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Nucleic acid therapy for metabolic-related diseases

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ABSTRACT

Metabolism is a general term for a series of ordered chemical reactions in an organism used to maintain life, mainly divided into anabolic and catabolic metabolism. Nucleic acid therapy can not only precisely up-regulate and down-regulate the expression of target genes but also correct mutated disease-causing genes, which demonstrates irreplaceable and outstanding advantages in the treatment of metabolism-related diseases and has been applied to the clinical treatment of metabolism-related diseases. In this review, we introduce the structures of several major nucleic acid drugs and the mechanism of nucleic acid therapy. Subsequently, we describe the mechanisms of various biomolecular and tissue metabolisms and the etiology of metabolic disorders, classified according to metabolic substrates. We analyze the signal pathways and potential targets affecting the metabolism of each substrate and describe the nucleic acid drugs applied to these targets and their delivery technologies. This review aims to provide new ideas and targets for treating these diseases by investigating the role played by metabolism in developing diseases and providing guidance for the selection and design of nucleic acid drugs.

1. Introduction

Metabolism is a biological process by which organisms exchange material and energy and is the most fundamental life activity. In turn, many diseases are accompanied by metabolic disorders. Multiple signal pathways regulate various metabolic pathways. Therefore, theoretically regulating critical targets in the signal pathways is expected to ameliorate metabolic disorders and thus contribute to treating metabolism-related diseases. Such as, according to the statistics of the International Diabetes Federation, 10,537 million adults had diabetes in 2021, of which more than 90% had diabetes mellitus type 2 [1]. However, at present, small molecule drugs are commonly used in clinical treatment, which have disadvantages such as high frequency of administration and large side effects, and new drugs are urgently needed to treat metabolic diseases.

Nucleic acid drugs are targeted drugs based on nucleic acid molecules that can be up-regulated, down-regulated, and edited at the DNA or RNA level [2]. In contrast to the limited number of targets of traditional small molecule and monoclonal antibody-targeted drugs, nucleic acid drugs can be applied to all targets, theoretically employing Watson-Crick base pairing [3]. In addition, nucleic acid drugs can regulate the mRNA of the disease-causing protein before its translation, and even integrate into the genome to change the disease-causing gene. Therefore, compared with common drugs such as small molecule drugs, nucleic acid drugs have durability in terms of drug administration. Furthermore, the design of nucleic acid drugs is relatively easy and has a high success rate due to the design of nucleic acid sequences based on target gene sequences. Therefore, nucleic acid drugs have irreplaceable and outstanding advantages in treating metabolism-related diseases [4,5].

This review begins with an introduction to the significant nucleic acid drugs, including their structures and nucleic acid therapeutic mechanisms. Considering that metabolic disturbances are not only associated with traditional metabolic diseases but also have relevance to other diseases, such as cardiovascular diseases, tumors, and rare diseases [6–9], this review is not classified by diseases but by metabolic substrates, such as glucose metabolism, lipid metabolism, protein metabolism and so on. This review focuses on the relationship between metabolism and disease, with the major signal pathways and key targets that affect metabolism.

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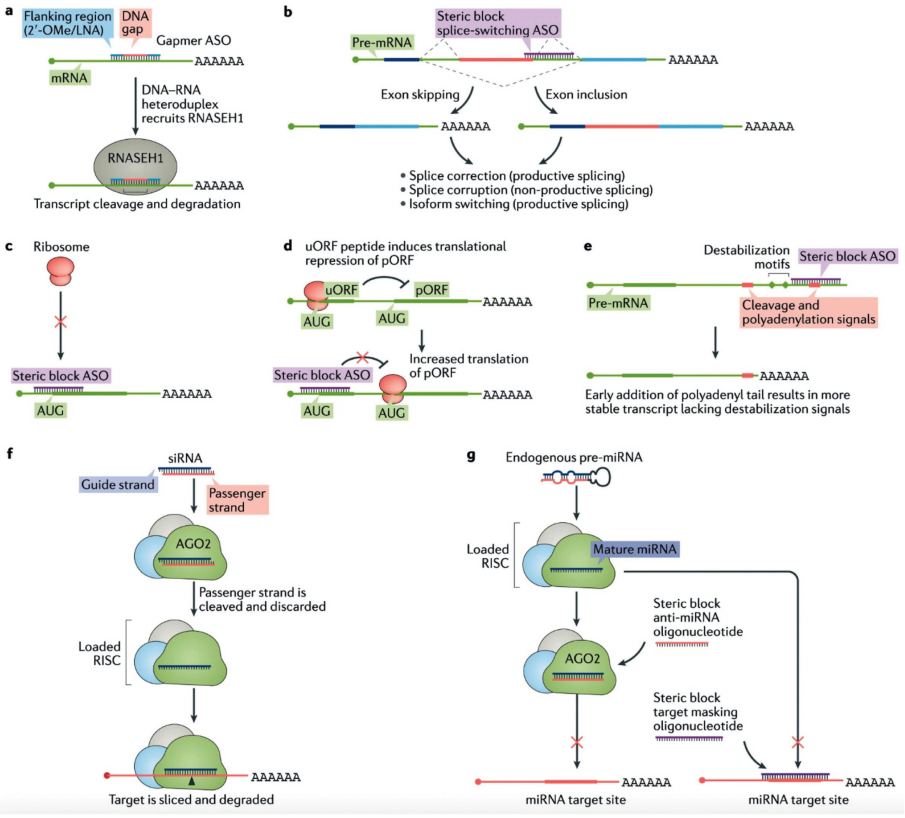

Fig. 1. Oligonucleotide-mediated gene regulatory mechanisms. (a–e) The regulatory mechanisms of ASO. (f) The regulatory mechanisms of siRNA. (g) The regulatory mechanisms of miRNA. Reproduced with permission [10]. Copyright 2020, Springer Nature Limited.

2. Classification of nucleic acid drugs

Nucleic acid drugs are a class of DNA or RNA drugs with specific base sequences, mainly including antisense oligonucleotide (ASO), small interfering RNA (siRNA), microRNA (miRNA), messenger RNA (mRNA), plasmid DNA (pDNA), clustered regularly interspaced short palindromic repeats (CRISPR), to achieve targeted therapy of disease by up-regulating or down-regulating the expression of disease targets or directly editing mutant disease-causing genes (Fig. 1) [10].

2.1. ASO

ASO is a short, single-stranded DNA (ssDNA)-like structure containing 12–30 deoxyribonucleotides, which binds to and mediates the degradation of target mRNAs through Watson-Crick base pairing to down-regulate the expression of target proteins [11,12]. There are two main mechanisms by which ASO regulates gene expression [13,14]. ASO forms a DNA-RNA hybrid strand with mRNA, which recruits ribonuclease H (RNase H) to degrade the target mRNA. ASO binds to the target mRNA in the cytoplasm to form a DNA-RNA complex, which recruits endogenous RNase H to degrade the target mRNA, thereby inhibiting the expression of pathogenic genes [15,16].

In 1998, Fomivirsen was approved by the Food and Drug Administration (FDA), which was the first nucleic acid drug used in the clinic [17]. The rapid development of genetics and gene sequencing technologies has advanced the study of disease-causing genes, allowing ASOs to target more mRNA to treat diseases. In addition, the optimization of chemical modification and delivery system has improved many shortcomings of ASO, such as poor targeting, insufficient biological activity, off-targeting and toxic side effects, which enables ASO to accurately target specific mutations for repair in addition to regulate the target genes specifically, and it has been widely used in the treatment of tumors, metabolic diseases, and neurodegenerative diseases [18–20].

