siRNA, with a molecular weight of about 13 kDa, recruits the RNA-induced silencing complex (RISC) to mRNA through base pairing, thereby inhibiting protein translation. The mRNA is targeted for cleavage through the catalysis of the RISC protein Ago2, a member of the Argonaute family. In addition, other Ago proteins (Ago1, Ago3, and Ago4) catalyze endonuclease-mediated degradation of non-specific mRNA by locating the bound mRNA in processing bodies (P-bodies).

Limitations of siRNA as a proprietary drug

- Naked siRNA is easily degraded by nucleases in blood, and its relatively high molecular weight, negative charge, and hydrophilicity make it difficult to penetrate cell membranes.
- siRNA tends to accumulate in the kidney and be excreted in urine or is captured by the reticuloendothelial system (RES), thus cannot effectively bind to target sequences.
- Naked siRNA can cause phagocytosis in the body's immune system in a length-dependent manner, and siRNA can also activate Toll-like receptors to trigger immune responses.
- Whether siRNA can successfully escape from endosomes affects its delivery efficiency in vivo.
- siRNA off-target effect.

The stability modification of siRNA can improve its delivery efficiency in vivo, thus reducing the required siRNA dosage and immunogenicity. In addition, bioinformatics tools are useful in screening and can effectively avoid the off-target effect of siRNA.

Chemical Modification of siRNA

Ribose modification

Modification at the 2' position of the glycosyl group. The most widely used ribose modifications include 2'-O-methylation (2'-OME), 2'-Fluro modification (2'-F), and 2'-O-methoxyethyl (2'-MOE), which can improve the nuclease resistance and thermal stability of the structure. At the same time, it can also promote the binding of siRNA with complementary mRNA and enhance the targeting efficiency of siRNA.

Phosphorothioate (PS) modification

The phosphodiester bond connecting the phosphate backbone of RNA is the chemical bond of nuclease action. PS modification of the vulnerable phosphorus atom can improve the nuclease resistance, pharmacokinetic properties, and serum stability of the modified nuclease, but high PS modification can lead to serious toxicities.

Base modification

Base modification can enhance the interaction between bases. The most commonly used base modification refers to introducing bromine or iodine at the 5' position of uracils, such as 5-bromouracil and 5-iodouracil.

N- & C-terminal modification

Terminal modifications include polyethylene glycol (PEG) modifications and cholesterol modifications, which are usually introduced into the 5' or 3' end of the sense sequence. PEG modification can increase particle size, shield negative charge, and reduce cytotoxicity, thus avoiding nuclease degradation and renal clearance of siRNA in vivo as well as reducing toxicity in vivo. Cholesterol-modified siRNA can prolong circulation time in vivo, enhance membrane permeability of siRNA, and promote cellular uptake.

siRNA Delivery

siRNA itself does not possess targeting ability. Meanwhile, unmodified naked siRNA presents a

low transfection efficiency in vivo and cannot achieve a high silencing efficiency. Hence, efficient delivery systems help siRNA better reach its target with improved bioavailability in vivo.

Liposomes

Liposomes are a kind of spherical carrier composed of a hydrophilic nucleus and hydrophobic phospholipid bilayer. It has good biofilm property and containment and can promote cell uptake and endosomal escape while avoiding nuclease degradation. The positive charge carried by cationic liposomes can neutralize the negative charge carried by siRNA to form a tightly structured complex that greatly extends the cycle time and facilitates the delivery of siRNA.

Peptide-siRNA Conjugates

Cell-penetrating peptide (CPP) is a short cationic peptide (<30 amino acids) consisting of arginine and lysine residues, which can deliver a variety of bioactive substances of different sizes and properties to cells through cell membranes. CPP and siRNA form non-covalent compounds through covalent bonding or charge interaction, which can enhance the stability and biological activity of siRNA, promote intracellular releases, and reduce immunogenicity.

Dynamic Polyconjugates (DPC)

DPC technology refers to aggregating the targeting ligands, PEG, siRNA, hydrophobic lipid, and many other ingredients required in the siRNA delivery system to the polymeric backbone containing the conjugation sites. