident feature of TEM, seriously limits the therapeutic efficiency of this oxygen-dependent strategy. Suppressing the overactivation of HIF-1 is of significant importance for adjusting hypoxic conditions in tumors. Therefore, we developed bioactivated nanodrugs, termed ptHDL/siHIF-ICG, to achieve *in situ* imaging and effective photogene therapy (Fig. 5A) [36]. Meanwhile, intense fluorescence appeared in the brain after 6 h and lasted for 24 h, indicating that ptHDL/siHIF-ICG exhibited excellent brain-targeting and tumor-homing ability (Fig. 5B). Collectively, the survival time of intracranial tumor-bearing mice was significantly prolonged, with negligible side effects (Fig. 5C).

Chemotherapy has played a leading role in cancer treatment in recent decades. As the first-line clinical agent, the therapeutic outcome of temozolomide (TMZ) treatment is limited by significant drug resistance and systemic toxicity. Reversing TMZ drug resistance is necessary for enhancing GBM clinical therapeutic effects. Shi et al. combined ApoE-functionalized nanoplatforms to co-deliver artesunate-phosphatidylcholine and TMZ (ApoE-ARTPC@TMZ), showing great potential for improving GBM sensitivity to TMZ and thus prolonging the survival time of GBM-bearing mice. Advanced NPs achieved precise GBM therapy with negligible side effects, showing great potential in clinical translation [37].

Although existing studies have confirmed the usefulness of a single chemotherapy regimen, its side effects cannot be ignored. Moreover, this method cannot easily maintain an ideal effect during recurrent tumor treatment. Recently, chemoimmunotherapy has emerged as a next-generation treatment modality for cancers owing to its unique properties, including high tumor-suppressing effects, good prognosis, and low systemic toxicity. Melittin, a membrane-penetrating peptide, has recently been shown to promote tumor cell lysis to sensitize dendritic cells and activate CD8+ T cells to trigger an immune response, providing an opportunity for improved therapeutic effects and prognosis. We developed bio-fabricated nanodrugs, named Mel-LNPs/MnAs, to achieve precise GBM diagnosis, tumor cell proliferation, and recurrence inhibition [38]. The median survival time (MST) of GL261-tumor-bearing mice was significantly prolonged, and the activation of CD8+ T cells improved approximately 2-fold after treatment (Fig. 5D-5F). The results of the GBM surgical resection model also proved that the size and scope of the tumor decreased, whereas the ratio of cytotoxic T lymphocytes increased, indicating that the establishment of immune protection triggered by our nanodrugs show great potential for the anti-recurrence of postoperative GBM (Fig. 5G-5I). Except for melittin, blockade therapy has become the first treatment option for many cancers and significantly changed the landscape of cancer therapy. However, severe immune escape caused by the high overexpression of PD-L1 and the independent mechanism-mediated immune activation are the two major barriers to the suppression of GBM recurrence and deterioration. Shi et al. designed dual-pathway biomimetic nanomedicines inspired by ApoE and achieved the co-delivery of TMZ and OTX015, termed ABNM@TMZ/OTX. The noticeable MST proliferation of GBM-bearing mice could be attributed to three key points: i)

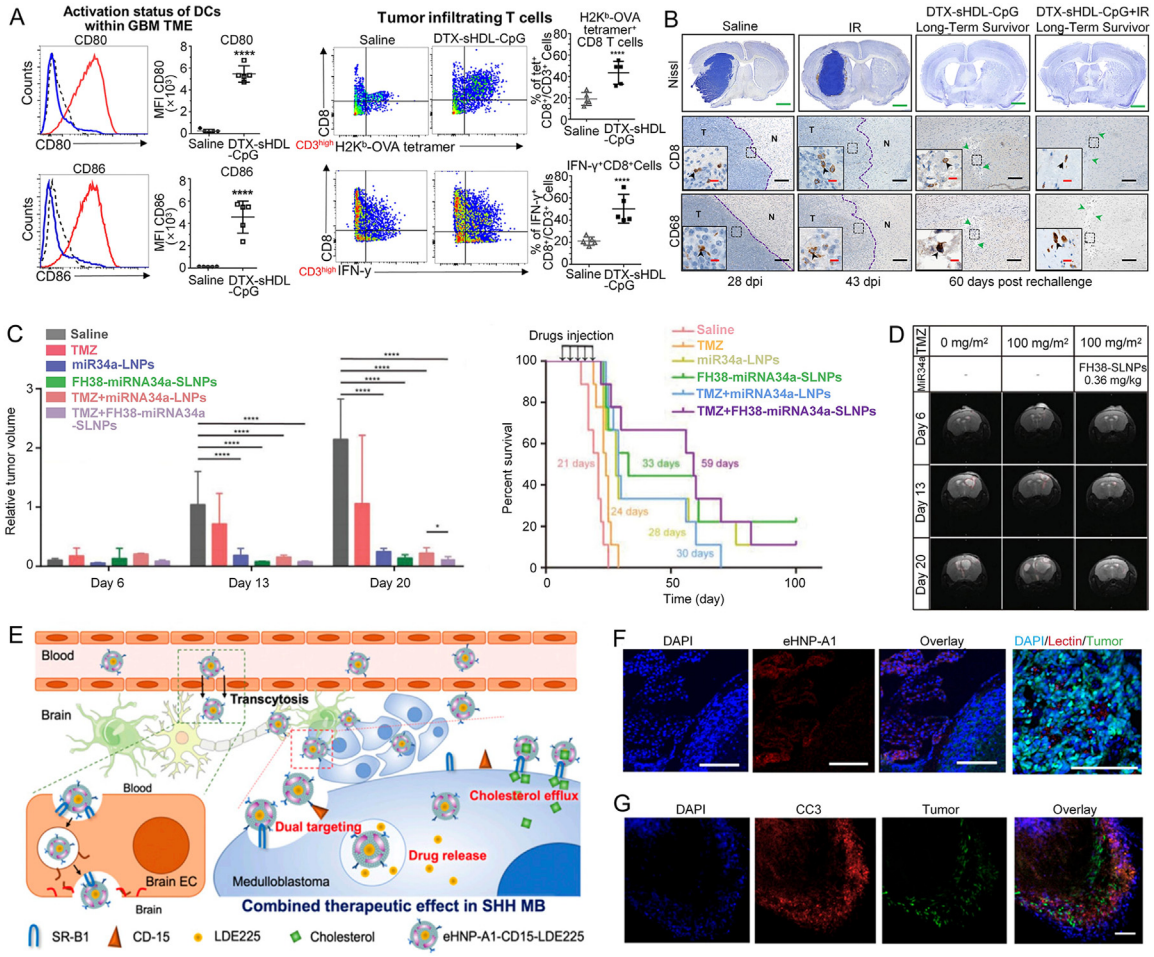

Fig. 6 – The therapeutic results of various kinds of lipoprotein-based DDSs in GBM. (A) Activation status of DCs and tumor-specific CD8+ T cells. (B) Nissl staining of brain sections. (C, D) *In vivo* anti-glioma activity of SLNPs. Reproduced from [45] Copyright 2020 WILEY-VCH. (E) Illustration of engineered biomimetic nanoparticles for SHH MB treatment. (F, G) *In vivo* capability for BBB-crossing and tumor cell accumulation. Reproduced from [51] Copyright 2019 PNAS.