2.2. siRNA

siRNA is a short, non-coding, double-stranded RNA with typically 21 nucleotides per strand [21]. The antisense strand is known as the guide strand, and the sense strand is known as the passenger strand. 19 base pairs typically bind the guide and passenger strands. The 3′ ends of both strands typically contain an overhang of 2 nucleotides each. The RNA-induced silencing complex (RISC), which consists of the Argonaute 2 protein (AGO2), the Dicer enzyme, and the transactivation response RNA binding protein, plays a crucial role in the RNAi process of siRNA [22,23]. The siRNA binds to RISC in the cytoplasm and is subsequently deconvoluted by RISC. The passenger strand is degraded, and the guide strand directs RISC to the target mRNA through Watson-Crick base pairing. AGO2 then cleaves the mRNA site corresponding to the 10–11 site of the guide strand. There is no protection group for the end group of the cleaved mRNA, and it is rapidly degraded by nuclease in the cell to silence the target gene [24,25]. siRNAs synthesized from natural nucleotides are unstable, highly electronegative, and challenging to be taken up by target cells [26,27]. Therefore, the delivery technology is crucial for siRNA to exert its drug effect. Currently, there are two main delivery approaches: carrier delivery and self-delivery [22,26]. Carrier delivery refers to the efficient delivery of siRNA by endowing the carrier with multiple functions, such as tissue/cell targeting, protection against enzymatic degradation of siRNA, endosomal escape, and response release. In contrast, self-delivery refers to the efficient *in vivo* delivery of siRNA by overcoming its natural defects through chemical modification and conjugation of the siRNA itself in a carrier-independent man-

ner [28,29]. Chemical modifications mainly refer to backbone, base, and 2′-ribose modifications, which improve stability and lipophilicity and reduce immunogenicity [3,30]. The conjugated molecules are protein, peptide, saccharide, and lipid molecules used for tissue/cellular targeting or to facilitate siRNA uptake.

2.3. miRNA

miRNAs are a class of endogenous non-coding single-stranded RNAs of approximately 20–25 nt in length [31]. When miRNAs undergo gene silencing, they usually first form primary miRNAs (pri-miRNAs) of 300–1000 nt, which are then cleaved in the nucleus by Drosha enzymes and cofactor DGCR8 into hairpin-shaped precursor miRNAs (pre-miRNAs) of about 70 nt. pre-miRNAs is transported to the cytoplasm by the transporter protein Exprotin-5 and sheared into mature miRNA by Dicer enzyme [32,33]. Like siRNAs, the guide strand in miRNAs can bind to RISC and be untwisted, and the other strand is rapidly degraded. RISC mediates the cleavage of the target mRNA to exert gene silencing. Unlike siRNAs, miRNAs act on the 3′ untranslated region of mRNAs (3′-UTR) and may generate mismatches, forming a complementary pair with the target mRNA completely or incompletely [34,35]. Thus, a miRNA can regulate the expression of multiple genes simultaneously. In addition, diseases can also affect the expression of endogenous miRNAs. Therefore, miRNAs can be widely used as disease diagnostic biomarkers and therapeutic agents [36,37].

2.4. mRNA

mRNAs are a class of single-stranded RNAs formed by transcribing a strand of DNA as a template, with a length of about 500–100,000 nt. mRNAs are mainly composed of cap, 5′ untranslated region, 3′ UTR, open reading frame (ORF), and polyadenosine (Poly(A)) residues [38,39]. The mRNA synthesized *in vitro* is also known as *in vitro* transcribed (IVT) mRNA. After entering the cytoplasm, IVT mRNA can bind to ribosomes due to its structural similarity to natural mRNA and express target proteins using an endogenous translation system to treat various diseases [40].

IVT mRNA has been applied to mRNA vaccines [41–44]. mRNA vaccines are directly translated and synthesized into corresponding antigenic proteins in the cytoplasm, thus inducing the body to produce a specific immune response and preventing diseases. Compared with traditional vaccines, mRNA vaccines are simple in design, have a short development cycle, and have a fast onset of action. Currently, researchers focus on improving the translation efficiency, stability, and longevity of mRNAs by modifying mRNAs and optimizing delivery technologies, among other ways [45,46]. Examples include optimizing the 5′ cap structure, adjusting the length of the 3′ Poly(A) tail, and modifying regulatory elements in the 5′ and 3′ untranslated regions to improve the translation efficiency of siRNAs. In addition, carrier delivery using lipid nanoparticles (LNP), polymeric nanoparticles (PNPs), and so on was used to improve mRNA's *in vivo* transport efficiency.

2.5. CRISPR

CRISPR was first discovered in 1987 in *Escherichia coli* [47]. In 2012, Doudna and Charpentier first discovered the mechanism of the CRISPR II system by combining CRISPR and CRISPR-associated protein9 (Cas9) consisting of the CRISPR/Cas9 gene editing tools [48]. They proved that Cas protein can precisely cleave double-stranded DNA under the guidance of dsRNA, which pushed forward the development of CRISPR technology. Compared to the previous two generations of gene editing tools, zinc-finger nuclease and transcription activator-like effector nuclease, the CRISPR/Cas system guides nucleic acid endonucleases through specific guide RNA (gRNA) to bind target DNA or RNA to cleave them, thus realizing more accurate, simple and efficient gene editing.

Makarova *et al.* [49] categorized existing CRISPR/Cas systems into type I, type II, and type III based on evidence from phylogenetic, genomic, and structural analyses. Each type of CRISPR/Cas edits different kinds of targets through other Cas proteins. These targets include dsDNA, ssDNA, RNA, and so on [49–51]. CRISPR/Cas9 is the most commonly used CRISPR/Cas system, mainly consisting of gRNA and Cas9 proteins [52]. Cas9 proteins are a multidomain DNA nucleic acid endonuclease, which is responsible for cutting the target genes to make double-strand breaks. When using the CRISPR/Cas9 system for gene editing, researchers combined CRISPR RNA (cRNA) and transactivating crRNA to design single guide RNA, which is used to guide the Cas9 protein to cut the target gene [52].

3. Common nucleic acid delivery carriers

Nucleic acid drugs can up-regulate, down-regulate and edit disease-related genes at the DNA or RNA level. In recent years, they have attracted extensive attention from researchers due to their unique advantages, and their research and development process has been accelerating. Compared with the limited number of targets of traditional small molecules and monoclonal antibody targeted drugs, nucleic acid drugs can be theoretically applied to all targets using Watson-Crick base pairing principle [53,54]. In addition, nucleic acid drugs have the ability to regulate before the synthesis of target proteins and are therefore persistent. Moreover, the design of nucleic acid drugs is relatively easy because the success rate of nucleic acid sequence design based on target gene sequence is higher than that of other drugs. Therefore, nucleic acid drugs have irreplaceable outstanding advantages in the treatment of genetic diseases, cancer, metabolic diseases and other diseases [4,55,56]. However, delivery technology is the main bottleneck restricting the clinical application of nucleic acid drugs.

During the delivery process, nucleic acid drugs will face the problems of nuclease degradation, multiple biological barriers (such as blood-brain barrier, cell membrane barrier, lysosome), poor targeting, short half-life, and low transfection efficiency [57,58]. Therefore, the use of specific vectors to deliver nucleic acid drugs is necessary. At present, the commonly used nucleic acid drug vectors can be divided into synthetic vectors and natural-derived vectors.

3.1. Synthetic nucleic acid carrier

Synthetic vectors can be precisely controlled for their chemical structure, size, shape, and surface properties, and can be designed into systems with specific targeting, biocompatibility, degradation rate, and drug release according to requirements, with high consistency between batches and easy to achieve large-scale production [55,58,59]. At present, inorganic nanoparticles, lipid nanoparticles, and polymer nanoparticles are common (Fig. 2) [60].