ApoE-mediated effective BBB penetration and precise tumor accumulation (approximately 1.67-fold higher than that of the control group), ii) enhanced TMZ sensitivity to tumor cells (with the strongest combination index value being 0.2 in GL261 cells); and iii) the combined ICD effect and reversed PD-1/PD-L1 to enhance the expression of immune cells [39].

CpG, a common and effective toll-like receptor 9 agonist, is vital in triggering immune rejection and inducing long-term immunity against gliomas. Kadiyala et al. constructed a dual-loaded drug delivery system using CpG as an immunotherapy agent with DOX-encased NPs [40]. Long-term immunological memory formed noticeable DC and T cell activation, resulting in 80% elimination in the tumors of GBM-bearing animals (Fig. 6A), significantly prolonging their survival time (Fig. 6B). The ability of CpG ODN to eliminate gliomas has also been demonstrated in another study. Scheetz et al. formulated a co-delivery HDL-like nanodisk vaccine consisting of CpG and tumor-specific neoantigens and showed that these NPs exhibit high BBB permeability and anti-tumor activity. When combined with PD-L1, orthotopic GL261 gliomas can be eliminated in 33% of mice because NeoAg-specific T-cell responses are activated.

In addition to CpG, several cytotoxic agents such as oxaliplatin, doxorubicin, and granzyme B can trigger the ICD effect to assist the emergence of anti-tumor immunity. Zhong et al. developed ApoE peptide-mediated systemic co-nanocarriers to deliver granzyme B (GrB) (ApoE-PS-GrB) and CpG ODN (ApoE-PS-CpG) to enhance immunotherapy in a LCPN glioma model. In the LDLR-mediated pathway, the two NPs could effectively cross the BBB, accumulate in tumor cells, and induce strong ICD to further stimulate cytokine production, DCs maturation, and CD8+ T cell infiltration. After treatment, tumor proliferation was significantly suppressed, and the survival rate of mice was enhanced by approximately 1.8-fold [41]. Although promising outcomes have been reported, sufficient immune response has not been elicited both in some preclinical and clinical therapies, mainly attributed to moderate drug concentration in the lesions and overexpression of negative immune regulators in the immunosuppressive tumor microenvironment. Therefore, the improvement of the cellular uptake of drugs, relying on

the specific nutrient uptake pathways of cancer cells to build a drug delivery platform, has attracted considerable attention. Macropinocytosis, a special endocytic process, is essential in ensuring the uptake of extracellular nutrients into tumors. Glioma-initiating cells (GICs) are usually the origins of glioma propagation and relapse. Developing a potent GIC-targeting strategy to promote drug internalization into GICs relying on receptor-stimulated macropinocytosis shows great potential for glioma treatment. As a novel therapeutic target, CXCR4 has been overexpressed in GICs and is important for their proliferation and metastasis [42]. Gao et al. developed tailored lipoprotein-like nanoparticles (SLNPs) decorated with an SDF1 mimic peptide, exhibiting a high affinity for the CXCR4 receptor for inhibiting glioma stemness and drug resistance. Consequently, SLNPs can accumulate at malignant sites. When loaded with miR34a, the nanoplatforms achieved satisfactory inhibition in the GIC-derived orthotopic mouse models (Fig. 6C and 6D) [43].

Medulloblastoma (MB) is the most malignant intracranial glioma, which mainly occurs in children under 14 years of age. Compared with those of other brain tumors, MB treatment is the most challenging because of its fast growth, varied genetic makeup, chemoresistance, poor BBB penetration, and tendency to spread outward along the CSF. However, conventional treatment methods may be dangerous for patients, including those with growth disorders, hormone imbalance, and serious neurocognitive dysfunction. Therefore, an alternative strategy both with sufficient brain targeting and low toxicity for the MB treatment is required. Hedgehon inhibitors, representing potential MB therapeutic agents, show great potential in suppressing cancer cell proliferation and tumorigenicity, thereby providing an opportunity to cure the disease. However, smoothened mutations caused by repeated administration significantly weaken the efficacy of such drugs [44]. To address this issue, Wang et al. recently designed novel ApoE-mimetic peptide-modified NPs by leveraging their highly brain-penetrating nature and encapsulated JQ1, a common hedgehon inhibitor, to specifically transport lesions [45]. Their results showed that ApoE-NPs enhance JQ1 accumulation in tumor cells by 8-fold after 24 h of administration and alleviate the burden of orthotopic MB tumors after 3-d treatment without significant side effects. Kim et al. developed a dual-functionalized DDS by employing ApoA1 and anti-CD15 to target tumors and loaded LDE-225 to regulate cholesterol, termed eHNP-A1-CD15-LDE225 (Fig. 6E) [45]. *In vivo* fluorescent imaging indicated that eHNP-A1 and eHNP-A1-CD15 exhibited remarkable anti-tumor efficacy, and cell viability decreased by 63% even without loading LDE-225 (Fig. 6F). After the therapeutic agent, LDE225, was loaded, *in vitro* experiments demonstrated that the volume of the tumor was reduced by 72% and the growth rate decreased significantly by increasing the expression of CC3, TUNEL, and cleaved PARP (Fig. 6G). The results revealed a promising role for eHNP-A1-CD15-LDE225 in the targeted therapy of MB.

4.4. *Other diseases*

As a common autoimmune disease, multiple sclerosis (MS) leads to recurrent damage to feelings and cognition, causing considerable burdens to patients. Although the pathogenesis of MS has not yet been elucidated, studies have reported that several inflammatory cells, such as monocytes and neutrophils, increase with the development of the disease. Moreover, monocytes play a critical role in the process of deterioration by accelerating neuronal injury and recruiting additional inflammatory cells to the CNS. Therefore, monocytes can be considered targets for MS treatment. As a receptor of HDL, SR-B1 was overexpressed in monocytes, and its expression was positively related to the level of inflammation. B, Lisen Lu A et al. proposed novel bio-nanocarriers, which were formed by HDL-mimicking peptide-phospholipid scaffold (HPPS) and selected experimental autoimmune encephalomyelitis (EAE) as an MS animal model to conduct a series of studies [46]. On the one hand, HPPS targets monocytes with high selectivity and specificity. After loading with fluorescent dye DiR-BOA, the NPs exhibited properties such as detecting early development of CNS inflammation in EAE mice. On the other hand, the bioconjugated curcumin-loaded HPPS (Cur-HPPS) resulted in great inhibition of other immune cell infiltration and a 3.01-fold decrease in EAE morbidity. The research results indicated that Cur-HPPS is a favorable nanomedicine for EAE treatment.