3.1.1. Lipid nanoparticles

Lipid nanoparticles are composed of one or more lipid bilayers [61] that can be used to encapsulate and deliver various types of drugs, including small molecule drugs, proteins, and nucleic acids. Lipid nanoparticles usually contain cholesterol, phospholipids and other components, which mimic the structure of natural cell membrane, so they have good biocompatibility [62]. Specific cell or tissue targeting can be achieved by surface modification, such as linking specific antibodies or ligands. Through the design selection of components and materials, drug stability can be improved and drug release can be controlled [63–66]. Li *et al.* [67] used cationic

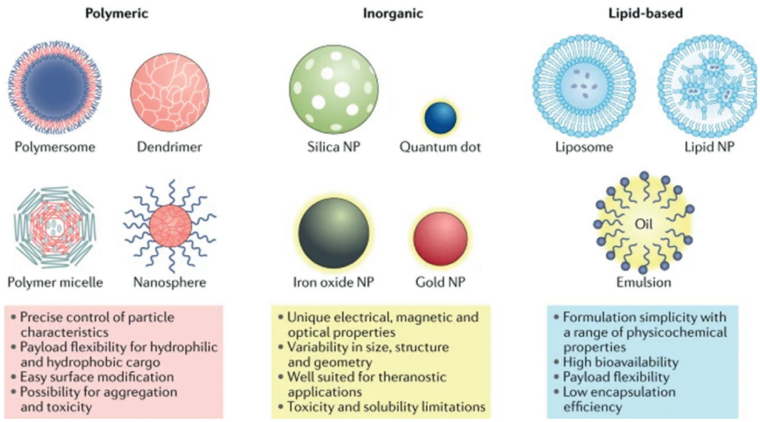

Fig. 2. Common types of synthetic vectors. Copied with permission [60]. Copyright 2020, Springer Nature Limited.

and pegylated lipids to construct a lipid nanoparticle drug delivery system to load siRNA of silenced HepG2 and modify cells to penetrate peptidyl octadecararginine to improve its cellular uptake efficiency. The stability of the system in the blood circulation was greater than that of free siRNA. It exhibits excellent gene silencing activity and tumor suppression with low immunogenicity and toxicity. Ramishetti *et al.* [68] constructed a library of sirNAloaded lipid nanoparticles based on novel ionizable amino lipids with different linker groups (*e.g.*, hydrazine, hydroxylamine and ethanolamine). Two effective lipid components were selected and their biodistribution characteristics were further evaluated by screening their delivery efficiency to leukocytes. It has good gene silencing ability and potential immune activation in mice, which provides theoretical guidance for a new way to deliver RNA to leukocytes.

3.1.2. Polymer nanoparticles

Polymer nanoparticles are nanoparticles composed of polymeric materials. Polymer materials can be natural polymers, such as proteins, polysaccharides, or they can be polymerized by artificial synthetic materials, which can be divided into multimers, aggregates, and dendrimers [69–71]. Polymer nanoparticles can precisely control their size by adjusting the composition and synthesis conditions of the polymer, and regulate various forms such as spherical, rod, and vesicle. In addition, polymer nanoparticles have good thermal stability, chemical stability and mechanical strength, which can be applied in different pathological environments and have broad application prospects [72]. Karlsson *et al.* [73] proposed the strategy of photocrosslinking biodegradable nanoparticles to improve the stability of the system and the rapid release of intracellular RNA. The siRNA vector containing ester bonds that are easy to degrade *in vivo* and disulfide bonds that are promoted by the disease microenvironment was designed. Compared with the non-crosslinked system, the sirna vector reduced the adsorption of serum proteins and prolonged the circulation *in vivo*, and the presence of secondary and tertiary amines could achieve effective endosomal escape. Moreover, it can achieve excellent siRNA-mediated gene knockdown in glioma and melanoma cells. After systemic administration, it also has a good gene knockdown effect in lung melanoma, and this system provides a new method for nucleic acid delivery technology.

3.2. Natural derived nucleic acid drug carriers

Natural-derived vectors are produced by the normal physiological activities of organisms and have high biocompatibility, low cytotoxicity and immunogenicity [74,75]. Natural-origin vectors can exploit specific mechanisms *in vivo* to achieve targeting to specific cells or tissues, thereby improving drug specificity and efficacy, but they may also be limited by heterogeneity between source batches, quality control issues during manufacturing, and possibly ethical concerns [74–77]. Therefore, when selecting and using vectors of natural origin, their characteristics and potential application limitations need to be comprehensively considered.

3.2.1. Exosomes

Exosomes are biological vesicles with a diameter of about 40–160 nm secreted by cells, which have a cell-like lipid bilayer structure and internal cavities, so they have been developed as carriers of a variety of drugs, such as small molecule drugs, protein drugs, and nucleic acid drugs [78–80]. Compared with synthetic drugs or carriers, exosomes have many irreplaceable advantages. (1) Exosomes are natural carriers for material and information exchange between cells, so they can deliver drugs to target cells more efficiently [81]. (2) Autologous derived exosomes have lower immunogenicity *in vivo* to avoid recognition and clearance by the immune system [82]. (3) Exosomes naturally carry a variety of bioactive molecules, such as RNA, DNA, proteins, sugars and lipids, which have shown excellent therapeutic effects on a variety of diseases [83–85]. (4) A variety of natural ligands are distributed on the surface of exosomes, which have natural homing functions for specific disease lesions [86]. Based on the above advantages, exosomes are widely used in various drug delivery and disease treatment. Didiot *et al.* [87] through hydrophobic modification of siRNA and modification of cholesterol at the 3′ end of the passenger chain, can be quickly inserted into the exosome membrane to realize the convenient and rapid preparation of exosome-loaded nucleic acid drug system. The investigators found that this system resulted in dose-dependent knockdown of Htt mRNA and protein in primary cortical neurons and enhanced uptake efficiency in refractory cells. Cui *et al.* [88] induced human induced pluripotent stem cells to produce Mesenchymal stem cells (MSC), extracted their exosomes to modify bone targeting peptide and loaded with siRNA that silently regulated Shn3 gene of osteogenic differentiation. The researchers found that this system could significantly silence Shn3 gene and reduce RANKL expression, thereby enhancing osteoblast formation and angiogenesis, demonstrating a potent anti-osteoporosis effect.

3.2.2. Apoptotic body

Apoptotic bodies are extracellular vesicles produced by germination shedding mechanism and autophagosome formation mechanism in the process of apoptosis, with a diameter of about 100–5000 nm, which are responsible for activating and executing the programmed death process of apoptosis [89,90]. Apoptosis is a genetically controlled cellular suicide process that is essential for maintaining balance and tissue homeostasis in multicellular organisms [91]. Apoptotic bodies contain several key components of the

apoptosis process [89,90,92,93]: Caspase family proteins are key proteins that regulate apoptosis and can activate related enzymes to degrade intracellular proteins. Apaf-1 protein can bind and activate the caspase-9 signaling pathway to promote the occurrence of apoptosis. It also contains nucleic acids, proteins, organelles and other substances in some cells, which have certain protocellular characteristics and have great potential in immunosuppression and anti-inflammatory therapy [93]. Li *et al*. [94] used adipose-derived apoptotic bodies to treat a full-thickness skin wound model in mice. Studies have shown that treatment with apoptotic bodies accelerates wound healing in mice and promotes the formation of granulation tissue and blood vessels in wound tissue. Further studies have shown that the treatment of apoptotic bodies can promote the M2 polarization of macrophages and reduce the inflammatory response, which may be related to miR-21–5p.

4. Glycometabolism

Saccharide is one of the primary sources of energy in living organisms, mainly in the form of glucose, which provides energy for life activities [95]. In addition, lipids and proteins can also be glycosylated to form glycolipids and glycoproteins, which play essential roles in maintaining stable structures of tissues and organs and promoting intercellular signal transmission [96]. Saccharide metabolism mainly refers to the process by which glucose, glycogen, and other saccharides are subjected to complex regulation in the body, which is broadly categorized into the following stages [97–99]: digestion and absorption of saccharides from food, glycolysis under hypoxic conditions, oxidative phosphorylation, pentose phosphate pathway and glycogen synthesis and breakdown [97,100].

Glucose metabolism is crucial for the homeostatic regulation of the body. Disorders of glucose metabolism promote the pathogenesis of diseases such as diabetes, hypoglycemia, and vascular lesions. Glucose metabolism disorder is also one of the main pathogenic factors of inflammation, cancer, and other diseases. Safe and effective treatment of glucose metabolism disorder is the focus and difficulty of current research.

4.1. Glucose transporter protein (GLUT)

GLUTs belong to the major facilitator superfamily. Currently, 14 GLUT isoforms have been identified, and GLUTs are mainly responsible for transporting glucose and fructose into the cells [101,102]. Abnormal function of GLUTs can lead to the disturbance of blood glucose levels in the body, which can cause the emergence of various metabolic diseases.

GLUT1 is the first glucose transporter protein widely distributed in the body. As the predominant glucose transporter protein in erythrocytes and epithelial cells, GLUT1 is crucial in maintaining blood glucose stability [103,104]. You *et al*. [105] directly injected anti-GLUT1 siRNA into the vitreous humor to inhibit the expression of GLUT1 to explore the effect of GLUT1 on diabetic retinopathy. siGLUT1 reduces retinal glucose concentration and ameliorates diabetic retinopathy by inhibiting GLUT1 expression to limit glucose transport. An inflammatory response accompanies most metabolic diseases. The immune response from inflammation stimulates GLUT1 expression, allowing macrophages to acquire sufficient glucose to maintain their function. Cornwell *et al*. [106] investigated the role of GLUT1-mediated glucose metabolism in the pro-inflammatory response of macrophages by siGLUT1. They found that the downregulation of GLUT1 decreased glucose uptake along with the levels of various pro-inflammatory factors. Thus, GLUT1 is a potential target for treating metabolic diseases by lowering inflammation levels.

GLUT4 is the predominant glucose transport protein in adipocytes and myocytes. GLUT4 is generally located in various intracellular organelles and is mainly regulated by insulin. GLUT4 can be rapidly transported to the cell membrane and consume glucose in the blood after being stimulated by insulin [107–109]. Lu *et al*. [110] found that miR-223 was closely related to glucose uptake in the heart by examining the expression levels of various miRNAs in the hearts of diabetic patients. After overexpressing miR-223 and down-regulating GLUT4 with siRNA, they found that miR-223 increased glucose uptake by GLUT4. This study provided a new therapeutic strategy for treating heart-related diseases caused by diabetes. Zhu *et al*. [111] explored the manner of glucose uptake in adipocytes. Insulin regulates glucose transport in adipocytes through the IRS1/PI3K/GLUT4 pathway. By examining glucose consumption in miR-146b-treated adipocytes, they found that miR-146b decreased GLUT4 content and glucose consumption. They further demonstrated that miR-146b inhibited the IRS1/PI3K/GLUT4 pathway, inhibiting adipocyte glucose uptake. This study suggested that miR-146b contributed to maintaining glucose homeostasis in adipocytes and provided a potential target for type 2 diabetes treatment.

Disorders of glucose metabolism are also common in cancer [112,113]. Glucose uptake in tumor tissues is much higher than in normal tissues, which is one of the essential reasons for the rapid proliferation of tumor cells. GLUT3, which is highly expressed in cancer cells, is now also one of the emerging targets for cancer treatment. Xu *et al*. [114] used PEG-PLA nanoparticles to effectively deliver siRNA into U87MG and U251 glioma cells and glioma stem cells derived from these tumor cells, which had significant glucose uptake reduction and glucose metabolism inhibition. Tumor cell proliferation and tumor growth were also significantly inhibited. This strategy can be used to treat a wide range of tumors and combined with other therapeutic approaches to enhance the therapeutic efficacy.

4.2. Ketohexokinase (KHK)

Excessive sucrose intake is also a critical factor that triggers metabolic diseases such as obesity, type 2 diabetes, and hyperlipidemia. Sucrose consists of one molecule of fructose and one molecule of glucose. KHK is expressed in the liver and kidney and is the rate-limiting enzyme in fructose metabolism. KHK is capable of converting fructose to fructose-1-phosphate. Fructose-1-phosphate is a substrate for the synthesis of fatty acids (FA). Excess fat in the liver exacerbates glucose metabolism disruption [115–118]. Inhibition of KHK expression contributes to reducing the lipogenesis of sugar and regulating glucose metabolism homeostasis.

Liu *et al*. [119] used anti-KHK ASO to knock down KHK in the liver to treat non-alcoholic fatty liver disease in rats. The results showed that the knockdown of KHK dramatically reduced blood glucose and lipids and alleviated insulin resistance, demonstrating that KHK is a potential target for treating glucose metabolism-related diseases. Strober *et al*. also explored the effects of different saccharides on adipogenesis by using KHK-targeted ASO, glucokinase-targeted ASO, and lactate dehydrogenase-targeted ASO, respectively. The results showed that all the different types of saccharides contributed to dyslipidemia. After knocking down the relevant targets using ASO, plasma triglyceride levels were significantly reduced.

KHK-targeted siRNAs have also been proven to have excellent therapeutic effects in type 2 diabetes. Noetzli *et al*. [120] synthesized *N*-acetylgalactosamine (GalNAc)-siKHK conjugates with high stability and low immunogenicity based on enhanced stabilization chemistry (ESC) technology and GalNAc conjugate delivery platform GalNAc-siKHK, accumulating in the liver after subcutaneous administration, decreased blood glucose and improved insulin sen-

sitivity in type 2 diabetic crab-eating monkeys. In particular, the GalNAc-siKHK conjugates demonstrated outstanding long-term efficacy and a high safety profile and have been supported to initiate clinical trials in obese healthy volunteers (NCT05761301).

4.3. Glucagon receptor (GCGR)

GCGR belongs to the class B G-protein-coupled receptor family. GCGR binds to the glucagon hormone and causes blood glucose to rise by promoting the breakdown of glycogen by hepatocytes, which plays a crucial role in maintaining glucose homeostasis [121,122]. GCGR is mainly expressed in the liver and kidney, with a small distribution in the cerebral cortex, adipose, gastrointestinal tract, and other tissues [123]. Glucagon produced by pancreatic α-cells can elevate blood glucose by promoting hepatic glycogenolysis. Under normal physiological conditions, insulin and other hormones inhibit glucagon secretion from pancreatic α-cells during hyperglycemia. In contrast, during hypoglycemia, pancreatic β-cells secrete less insulin, eliminating the inhibitory effect on pancreatic α-cells, and glucagon secretion is increased to stabilize glucose metabolism *in vivo* [124,125]. Therefore, GCGR, as a receptor for glucagon, is an essential target for treating obesity, diabetes, hypoglycemia, and other diseases through glucose homeostasis *in vivo*.

Neumann *et al*. [126] treated type 1 and type 2 diabetes mellitus by combination of siGCGR and leptin. They delivered the drugs to the liver *via* lipid nanoparticles. Blood glucose levels were significantly reduced, glucose metabolism and oral glucose tolerance were significantly improved, and plasma ketone levels were normalized after combined treatment with siGCGR and leptin. These results suggested that GCGR is a promising target for type 1 and type 2 diabetes mellitus. Han *et al*. [127] utilized siRNA silencing GCGR to investigate the effect of glucose metabolism on the regulation of lipid metabolism. Knockdown of hepatic GCGR significantly reduced blood glucose levels but also caused an increase in total cholesterol. Further studies showed that siGCGR caused an increase in low-density lipoproteins (LDL) levels but did not alter very low-density lipoproteins (VLDL) and high-density lipoproteins (HDL) levels. These results demonstrated that siRNA-mediated knockdown of GCGR inhibited hepatic glucagon signaling and affected both glucose and lipid metabolism.