Because of lifelong and irreversible dysfunction, secondary damage in traumatic brain injury (TBI) has gained traction. Cyclosporine A (CsA), one of the most common therapeutic agents, displays excellent features both in maintaining the integrity of mitochondrial function and improving the utilization of oxygen. However, inefficient drug concentration in brain lesions is hindered by poor water solubility and poor BBB penetration. To address these adverse effects and enhance bioavailability without significant side effects, Chen et al. constructed a drug delivery system by employing a MAP peptide with CsA-loaded NPs (CsA-MCRL) [47]. *In vivo* experimental results showed that CsA-MCRL exhibits excellent targeting and therapeutic capabilities. Compared with healthy tissues, lesions exhibited 24-fold accumulation, and their levels achieved time-dependent enhancement. Moreover, such nanomedicine achieved a good curative effect only at a dose of one-sixteenth that of CsA. Their results demonstrated that CsA-MCRL can be a potential nanomedicine for TBI treatment. In addition to independent chemotherapy, combination therapy has attracted considerable attention for the treatment of complex diseases. The conventional TBI therapeutic pattern of mild hypothermia (MH) is limited by its anti-inflammatory and neuroprotective effects. Jiang et al. combined MH and rHDL to improve the efficiency of TBI treatment. As expected, four types of proinflammatory factors were significantly suppressed in bEnd.3, BV2, and HT22 cells after combination therapy with sequential rHDL and mild hypothermia. Compared with saline treatment in an animal model of cortical contusion injury, the expressions of glial fibrillary acidic protein in the cortical and hippocampal were 2.2-fold and 1.7-fold lower, respectively. These results suggest promising cooperative treatment patterns for patients with TBI [48].

Melanomas are molecularly heterogeneous and immunogenic tumors, which pose a severe risk of brain metastasis and threaten numerous lives worldwide. Several

drugs have been developed to curb this deterioration. For example, BRAF, a serine/threonine kinase, plays an important role in melanoma metastasis by providing a crucial key point and promoting the rapid development of related inhibitors (such as dabrafenib). Moreover, immune checkpoint inhibitors, such as PD-1/PD-L1, exhibit excellent therapeutic effects in such disorders. However, this drug exhibits little ability to treat melanomas with brain metastasis because of poor BBB and BBTB penetration. Therefore, novel vehicles for drug delivery into the brain are required. Bjerkvig et al. recently constructed a nanocarrier-modified K16ApoE to achieve high BBB permeability and lesion-targeting capability. Their results showed that combination therapy with K16ApoE and dabrafenib has high therapeutic efficacy, representing a promising method for inhibiting the brain metastasis of melanoma and prolonging survival time [49].

5. Further developments

To achieve clinical translation, the processes and outcomes of the biological environment interacting with nanomedicine should be considered, especially the CRITID cascade [50]. Nanomedicines should maintain a relatively stable state after entering the life system, such as avoiding reactions with proteins in the body that can lead to the aggregation, fragmentation, oxidation, or disintegration of these vulnerable particles. When drugs enter the body, however, proteins in bodily fluids (such as blood) quickly adsorb onto the surface of particles and form protein crowns, and then alter their fate by influencing their physicochemical properties. Due to the EPR effect or active targeting ability, these nanomedicines will further migrate to the lesion area (such as tumor tissue, inflammatory lesion site, etc.). By utilizing certain characteristics of the lesion site, such as low pH, hypoxia, high ROS expression, and overexpression of some specific membrane proteins, properties of lesions-homing and timed-release of nanomedicines will be improved significantly.

5.1. *Protein corona-produced lipoprotein nanocarriers*

A protein corona is a dynamic multilayer protein structure on the surface of NPs, formed by the rapid adsorption and accumulation of various proteins (such as albumin, apolipoprotein, and fibrinogen) after entering the intercellular environment. As the interface between NPs and cells or molecules, a protein corona is essential to the biological effects of NPs and even affects their therapeutic efficiency. More than 90% of NPs are removed by the mononuclear phagocytic system *in vivo*, causing serious off-target effects and toxicity. However, monocyte phagocytosis of NPs may be affected by the formation of a protein corona, thus extending or shortening their blood circulation period and presenting opportunities or challenges to drug delivery design. There are four directions are proposed to discuss the influence of protein deposition on NPs, mainly classifying as follows: (i) targeting ligands are shrouded to cause reduced binding capability with tumor receptor, (ii) bioavailability is declined owing to enhanced uptake of macrophage, (iii) promote NP hydrodynamic size increasement, and (iv) deep penetration with tumor tissue is avoided by inducing NP aggregation. By changing its surface characteristics, the protein corona of NPs may affect the crossing of several biological barriers (*e.g.*, BBB), cell uptake, and targeting capability. Therefore, lipid NPs have been decorated by incubation with ApoE4 to enhance the accumulation of therapeutic agents in the brain [51]. The design principle is dependent on the high affinity between ApoE and specific receptors in the BBB. For instance, the modification of ApoE4 into polysorbate 80-stabilized lipid NPs produces an artificial protein corona, improving the BBB-penetrating capability 3-fold higher than that of naked NPs.

Tf, a common target molecule with a high affinity for TfR, is used to modify the surface of nanocarriers to improve BBB penetration and tumor-targeting accumulation. The receptor-mediated transmembrane transport in the BBB is usually divided into three parts: the targeted molecules on the surface of NPs are recognized by cell membrane proteins for endocytosis, intracellular transport of NPs, and efflux of NPs. However, most studies have focused on the impact of protein crowns on the recognition of targeted molecules and their receptors, but little is known about their impact on the BBB transport process. In addition, the ability of NPs to target brain tumor cells after crossing the BBB has not been elucidated. Recently, Gao et al. investigated the effect of Tf-modified NPs across the BBB and subsequent brain tumor accumulation after shrouding in protein crowns from three aspects. *In vitro* formation of protein crowns abolished Tf-mediated receptor-specific recognition, lysosome escape, and transmembrane transport. Meanwhile, receptor recognition and lysosomal escape were retained *in vivo*; however, a protein corona attenuated these effects. For example, the results showed that NP-modified Tf covered by a corona reduced endothelial permeability by approximately 2-fold. However, both Tf-NPsVtr (1.7-fold higher) and Tf-NpsViv (1.2-fold higher) exhibited improved tumor-targeting abilities compared with transPEG-NPsVtr and transPEG-NpsViv. Researchers have found that Apo A-I protein is more conducive to NP transport in the BBB than other proteins. This study provided new insights for solving the problems of limited BBB-penetrating efficiency and tumor-homing accumulation in brain-targeting nano-DDSs [52].

5.2. *Stimuli-responsive lipoprotein nanocarriers*

Ultrasound, a simple, economic, and non-invasive method, has significantly facilitated drug delivery efficacy. It shows high tissue penetration and great potential for controlling drug release. Some studies have shown that the abnormal vascular systems of tumors and their microenvironment limit the effective systemic delivery of chemotherapy drugs. Non-invasive ultrasound can penetrate the human body, safely and reversibly increase the permeability of the tumor blood vessel wall, focus on the tumor site, and promote the accumulation of anticancer drugs. Xiong et al. investigated the effect of IR-780-loaded rHDL NPs on ultrasound stimulation. rHDL-like NPs can selectively deliver anticancer drugs to tumor cells depending on the overexpression of SR-B1 receptors. IR-780 iodine, a lipophilic NIR fluorescence imaging dye, has excellent tumor targeting and near-infrared

imaging potential. Their results showed that the ultrasound-stimulated method can improve the efficiency of drug delivery and tumor detection.

In addition, transient, non-invasive, and localized BBB openings can be achieved when microbubbles are exposed to focused ultrasound. This provides an approach for the brain-targeted delivery of therapeutic agents. DHA, a bioactive omega-3 fatty acid, has neuroprotective functions and is vital in brain functioning. Mulik et al. reported the engineering of a model DHA or fluorescent probe DiR-loaded LDL NPs with both strong localization of fluorescence signals and brain-targeting functions. After intravenous administration of LDL-DiR or LDL-DHA, BBB opening was observed using pulsed ultrasound in a localized brain region in a rat model. LDL-DHA produced 2-fold more DHA in the exposed regions of the brain, without increased tissue damage [53].

Recently, most studies have demonstrated the use of energy generated by external radiation to achieve or promote treatment efficacy. For example, photothermal therapy, which is commonly constructed by local irradiation with NIR irradiation, has emerged as a promising strategy for various diseases, owing to its feeble side effects. Compared with other irradiation methods, NIR irradiation has significant advantages in deep tissue penetration and spatial precision control. In AD treatment, the local hyperthermia released by NIR irradiation can effectively enhance the decomposition of Aβ. Martins et al. proposed a novel multifunctionalized NP for photothermal therapy using a gold core encapsulated in DMPC. To improve BBB permeability, ApoE was modified on the DMPC. After 15 min NIR irradiation, the tightness and quantity of Aβ exhibited significant reduction and degradation and even disappeared completely, demonstrating that local heat boosted by NIR irradiation can further enhance the decomposition effect of Aβ aggregate. In addition to NIR, infrared irradiation exhibits excellent performance in the treatment of disorders. As mentioned earlier, Kadiyala et al. developed a comprehensive method, chemoimmunotherapy combined with infrared, which exhibited remarkable advantages in inhibiting glioma growth. When the two therapies were combined, the degree of tumor regression increased from 40% to 80%. Furthermore, mice treated with infrared irradiation can obtain good long-term immune memory, and their survival time can be significantly prolonged [54].

5.3. *Absorb-responsive lipoprotein nanocarriers*

Recently, many studies have focused on the exploitation of "conjugated lipoprotein nanocarriers," directly embedding apolipoproteins or apolipoprotein biomimetic peptides on the carrier surface and then actively targeting lesions. For example, solid lipid NPs functionalized with ApoE possess highly efficient infiltration properties in the brain; ApoA-I-mimicking peptide (D4F)-modified NPs show substantial potential in tumor homing and penetration. However, the formation of a protein corona may present significant obstacles to the clinical translation of ideal drug candidates. Fortunately, in addition to some inert plasma proteins, numerous functional proteins are still adsorbed on the surface of nanocarriers.

Generally, two functional regions exist in exchangeable apolipoproteins (such as ApoE, ApoA1, and ApoJ): lipid-binding and receptor-binding regions. Moreover, one of the mechanisms of Aβ clearance in AD relies on linking the lipid-binding region and exposing the receptor-binding region, followed by crossing the BBB and discharging into peripheral blood via receptor-mediated transport. Peripheral ligands can also be transported to the brain via the bidirectional transport of related receptors. These theories may provide an opportunity to select the binding modes of apolipoproteins on nanosurfaces to achieve brain-targeted delivery. Recently, Zhan et al. developed a novel brain-targeted liposome modified with Aβprotein biomimetic nontoxic peptide to achieve highly efficient brain targeting. In blood circulation, functionalized liposomes can actively absorb the lipid-binding region of targeted endogenous apolipoproteins and the receptor-binding area is exposed on the liposome surface. Furthermore, when loading doxorubicin, the conjugates (SP-sLip/DOX) exhibited significant enhancement in both lesion-targeting biodistribution and anti-tumor efficacy (gliomas and medulloblastoma), prolonging the median survival period of model mice. Although DDSs (without modification of brain-targeting ligands) have rarely been reported to achieve BBB permeability depending only upon absorbing apolipoproteins after entering the blood, their design ideas and results have paved a new avenue to facilitate the development and clinical translation of nanomedicine [55].

6. Perspectives and conclusion

In the past decades, the continual development of biomimetics has inspired scientists to exploit endogenous or semi-endogenous nanocarriers, such as lipoproteins, exosomes, and bacteria, for disease treatment. Transporter agents range from conventional chemical drugs and contrast agents to biological medicines and photodynamic agents. Modification methods include the use of inorganic substances, polymers, small peptides, and macromolecular proteins. Diseases being treated have also broadened from common neurodegenerative diseases to gliomas and other autoimmune diseases. When combined with assorted modification methods and other advanced technologies, lipoprotein-based nanocarriers can effectively address the problem of eliminating obstacles in the treatment of diverse diseases.

As drug carriers, lipoproteins have drawn considerable attention because of their appreciable loading ability, long circulation time, inherent biocompatibility, favorable non-immunogenic character, and, most importantly, natural lesion-targeting capability.

Based on previous studies, we envisaged that the development trends of lipoprotein-induced nanocarriers can be summarized into two major issues: "self-transformation" and "associated-engineering." The former involves four key points that may reverse the adverse situation. First, the choice of more suitable core fillers should be explored to achieve high stability. The eutectic mixture of lauric acid and stearic acid (melting point of approximately 39 °C) can be used as the core materials of rLDL to synergistically participate in drug

delivery. Compared with cholesterol, such eutectic mixtures significantly control drug release on demand through metabolism and provide assistance to prevent lysosomal degradation with high security [56]. After consolidation, the crystallinity of eutectic mixtures is reduced and the drug-loading capacity of rLDL is further improved. Second, quality heterogeneity and uncontrollability are common concerns in the scale-up manufacturing and bench-to-bedside clinical translation of these nonocarriers. As endogenous substances, the membrane components of lipoproteins include various phosphoric acids, which are easily oxidized and adhere to other attachments, resulting in carrier collapse and drug leakage after long-term transportation or storage. Recently, most studies have demonstrated that DMPC, DOPC, and DOPE, which are commonly used lipid membrane materials, have excellent membrane-forming ability and stability and can form ideal lipoprotein-based carriers after binding with apolipoproteins. Meanwhile, the *in vivo* evaluation of lipoprotein nanocarriers should be systematically implemented and improved. Although the efficiency and safety of lipoprotein-induced DDSs have been demonstrated in the laboratory, the changes in normal tissues after long-term administration cannot be easily observed because the cycle of such experiments is relatively short. To further promote the clinical application of lipoprotein-based DDSs, systematic long-term investigations should be encouraged. Third, receptor-mediated endocytosis is a major pathway that endows lipoproteins and lipoprotein-inspired DDSs with enhanced BBB penetration and lesion-targeting capability. As mentioned earlier, LDLR, SR-BI, or other receptors (such as folate receptors) are often overexpressed on the membrane surface of brain endothelial and tumor cells, causing NP cell internalization through binding with apolipoproteins. However, full-length natural apolipoproteins are often unstable, have high molecular weights, and are difficult to purify. Complex pathological changes may lead to a loss or insensitivity of receptors at the lesion site, further contributing to reduced distribution and unwanted side effects. Therefore, the exploration of biomimetic and multifunctional hybrid peptides using apolipoproteins is crucial. Most studies on biomimetic peptides have focused on ApoA-I (such as D4F) and ApoE (such as COG112, COG133, COG1410, and Ac-hE 18A-NH2). To guarantee maximum therapeutic efficacy, biomimetic peptides equipped with other functional peptides, including targeting peptides (TfR, NGR, Angiopep-2, and M2pep), penetrating peptides (iRGD, CPP44, and Mel), and therapeutic peptides (GR, α-M and GM1), should be further developed [57]. Finally, simple chemotherapy does not hinder disease progression, whereas other management strategies, such as thermomagnetic and