4.4. Other emerging targets

Glycolysis converts nonsaccharide precursors (*e.g.*, lactate, gluconeogenic amino acids) into sugars [128]. Unregulated gluconeogenesis leads to a significant increase in hepatic glucose, which is one of the critical factors in disorders associated with disturbed glucose metabolism, mainly causing hyperglycemia and type 2 diabetes. Among them, phosphoenolpyruvate carboxykinase-1 (PCK-1) is one of the critical rate-limiting enzymes of gluconeogenesis. Downregulation of PCK-1 improves glucose homeostasis *in vivo*. Singh *et al*. [129] synthesized graphene nanosheets with liver specificity loaded with siPCK-1 to treat type 2 diabetic mice. siPCK-1 blocked the hepatic gluconeogenesis process, which improved insulin sensitivity and restore glucose homeostasis. This study provided an alternative strategy for the therapeutic approach to T2DM. The transcription factor TORC2 is a significant regulator of gluconeogenesis. Therefore, down-regulation of TORC2 expression is expected to treat glucose metabolism-related diseases. Saberi *et al*. used a novel lipid nanoparticle system to deliver siTORC2 [130]. siTORC2 reduced gluconeogenesis, inhibited fasting hepatic glucose production and hyperglycemia, improved hepatic and skeletal muscle insulin sensitivity, and improved Zucker diabetic fatty (ZDF) rats with systemic hyperglycemia. These results demonstrate the importance of TORC2 in regulating glucose metabolism and illustrate a method for targeting hepatic delivery of siRNA.

Glycolysis is the first stage of glucose catabolism [131]. In tumors, the high level of glycolysis and oxidative phosphorylation is one of the hallmarks of disturbed glucose metabolism. Pyruvate kinase M2 (PKM2) is the rate-limiting enzyme of glycolysis. Huang *et al*. developed tumor-targeted bionic metal-organic framework delivery of siPKM2 [132]. siPKM2 effectively blocked glycolytic pathways in tumor model mice, which resulted in superior anticancer effects. They also performed magnetic resonance imaging based on the release of Mn^{2+} under pathological conditions, enabling *in vivo* detection of the therapeutic process. These results demonstrate the great promise of siRNAs in treating tumors by inhibiting abnormal glucose metabolism through the regulation of glycolysis.

Islet homeostasis protein (IHoP) and glucagon are overexpressed in pancreatic islets of diabetic patients. Activated IHoP induces glucagon secretion to regulate glucose homeostasis [133]. Oh *et al*. [134] inhibited IHoP expression by siIHoP in type 1 diabetic mice, which reduced glucagon secretion. siIHoP maintained normoglycemia in mice for 35 weeks after treatment and promoted pancreatic islet β-cell recovery, which inhibited the progression of diabetes.

Factor-related apoptosis (Fas) and its ligand (FasL) are type II transmembrane proteins. They interact with tumor necrosis factor receptor-1 and cause apoptotic cell death. Fas-FasL interaction plays an essential role in the pathogenesis of diabetes. It triggers the interaction between pancreatic β-cells and T-cells, which leads to apoptotic islet β-cell death [135]. Jeong *et al*. [136] used linear polyethyleneimine carriers to deliver siFas to prevent diabetes. The onset of diabetes was delayed up to 40 days in siFas-treated mice compared to untreated mice. This study demonstrated that Fas-FasL interaction was a potential target for diabetes prevention.

5. Lipid metabolism

Lipids are one of the main components of food and are essential components that form cellular biomembranes [137]. In addition, lipids can be used for energy storage and metabolism and participate in many cellular activities as signaling molecules. Therefore, regulation of lipid metabolism is crucial for maintaining physiological homeostasis [138]. Dysregulation of lipid metabolism is one of the prominent alterations in many diseases, such as obesity, cardiovascular diseases, and hyperlipidemia. In addition, dysregulated lipid metabolism is the most prominent metabolic alteration in cancer. Therefore, lipid metabolism regulation has potential in the treatment of many diseases.

Lipid metabolism is dynamically regulated at multiple levels, including digestion, absorption, storage, and mobilization of dietary fat; synthesis and catabolism of phospholipids; synthesis and conversion of cholesterol; formation and transport of plasma lipoproteins (Fig. 3) [138–142]. In the intestine, ingested lipids are emulsified and broken down, and small intestinal cells internalize them into the bloodstream. The liver plays a central role in lipid metabolism. It eliminates some chylomicrons (CM) and employs lipoproteins and glucose to promote lipogenesis, thus ensuring lipid metabolic homeostasis through mutual regulation with extrahepatic tissues [141–143].

When lipid metabolic processes are disrupted, there is an increase in serum total cholesterol (TC), triglycerides (TG), and LDL and a decrease in HDL, which leads to the development of diseases, including atherosclerosis (AS), hypercholesterolemia (HC), non-alcoholic fatty liver disease (NAFLD), and obesity [144,145]. Current therapeutic strategies aim to lower plasma lipid levels while reducing inflammation and oxidative stress at affected sites[146,147]. Nucleic acid therapy is an emerging approach that

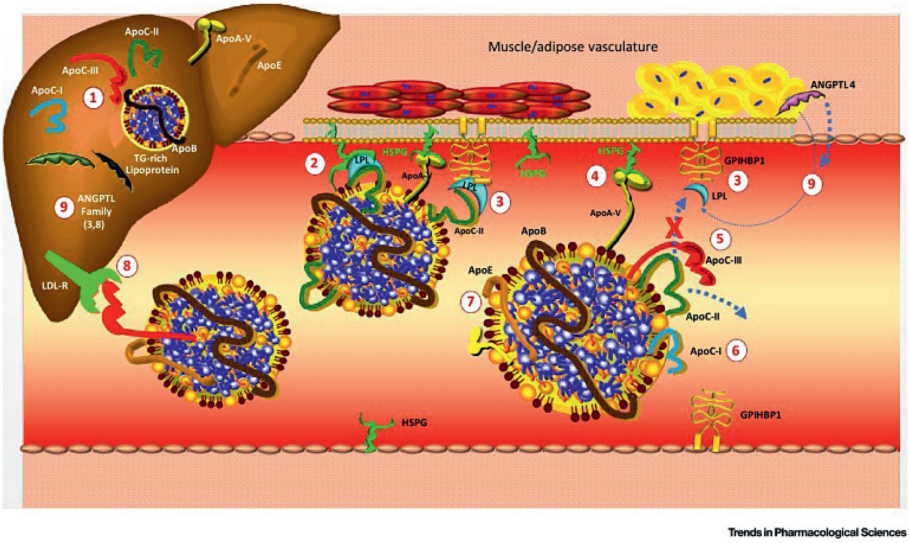

Fig. 3. Regulation of Triglyceride-Enriched Lipoprotein Lipolysis. Copied with permission [142]. Copyright 2018, Elsevier Ltd.

shows great promise, as it can regulate essential targets related to lipid metabolism, such as apolipoprotein B (ApoB), the preprotein convertase Bacillus subtilis protease 9 (PCSK9), angiopoietin-like protein 3 (ANGPTL3), and apolipoprotein C-III (ApoC-III). Numerous clinical trials and marketed drugs have showcased the effectiveness of nucleic acid drug therapy in treating diseases linked with lipid metabolism disorders.

5.1. ApoB

Dyslipidaemia is mainly caused by increased production of LDL, leading to elevated plasma levels of TG, which can lead to various diseases [148]. ApoB includes two types of ApoB, ApoB100 and ApoB48, which are mainly synthesized in the liver. ApoB48 is one of the apolipoproteins that make up CM, and ApoB100 is one of the apoproteins that make up LDL and VLDL [149]. Down-regulation of ApoB reduces the assembly and secretion of VLDL and can significantly reduce dyslipidemia. However, it is also important to note that excessive inhibition of ApoB may lead to the accumulation of TG in the liver, ultimately resulting in steatosis [149–151].