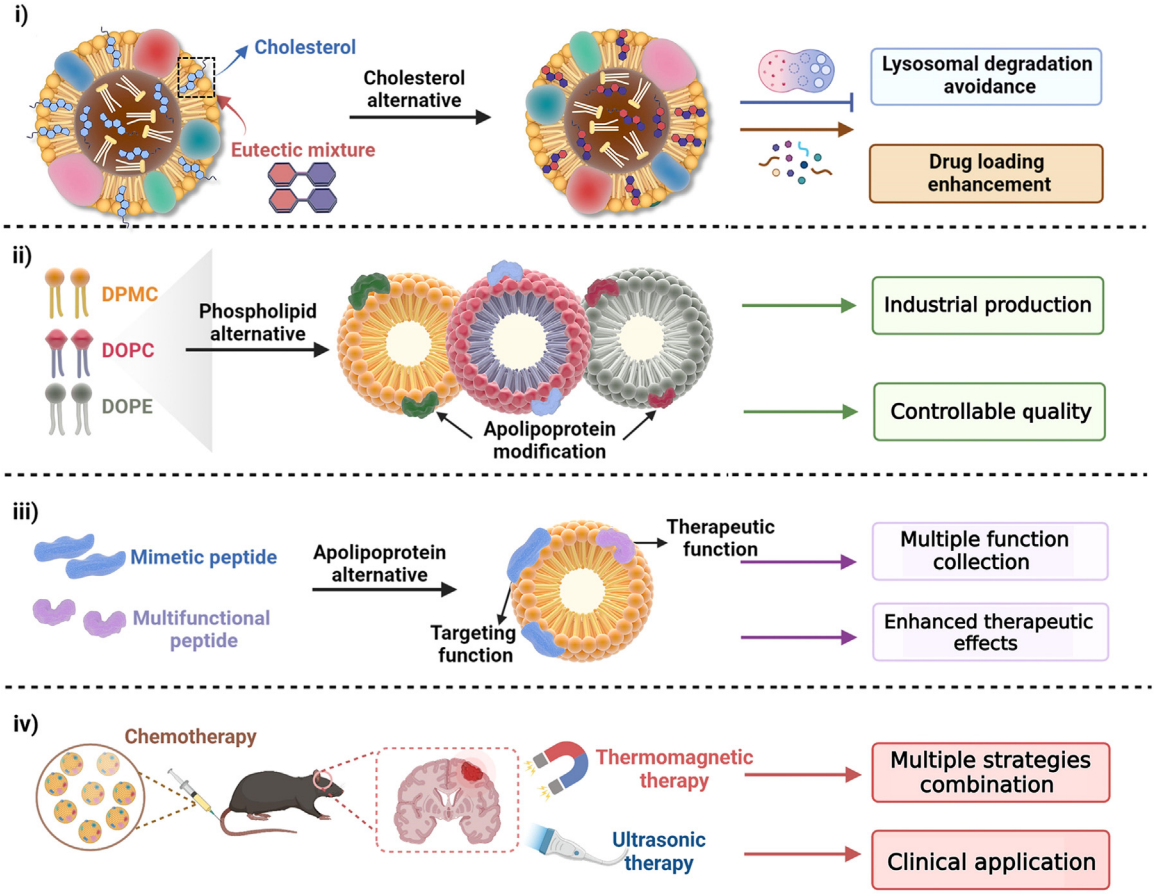

Fig. 7 – Schematic diagram of "Self-transformation". The strategies major include four key issues as follows: (i) cholesterol alternative; (ii) phospholipid alternative and apolipoprotein modification; (iii) multifunctional peptides modification; (iv) multiple therapy combination.

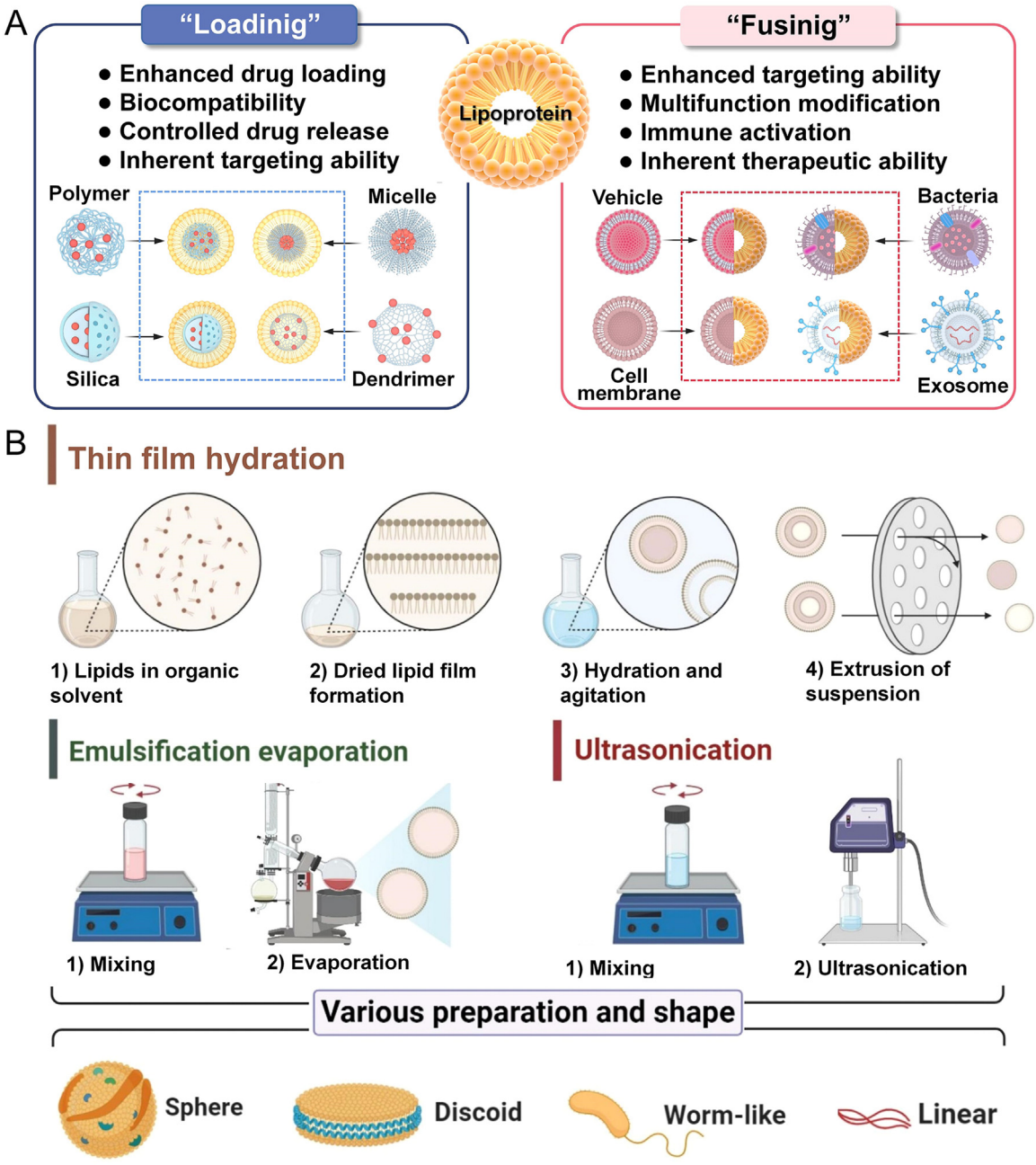

Fig. 8 – Construction of comprehensive lipoprotein-based nano-DDSs. (A) Schematic diagram of "Associated-engineering" by combining with other nanoplatforms. (B) Preparation strategies and different shapes of lipoprotein-based nano-DDSs.

ultrasonic therapy, provide external stimulation to highly facilitate curative effects through enhanced lesion targeting, imaging diagnosis, and tumor and inflammatory substance elimination (Fig. 7).

Thus far, the construction, development, and evaluation of brain-targeting synthetic nano-DDSs have led to significant progress in precise personalized therapy. However, the gap between laboratory and clinical results is considerable owing to systematic toxicity and off-target effects. Lipoproteins are natural or bioinspired nanocarriers that can replace exogenous vehicles for drug delivery. However, limited drug-loading and inadequate lesion-targeting abilities are severe limitations that need to be addressed. Therefore, the combination of lipoproteins with synthetic NPs or other bioactivated nanocarriers may be an optimal solution for achieving maximal potent drug delivery. Combining with synthetic NPs can enhance drug loading and achieve drug release on demand with biocompatibility and inherent BBB-penetrating and lesion-targeting abilities. Exosomes and cell membranes, which are the most promising candidates for drug delivery, share inherent properties with BBB crossing and cell homing and have received considerable attention in recent years. However, complex internal components and limited hydrophobic-drug loading may hinder their

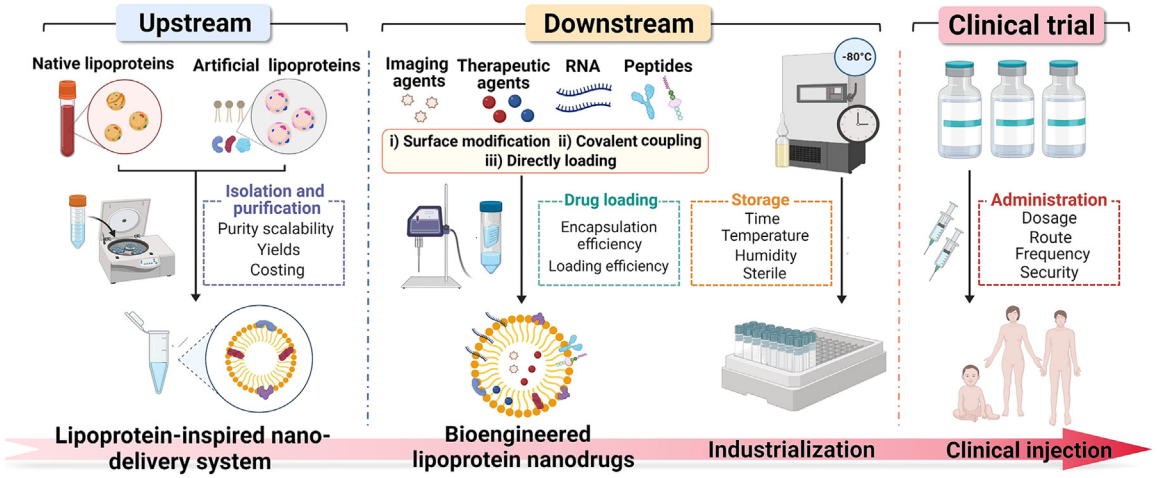

Fig. 9 – The development and critical issues of lipoprotein-based nano-DDSs can be generally subdivided into upstream and downstream processing and preclinical testing.

application. As a novel vehicle, bacteria possess natural innate immune activation; however, excessive stimulation may also lead to severe inflammatory storms. Therefore, combining bio-activated nanocarriers for lipoprotein "associated-engineering" can strengthen brain-targeting accumulation and therapeutic ability and address limitations such as low loading of hydrophobic drug and potential insecurity (Fig. 8A). Meanwhile, simplifying the preparations to the maximum extent possible and selecting various shapes, such as discoid, worm-like, and linear, to improve systemic circulation time and adhesion of the lesion site also show potential for improving therapeutic efficiency (Fig. 8B).

As drug carriers, lipoproteins have drawn considerable interest owing to their appreciable loading ability, long circulation time, inherent biocompatibility, favorable non-immunogenic character, and, most importantly, natural lesion-targeting capability. Some lipoprotein products without additional therapeutic agents have been clinically tested for atherosclerosis and metabolic diseases. For example, CSL-111 and CSL-112, which mainly comprise phospholipids and ApoA-I purified from human plasma, can affect cholesterol transport and elimination. According to the results of phase II clinical trials, CSL-112 yields better outcomes than CSL-111 in terms of patient tolerance and safety. Currently, CSL-112 (*e.g.*, NCT03473223) has entered phase III clinical trials for the treatment of acute coronary syndrome. However, several issues need to be considered in the field of clinical translation. First, it is limited to acquiring natural lipoproteins in extremely large quantities, because most of them are isolated from blood. Furthermore, because of the complexity of biochemistry properties, a highly reliable production technology is required. Second, the tendency to aggregate during long-term drug storage has raised concerns and may induce unexpected safety problems. Third, inner components may have potential side effects. Generally, drugs along with cholesteryl esters are loaded into the core together to maintain particle stability. However, with the long-term release of such compounds, atherosclerotic plaques can be formed because of the increased total cholesterol levels in the blood. Meanwhile, the high drug-loading ability is an essential precondition for adequate accumulation of lesions at an efficient concentration. Finally, ineluctable off-targeting effects and tissue toxicity are still two detrimental factors that need to be addressed to obtain excellent clinical results (Fig. 9). With progress in science and technology, we believe that the application of lipoproteins as drug carriers can emerge in biomedicine.

Conflicts of interest

The authors declare that they have no conflicts of interest

Acknowledgements

The authors acknowledge financial support from the National Natural Science Foundation of China (No. 82274104, 82074024, 82374042), the Open Project of Chinese Materia Medica First-Class Discipline of Nanjing University of Chinese Medicine (No. 2020YLXK019) and Young Elite Scientists Sponsorship Program by CACM (No. 2021-QNRC2-A01).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ajps.2023.100857.