Soutschek *et al.* [152] injected chemically modified siApoB intravenously to verify the role of ApoB in regulating lipid metabolism. After administration, ApoB mRNA expression levels were reduced in the liver and jejunum, plasma ApoB levels were reduced, and total cholesterol levels were significantly decreased. They further demonstrated that siApoB can also silence human ApoB mRNA in a transgenic mouse model. These experimental results demonstrate that ApoB is one of the significant targets of nucleic acid therapy for lipid metabolism and also predict the potential of siRNA for treating such diseases. Altangerel *et al.* [153] designed 6-amino-6-deoxy-curdlan (6AC-100PEG) material to deliver siApoB. siRNA/6AC-100PEG was mainly targeted to mouse liver and lungs, inhibiting about 50% of ApoB mRNA expression in the liver and decreasing plasma levels of ApoB100 protein by about 55%, thereby attenuating plasma total cholesterol levels. Lee *et al.* [154] successfully developed a cysteamine-modified gold nanoparticle (AuCM)/siRNA/PEI/hyaluronic acid (HA) complex to deliver siApoB using HA receptor-mediated endocytosis. siApoB treatment resulted in a significant reduction in the ApoB mRNA level in liver tissues, which demonstrated the complex's excellent specific gene silencing effect and was higher than that of commercial siRNA/Lipofectamine 2000. The AuCM/siRNA/PEI/HA complex provides a successful model for treating lipid metabolism disorders and siRNA-specific targeting therapy. Conlon *et al.* [155] treated mice with anti-ApoB ASO for six weeks, which reduced the levels of VLDL and TC in mice. Endoplasmic reticulum autophagy occurred after six weeks of treatment and increased FA oxidation. Inhibition of ApoB synthesis traps lipids transferred to the endoplasmic reticulum by microsomal triglyceride transfer protein (MTP), which induces endoplasmic reticulum stress, triggering endoplasmic reticulum autophagy and lysosomal lipolysis of TGs, showing a favorable lipid inhibitory effect. Mipomersen is an FDA-approved ASO drug targeting ApoB. Reyes-Soffer *et al.* [156] found that Mipomersen significantly reduced ApoB in VLDL, intermediate-density lipoprotein (IDL), and LDL in healthy volunteers. They found that the drug reduced hepatic secretion of VLDL by increasing VLDL clearance rather than reducing VLDL to reduce plasma levels of LDL and VLDL, thereby potentially avoiding hepatic steatosis.

5.2. PCSK9

PCSK9 is a serine protease encoded by the PCSK9 gene, which is mainly produced by the liver and is also expressed in the intestine, heart, pancreas, and other organs [157]. PCSK9 can bind to and degrade the receptor for low-density lipoprotein cholesterol (LDL-C), thereby interrupting LDL-C uptake by hepatic parenchymal cells. PCSK9 also binds specifically to the LDL receptor (LDLR) cell surface to form a complex and transport it to the lysosome, leading to accelerated degradation of the LDLR, resulting in elevated LDL-C levels [158–160]. In addition, PCSK9 is not only associated with autosomal dominant hypercholesterolemia but can also effectively regulate lipid metabolism *in vivo*, thereby influencing the occurrence of many cardiovascular diseases [159,161]. Monoclonal antibodies and siRNA drugs have been marketed against this target.

Yang *et al.* [162] investigated lipid nanoparticle delivery of anti-PCSK9 ASO for efficient silencing of PCSK9 mRNA *in vitro* and *in vivo*. The screened lipid nanoparticles (306-O12B-3) delivered anti-PCSK9 ASO *in vivo* in mice, significantly reducing PCSK9 levels and, consequently, serum cholesterol levels. In addition, this nucleic acid drug carrier has an excellent biosafety profile. Therefore, the 306-O12B-3 material could be a good candidate for nucleic acid drug delivery and the delivery of anti-PCSK9 ASO for lipid reduction. Cholesterol can be conjugated to the nucleic acid drugs to deliver the drugs precisely to the liver. However, cholesterol-conjugated ASOs accumulate mainly in non-

parenchymal cells (NPCs). Wada *et al*. [163] designed several types of cholesterol-conjugated anti-PCSK9 ASOs to increase hepatic accumulation and investigated their effects on pharmacological parameters. Most cholesterol conjugations significantly increased ASO accumulation in the liver and reduced plasma lipid levels, but the cellular tropism varied. The disulfide bond-containing conjugates accumulated predominantly in NPCs, whereas the hexamethylene succinimide-containing conjugates accumulated predominantly in hepatocytes. This study may guide the design of ASO conjugates.

Inclisiran (Leqvio), a siPCSK9-based nucleic acid drug co-developed by Novartis and Alnylam Pharmaceuticals, was approved by the European Union in 2020 for the clinical treatment of hypercholesterolemia [164]. Inclisiran was based on Alnylam Pharmaceuticals' GalNAc conjugate delivery platform, and enhanced stabilization chemistry technology was developed to improve the stability of siPCSK9 and promote accumulation in the liver. Inclisiran has demonstrated excellent long-lasting efficacy with 3–6 months dosing intervals, reversing the traditional treatment paradigm for hypercholesterolemia. Gennemark *et al*. [165] developed an oral anti-PCSK9 ASO drug. Anti-PCSK9 ASO was formulated into tablets after conjugation of GalNAc, and sodium decanoate was added as an osmotic enhancer. After oral administration, the GalNAc-ASO conjugates achieved 7% hepatic bioavailability, approximately five times higher than plasma bioavailability.

5.3. ANGPTL3

ANGPTL3 belongs to a family that regulates angiogenesis and lipid metabolism and is almost exclusively secreted by the liver. The amino terminus of ANGPTL3 has a homologous coiled-coil domain that mediates formation. Its carboxyl terminus has a fibrinogen-like domain that mediates ligand activity [166,167]. ANGPTL3 reversibly binds to and inhibits lipoprotein lipase (LPL) activity [168]. LPL allows the hydrolysis of triglycerides of CM and VLDL to free FA and glycerol monoesters. In addition, ANGPTL3 inhibits endothelial lipase (EL) activity [169]. EL is located in the lumen of vascular endothelial cells and is mainly involved in the hydrolysis of plasma high-density lipoprotein cholesterol (HDL-C). Therefore, inhibition of ANGPTL3 activity can inhibit both LPL and EL, thereby lowering plasma lipid levels, and is a promising target for treating diseases caused by dyslipidemia.

Bell *et al*. [170] used anti-ANGPTL3 ASO to treat mice with moderate to high levels of hyperlipidemia and investigated the effect of ANGPTL3 inhibition on reverse cholesterol transport (RCT). RCT refers to the transport of cholesterol from peripheral tissues back to the liver and is effective in reducing plasma lipid levels. They found that different doses of ANGPTL3 ASO promoted RCT in hyperlipidaemic mice. Although HDL-C was only reduced in a moderate hyperlipidemia model, HDL plasma clearance and hepatic uptake increased. These results demonstrate that inhibition of ANGPTL3 reduces plasma lipid levels and improves HDL-mediated RCT, further ameliorating the associated disease. Wang *et al*. [171] developed a GalNAc-conjugated siANGPTL3 and evaluated its efficacy in a mouse model and a dyslipidaemic monkey model of spontaneous metabolic syndrome. The GalNAc-siANGPTL3 conjugates significantly reduced plasma total cholesterol levels, and the effect was sustained for 15 weeks, demonstrating the long-lasting efficacy of siRNA therapy. GalNAc-siANGPTL3 conjugates also attenuated the weight of dyslipidaemic monkeys. These results suggest that reducing ANGPTL3 expression prevents hyperlipidemia and atherosclerosis by downregulating ANGPT3 to promote lipolytic metabolism and clearance of TG-rich lipoproteins.

Several nucleic acid drugs targeting ANGPTL3 are currently in clinical trials or approved for marketing. ARO-ANG3, an siRNA targeting ANGPTL3, has been granted orphan drug designation by the FDA for the indication of homozygous familial hypercholesterolemia and is currently initiating a phase II clinical trial in this indication (NCT05217667) [172]. Treatment with ARO-ANG3 has been shown to reduce plasma TG by 65%–45%, LDL-C by 54%–14%, and HDL-C by 37%–12%, and can effectively treat hypercholesterolemia with no significant dose-response [173].