REFERENCES

[1] Jin W, Shi K, He W, Sun J, Van Kaer L, Shi F, et al. Neuroblast senescence in the aged brain augments natural killer cell cytotoxicity leading to impaired neurogenesis and cognition. Nat Neurosci 2021;24(1):61–73.
[2] Xia X, Zhou Y, Gao H. Prodrug strategy for enhanced therapy of central nervous system disease. Chem Commun (Camb) 2021;57(71):8842–55.

[3] Gavilan H, Avugadda SK, Fernandez-Cabada T, Soni N, Cassani M, Mai BT, et al. Magnetic nanoparticles and clusters for magnetic hyperthermia: optimizing their heat performance and developing combinatorial therapies to tackle cancer. Chem Soc Rev 2021;50(20):11614–67.
[4] El AS, Mager I, Breakefield XO, Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. Nat Rev Drug Discov 2013;12(5):347–57.
[5] Zhu Y, Ouyang Z, Du H, Wang M, Wang J, Sun H, et al. New opportunities and challenges of natural products research: when target identification meets single-cell multiomics. Acta Pharm Sin B 2022;12(11):4011–39.
[6] Tang J, Baxter S, Menon A, Alaarg A, Sanchez-Gaytan BL, Fay F, et al. Immune cell screening of a nanoparticle library improves atherosclerosis therapy. Proc Natl Acad Sci USA 2016;113(44):E6731–40.
[7] Ju H, Kim D, Oh YK. Lipid nanoparticle-mediated CRISPR/Cas9 gene editing and metabolic engineering for anticancer immunotherapy. Asian J Pharm Sci 2022;17(5):641–52.
[8] Petri B, Bootz A, Khalansky A, Hekmatara T, Muller R, Uhl R, et al. Chemotherapy of brain tumour using doxorubicin bound to surfactant-coated poly(butyl cyanoacrylate) nanoparticles: revisiting the role of surfactants. J Control Release 2007;117(1):51–8.
[9] Song Y, Hu C, Fu Y, Gao H. Modulating the blood-brain tumor barrier for improving drug delivery efficiency and efficacy. View (Beijing) 2022;3(1):20200129.
[10] Niu X, Chen J, Gao J. Nanocarriers as a powerful vehicle to overcome blood-brain barrier in treating neurodegenerative diseases: focus on recent advances. Asian J Pharm Sci 2019;14(5):480–96.
[11] Tenchov R, Bird R, Curtze AE, Zhou Q. Lipid nanoparticles horizontal line from liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. ACS Nano 2021;15:16982–7015.
[12] Tiwari SK, Agarwal S, Seth B, Yadav A, Nair S, Bhatnagar P, et al. Curcumin-loaded nanoparticles potently induce adult neurogenesis and reverse cognitive deficits in Alzheimer's disease model via canonical wnt/beta-catenin pathway. ACS Nano 2014;8(1):76–103.
[13] Yang Y, Yang T, Chen F, Zhang C, Yin B, Yin X, et al. Degradable magnetic nanoplatform with hydroxide ions triggered photoacoustic, MR imaging, and photothermal conversion for precise cancer theranostic. Nano Lett 2022;22:3228–35.
[14] Niu D, Li Y, Shi J. Silica/organosilica cross-linked block copolymer micelles: a versatile theranostic platform. Chem Soc Rev 2017;46:569–85.
[15] Sharma AK, Gothwal A, Kesharwani P, Alsaab H, Iyer AK, Gupta U. Dendrimer nanoarchitectures for cancer diagnosis and anticancer drug delivery. Drug Discov Today 2017;22:314–26.
[16] Kumar V, Butcher SJ, Oorni K, Engelhardt P, Heikkonen J, Kaski K, et al. Three-dimensional cryoEM reconstruction of native LDL particles to 16a resolution at physiological body temperature. Plos One 2011;6(5):e18841.
[17] Paolino D, Cosco D, Gaspari M, Celano M, Wolfram J, Voce P, et al. Targeting the thyroid gland with thyroid-stimulating hormone (TSH)-nanoliposomes. Biomaterials 2014;35(25):7101–9.
[18] Rosenblum D, Joshi N, Tao W, Karp JM, Peer D. Progress and challenges towards targeted delivery of cancer therapeutics. Nat Commun 2018;9(1):1410.
[19] Kaiser K, Gyllborg D, Prochazka J, Salasova A, Kompanikova P, Molina FL, et al. Wnt5a is transported via lipoprotein particles in the cerebrospinal fluid to regulate hindbrain morphogenesis. Nat Commun 2019;10(1):1498.
[20] Wang R, Gu X, Zhou J, Shen L, Yin L, Hua P, et al. Green design "bioinspired disassembly-reassembly strategy" applied for improved tumor-targeted anticancer drug delivery. J Control Release 2016;235:134–46.
[21] Ding Y, Han Y, Wang R, Wang Y, Chi C, Zhao Z, et al. Rerouting native HDL to predetermined receptors for improved tumor-targeted gene silencing therapy. ACS Appl Mater Interface 2017;9(36):30488–501.
[22] Wang R, Han Y, Sun B, Zhao Z, Opoku-Damoah Y, Cheng H, et al. Deep tumor penetrating bioparticulates inspired burst intracellular drug release for precision chemo-phototherapy. Small 2018;14(12):e1703110.
[23] Tian M, Ticer T, Wang Q, Walker S, Pham A, Suh A, et al. Adipose-derived biogenic nanoparticles for suppression of inflammation. Small 2020;16(10):e1904064.
[24] Wang R, Zhang C, Li J, Huang J, Opoku-Damoah Y, Sun B, et al. Laser-triggered polymeric lipoproteins for precision tumor penetrating theranostics. Biomaterials 2019;221:119413.
[25] Smith ES, Porterfield JE, Kannan RM. Leveraging the interplay of nanotechnology and neuroscience: designing new avenues for treating central nervous system disorders. Adv Drug Deliv Rev 2019;148:181–203.
[26] Song Q, Huang M, Yao L, Wang X, Gu X, Chen J, et al. Lipoprotein-based nanoparticles rescue the memory loss of mice with Alzheimer's disease by accelerating the clearance of amyloid-beta. ACS Nano 2014;8(3):2345–59.
[27] Song Q, Song H, Xu J, Huang J, Hu M, Gu X, et al. Biomimetic ApoE-reconstituted high density lipoprotein nanocarrier for blood-brain barrier penetration and amyloid beta-targeting drug delivery. Mol Pharm 2016;13(11):3976–87.
[28] Huang M, Hu M, Song Q, Song H, Huang J, Gu X, et al. GM1-modified lipoprotein-like nanoparticle: multifunctional nanoplatform for the combination therapy of alzheimer's disease. ACS Nano 2015;9(11):10801–16.
[29] Karran E, Mercken M, De Strooper B. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. Nat Rev Drug Discov 2011;10(9):698–712.
[30] Zhang H, Jiang W, Zhao Y, Song T, Xi Y, Han G, et al. Lipoprotein-inspired nanoscavenger for the three-pronged modulation of microglia-derived neuroinflammation in Alzheimer's disease therapy. Nano Lett 2022;22(6):2450–60.
[31] Zhang H, Zhao Y, Yu M, Zhao Z, Liu P, Cheng H, et al. Reassembly of native components with donepezil to execute dual-missions in Alzheimer's disease therapy. J Control Release 2019;296:14–28.
[32] Li Xin, Jiang Xiao-wen, Chu Hai-xiao, Zhao Qing-chun, Huai-wei Ding, Cai Chao-hong. Neuroprotective effects of kukoamine A on 6-OHDA-induced Parkinson's model through apoptosis and iron accumulation inhibition. Chin Herbal Med 2021;13(1):105–15.
[33] Zhang Ru-yi, Zhang Xu, Zhang Lan, Wu Yan-chuan, Sun Xue-jing, Li Lin. Tetrahydroxystilbene glucoside protects against sodium azide-induced mitochondrial dysfunction in human neuroblastoma cells. Chin Herbal Med 2021;13(2):255–60.
[34] Rajora MA, Ding L, Valic M, Jiang W, Overchuk M, Chen J, et al. Tailored theranostic apolipoprotein E3 porphyrin-lipid nanoparticles target glioblastoma. Chem Sci 2017;8(8):5371–84.
[35] Jiang Y, Zhang J, Meng F, Zhong Z. Apolipoprotein E peptide-directed chimeric polymersomes mediate an ultrahigh-efficiency targeted protein therapy for glioblastoma. ACS Nano 2018;12(11):11070–9.
[36] Wang R, Wang X, Li J, Di L, Zhou J, Ding Y. Lipoprotein-biomimetic nanostructure enables tumor-targeted penetration delivery for enhanced photo-gene therapy towards glioma. Bioact Mater 2022;13:286–99.