6. Amino acid metabolism

Amino acids are organic compounds comprising amino and carboxyl groups in their molecules, serving as the fundamental building blocks of proteins and constituting a crucial constituent of organisms [174]. Proteins comprise 22 principal amino acids. Amino acids are typically categorized into non-polar and polar types based on their side chain structure. Additionally, they may be classified as essential or non-essential according to the human body's needs [175,176]. There are 20 essential amino acids, all under the α-type classification. Except for glycine, the α-carbon atoms of amino acids are typically asymmetric, resulting in most amino acids possessing stereoisomers in the form of D-type and L-type configurations [177–178]. Compared to L-type amino acids, D-type amino acids have a limited range of functions and play a role in certain aspects of growth, development, and regulation of the endocrine and nervous systems [179].

Amino acid metabolism is a complex process. The digestion of proteins in food commences in the stomach, whereby pepsin breaks down some of the proteins into peptides despite the food's short residence time. The small intestine is the primary organ for protein digestion [180]. The pancreas secretes a range of proteases and peptidases into the small intestine. These enzymes break down incompletely digested proteins and polypeptides into amino acids or small peptide chains composed of several amino acids. These smaller molecules are then absorbed by the mucosal cells in the intestine [181]. Once absorbed, the amino acids travel through the hepatic portal vein to the liver. Some of the amino acids are catabolized or synthesized into proteins in the liver, while others are distributed from the bloodstream to various organs or tissues to perform their function [182,183]. Both anabolic and catabolic metabolism of amino acids are crucial for maintaining physiological homeostasis in the human body. Anabolic metabolism of amino acids mainly involves the synthesis of amino acids and their participation in life activities [184]. The raw materials for amino acid synthesis come from the intermediates of glycolysis, the tricarboxylic acid cycle, and other processes. Different amino acids' synthesis pathways are different and regulated by different enzymes. In addition to their role as substrates in physiological activities, amino acids predominantly form peptides and proteins that comprise various components such as hormones, enzymes, and tissues. Amino acid catabolism primarily involves transamination, deamination, decarboxylation, carbon skeleton metabolism, and the urea cycle (Fig. 4) [185,186]. These processes mainly generate substances such as α-keto acids, which serve as intermediate products of glycolysis. The body predominantly produces α-keto acids, which produce energy in oxidation reactions to generate CO_2, water, and ATP. Additionally, it synthesizes sugars and lipids.

Under normal circumstances, amino acid production and metabolism are in dynamic equilibrium. However, disturbances in amino acid metabolism can cause amino acid deficiency or abnormal aggregation in the body, resulting in disorders such as phenylketonuria, congenital hyperammonemia, abnormal tryptophan transport, and albinism [187–190]. Maintaining a balanced amino acid metabolism is crucial to prevent these disorders. Developing other metabolic conditions, including cardiovascular disorders, immune-related ailments, and cancers, can lead to amino acid metabolism abnormalities, harming the body [191–193]. Therefore, modulating the metabolism of amino acids related to diseases proves a practical approach to treating several metabolic diseases.

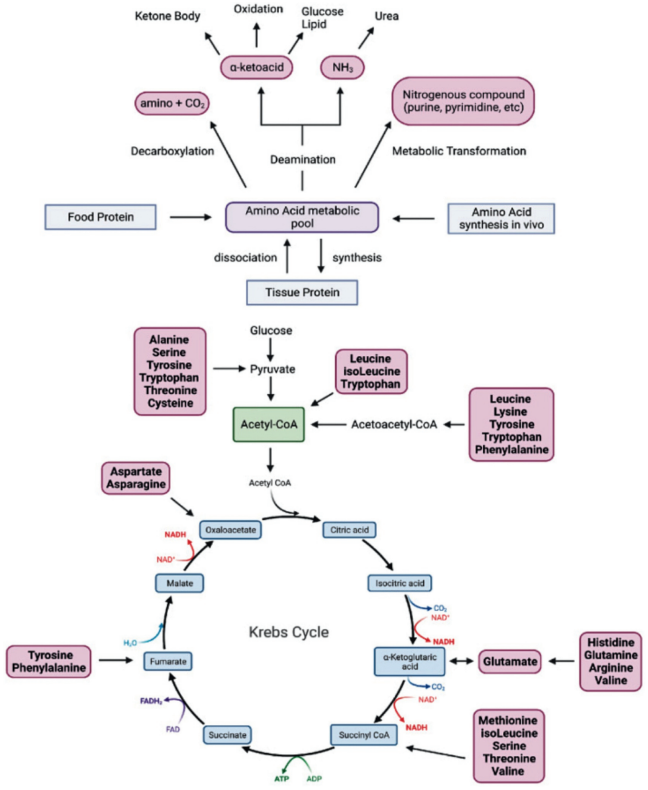

Fig. 4. Overview of amino acid metabolism. Copied with permission [185]. Copyright 2023, West China Hospital, Sichuan University.

6.1. Sodium-coupled neutral amino acid transporters (SNATs)

SNATs belong to the SLC38 family, and there are currently 11 isoforms can transport small neutral amino acids in a sodium-dependent manner and participate in the mTOR signal pathway to play important physiological roles [194,195]. Based on their functional characteristics and regulatory modes, SNATs are classified into System A and System N [196,197]. SNATs are widely distributed in the human body, mainly in brain and spinal cord neurons, and are expressed in organs such as the liver, kidneys, spleen, and lungs [198]. Therefore, SNATs are effective targets for the regulation of amino acid metabolism.

Bevilacqua *et al.* [199] used siSNAT2 to inhibit amino acid transport to explore the role of SNAT2 in cellular hyperosmotic stress. When the organism is under severe hyperglycemia, hypernatremia, and other pathologies, the extracellular osmotic pressure increases, and the cell undergoes water loss, leading to cellular crumpling and cytoskeletal disruption. Intracellular ions and biomolecules accumulate, and genetic material is destroyed due to elevated concentrations, resulting in severe consequences for human health. Researchers applied siSNAT2 to fibroblasts under hypertonic stress to inhibit SNAT2 expression. The decrease in total intracellular amino acids and the slower recovery of cell volume demonstrated the role of SNAT2 in amino acid transport. They predicted that SNAT2 is a crucial factor in regulating cellular osmotic pressure. Thus, SNAT2 could be a target for regulating amino acid metabolism. Dikalova *et al.* used SNAT1 siRNA to explore its effects on chronic hypoxia-induced pulmonary hypertension [200]. Impaired nitric oxide (NO) signaling may lead to chronic hypoxia, which induces the development of pulmonary hypertension. L-Citrulline has been shown to modulate NO production. siSNAT1 reduces L-citrulline uptake and inhibits NO production. This study provides methodological implications for exploring the mechanism of action of amino acid-regulated diseases. Modulation of L-citrulline transport may be a viable therapeutic approach to modulate NO production in various cardiovascular diseases.

6.2. Mammalian target of rapamycin (mTOR)

mTOR is a critical protein in the mTOR signal pathway, which mainly regulates physiological activities such as cell proliferation, metabolism, and protein synthesis [201]. mTOR is a highly conserved serine/threonine kinase that forms two complexes, mTORC1 and mTORC2. When activated, the mTOR signal pathway upregulates the anabolic processes, such as translation of mRNAs, amino acid biosynthesis, and simultaneously inhibits catabolic processes, such as cellular autophagy [202,203]. Many studies have demonstrated that amino acid metabolism is positively correlated with the mTOR signal pathway. Modulating the mTOR signal pathway and its upstream and downstream signaling molecules is a promising strategy for treating disease-induced abnormalities in amino acid metabolism.

Insulin-like growth factor 1 (IGF-1) is one of the critical factors for fetal growth, and phosphorylation of Insulin-like growth factor-binding protein 1 (IGFBP-1) reduces the bioavailability of IGF-1. Leucine plays an essential role in this process. Leucine reduction strongly induces hyperphosphorylation of IGFBP-1, leading to fetal growth restriction (FGR), further reducing the bioavailability of amino acids and IGF-1 and promoting disease progression [204]. Malkani *et al.* [205] used either siRNAs that inhibit mTOR (siRICTOR) or activate mTOR siRNA (siDEPTOR) for treatment and found that activation of mTOR had the effect of attenuating IGFBP-1 phosphorylation and ameliorating FGR due to reduced amino acid utilization. Liu *et al.* [206] used siRNA to knock down solute carrier family 3 member 2 (SLC3A2) and mTOR1 to study the effects of endoplasmic reticulum stress induced by unfolded protein response (UPR) on rat cardiomyocytes and neuronal cells. They found that the knockdown of SLC3A2 and mTOR1 attenuated endoplasmic reticulum stress, reduced amino acid transport, and enhanced apoptosis. They also demonstrated that SLC3A2 is a complex multifunctional signaling protein that regulates the upstream signaling molecules of mTOR1, providing a potential target for treating endoplasmic reticulum stress-related diseases. The amino acid transporter proteins alanine–serine-cysteine transporter 2 (ASCT2) and L-type amino acid transporter 1 (LAT1) are also known to activate the mTOR signal pathway [207,208]. They enhance the metabolism of amino acids and other substances, which is more pronounced in cancers with hyper-enhanced metabolism. Bothwell *et al.* [209] knocked down the *ASCT2* and *LAT1* genes by short hairpin RNA (shRNA) and CRISPR-Cas9, respectively, to explore the effect of reduced amino acid transporter on the proliferation of tumor cells. They found that both methods significantly reduced glutamine or leucine transport but failed to inhibit the mTOR signal pathway significantly and that the effects of the mTOR signal pathway on amino acid metabolism are regulated by various factors, which could guide the selection of therapeutic targets for amino acid metabolism-related diseases.

6.3. Phenylalanine hydroxylase (PAH)

Phenylketonuria (PKU) is caused by a genetic mutation resulting in a defect in PAH, also known as PAH deficiency, mainly expressed in the liver. PAH converts phenylalanine (Phe) to tyrosine (Tyr) with the participation of tetrahydrobiopterin (BH4), which synthesizes substances involved in normal physiological activities. This process synthesizes substances such as thyroxine and epinephrine, which are involved in normal physiological activities. PAH defects cause an abnormal accumulation of Phe and Tyr deficiency in the

body, resulting in symptoms such as growth retardation, mental retardation, and generalized skin hypopigmentation [210,211]. Harding *et al.* [212] used recombinant adeno-associated viral vectors (rAAV2/8) and constructed a liver-specific promoter for delivery of PAH cDNA to increase regular PAH expression. Mice injected with rAAV2/8 showed a significant decrease in serum Phe levels, an increase in Tyr levels, and a reduction in symptoms in PKU mice. This study demonstrated that AAV2/8-mediated targeted liver gene therapy is a promising new therapeutic approach for PKU and related inborn errors of metabolism disorders. Perez-Garcia *et al.* [213] used LNP as a nucleic acid drug carrier loaded with hPAH mRNA to treat mice with PKU. hPAH mRNA was delivered by LNP to the liver to replenish PAH. This system reduced Phe levels and delayed PKU progression without adverse clinical symptoms. Compared with viral vector therapy, mRNA drugs act in the cytoplasm and are biologically safe. Therefore, mRNA replacement therapy is expected to be a new direction for PKU treatment.

7. Conclusion

Abnormal metabolism is closely associated with many diseases, including metabolic disorders, tumors, and rare diseases. Therefore, restoring metabolic homeostasis is a potential strategy for treating many diseases. Metabolism is regulated by multiple signal pathways and their essential proteins, which implies that targeted therapies are a rational strategy to alleviate metabolic disorders. After years of painstaking research accumulation by scientists, nucleic acid drugs have met their targeted therapeutic potential and irreplaceable advantages in the clinical treatment of various diseases. However, the research on nucleic acid drugs in metabolism-related diseases must be paid more attention.

The research of nucleic acid drugs in metabolism-related diseases is not only an in-depth exploration of the disease mechanism, but also an active pursuit of innovative therapeutic methods. The core of its research focuses on two major aspects, revealing the infinite possibilities of nucleic acid drugs in the treatment of metabolic diseases.

First, pathologists and molecular biologists work closely together to fully consider the impact of metabolic disorders in the study of disease pathology, and strive to find more potential preclinical research targets. Through in-depth research on the relationship between metabolic disorders and the occurrence and development of diseases, they not only provided theoretical support for the development of nucleic acid drugs, but also laid a solid foundation for subsequent clinical trials.

Pharmacologists, chemists, and materials scientists also play a key role in the development of nucleic acid drugs. By continuously optimizing sequence design, synthesis technology and delivery platform of nucleic acid drugs, they strive to improve the stability, specificity and bioavailability of drugs to ensure the optimal efficacy of drugs *in vivo*. It is worth mentioning that with the rapid development of computer simulation technology, its application in nucleic acid drug research and development is increasingly extensive. Using computer simulation technology, scientists can predict the interaction between drugs and targets at the early stage of drug development and evaluate the efficacy and safety of drugs, thus greatly shortening the research and development cycle and reducing research and development costs.

In terms of drug delivery technology, although the *in vivo* delivery technology of nucleic acid drugs targeting the liver has been relatively mature, the extrahepatic drug delivery technology, especially the intracerebral drug delivery, still faces many challenges. Due to the strict limitations of the blood-brain barrier, many nucleic acid drugs are difficult to penetrate effectively and act on the brain. Therefore, how to break through the blood-brain barrier and realize effective extrahepatic delivery of nucleic acid drugs has become a hot and difficult topic in current research. In order to fully realize the potential of nucleic acid drugs in the treatment of metabolic related diseases, we need scientists in different fields to work closely together to build a drug research and development platform of "disease pathology-metabolic target-nucleic acid drug design-delivery technology" (Fig. 5).

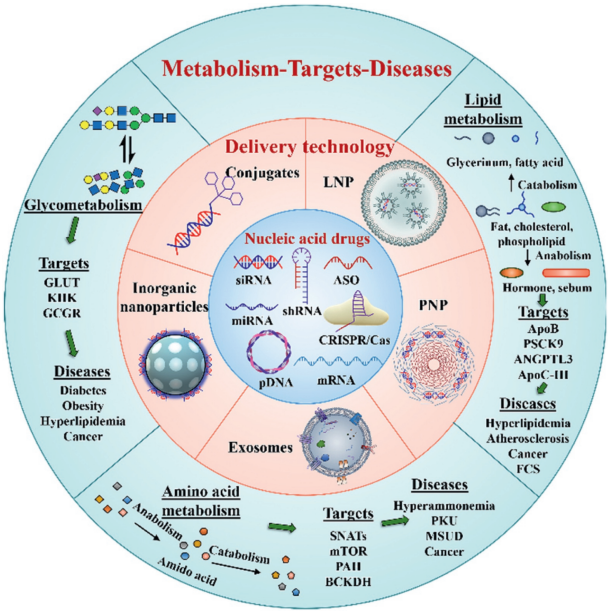

Fig. 5. Drug research and development platform of "disease pathology-metabolic targets-nucleic acid drug design-delivery technologies".

In summary, nucleic acid drugs have great potential in the targeted therapy of metabolism-related diseases. Through interdisciplinary cooperation, the application of computer simulation technology and the exploration of personalized treatment strategies, we are confident that nucleic acid drugs will become a new tool for the treatment of metabolism-related diseases, and make greater contributions to human health.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Jing Guo: Writing – original draft, Investigation. **Zhi-Guo Lu:** Writing – review & editing, Writing – original draft, Supervision. **Rui-Chen Zhao:** Software, Conceptualization. **Bao-Ku Li:** Supervision. **Xin Zhang:** Visualization, Funding acquisition, Formal analysis.

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