[37] Ismail M, Yang W, Li Y, Chai T, Zhang D, Du Q, et al. Targeted liposomes for combined delivery of artesunate and temozolomide to resistant glioblastoma. Biomaterials 2022;287:121608.
[38] Wang R, Zhang X, Huang J, Feng K, Zhang Y, Wu J, et al. Bio-fabricated nanodrugs with chemo-immunotherapy to inhibit glioma proliferation and recurrence. J Control Release 2023;354:572–87.
[39] Liu Y, Wang W, Zhang D, Sun Y, Li F, Zheng M, et al. Brain co-delivery of first-line chemotherapy drug and epigenetic bromodomain inhibitor for multidimensional enhanced synergistic glioblastoma therapy. Exploration (Beijing) 2022;2(4):20210274.
[40] Kadiyala P, Li D, Nunez FM, Altshuler D, Doherty R, Kuai R, et al. High-density lipoprotein-mimicking nanodiscs for chemo-immunotherapy against glioblastoma multiforme. ACS Nano 2019;13(2):1365–84.
[41] Wei J, Wu D, Shao Y, Guo B, Jiang J, Chen J, et al. ApoE-mediated systemic nanodelivery of granzyme B and CpG for enhanced glioma immunotherapy. J Control Release 2022;347:68–77.
[42] Ramirez C, Hauser AD, Vucic EA, Bar-Sagi D. Plasma membrane v-atpase controls oncogenic RAS-induced macropinocytosis. Nature 2019;576(7787):477–81.
[43] Jiang G, Chen H, Huang J, Song Q, Chen Y, Gu X, et al. Tailored lipoprotein-like miRNA delivery nanostructure suppresses glioma stemness and drug resistance through receptor-stimulated macropinocytosis. Adv Sci (Weinh) 2020;7(5):1903290.
[44] Wang Q, Kumar V, Lin F, Sethi B, Coulter DW, Mcguire TR, et al. ApoE mimetic peptide targeted nanoparticles carrying a BRD4 inhibitor for treating medulloblastoma in mice. J Control Release 2020;323:463–74.
[45] Kim J, Dey A, Malhotra A, Liu J, Ahn SI, Sei YJ, et al. Engineered biomimetic nanoparticle for dual targeting of the cancer stem-like cell population in sonic hedgehog medulloblastoma. Proc Natl Acad Sci U S a 2020;117(39):24205–12.
[46] Lu L, Qi S, Chen Y, Luo H, Huang S, Yu X, et al. Targeted immunomodulation of inflammatory monocytes across the blood-brain barrier by curcumin-loaded nanoparticles delays the progression of experimental autoimmune encephalomyelitis. Biomaterials 2020;245:119987.
[47] Chen L, Song Q, Chen Y, Meng S, Zheng M, Huang J, et al. Tailored reconstituted lipoprotein for site-specific and mitochondria-targeted cyclosporine a delivery to treat traumatic brain injury. ACS Nano 2020;14(6):6636–48.
[48] Huang J, Zhu H, Yu P, Ma Y, Gong J, Fu Y, et al. Recombinant high-density lipoprotein boosts the therapeutic efficacy of mild hypothermia in traumatic brain injury. ACS Appl Mater Interfaces 2023;15(1):26–38.
[49] Aasen SN, Espedal H, Holte CF, Keunen O, Karlsen TV, Tenstad O, et al. Improved drug delivery to brain metastases by peptide-mediated permeabilization of the blood-brain barrier. Mol Cancer Ther 2019;18(11):2171–81.
[50] Ruan S, Zhou Y, Jiang X, Gao H. Rethinking critid procedure of brain targeting drug delivery: circulation, blood brain barrier recognition, intracellular transport, diseased cell targeting, internalization, and drug release. Adv Sci (Weinh) 2021;8(9):2004025.
[51] Ma X, Song Q, Gao X. Reconstituted high-density lipoproteins: novel biomimetic nanocarriers for drug delivery. Acta Pharm Sin B 2018;8(1):51–63.
[52] Xiao W, Wang Y, Zhang H, Liu Y, Xie R, He X, et al. The protein corona hampers the transcytosis of transferrin-modified nanoparticles through blood-brain barrier and attenuates their targeting ability to brain tumor. Biomaterials 2021;274:120888.
[53] Mcconathy WJ, Nair MP, Paranjape S, Mooberry L, Lacko AG. Evaluation of synthetic/reconstituted high-density lipoproteins as delivery vehicles for paclitaxel. Anticancer Drugs 2008;19(2):183–8.
[54] Kuai R, Subramanian C, White PT, Timmermann BN, Moon JJ, Cohen MS, et al. Synthetic high-density lipoprotein nanodisks for targeted with alongolide delivery to adrenocortical carcinoma. Int J Nanomedicine 2017;12:6581–94.
[55] Kuai R, Ochyl LJ, Bahjat KS, Schwendeman A, Moon JJ. Designer vaccine nanodiscs for personalized cancer immunotherapy. Nat Mater 2017;16(4):489–96.
[56] Mcmahon KM, Plebanek MP, Thaxton CS. Properties of native high-density lipoproteins inspire synthesis of actively targeted *in vivo* siRNA delivery vehicles. Adv Funct Mater 2016;26(43):7824–35.
[57] Nakayama T, Butler JS, Sehgal A, Severgnini M, Racie T, Sharman J, et al. Harnessing a physiologic mechanism for siRNA delivery with mimetic lipoprotein particles. Mol Ther 2012;20(8):1582–9.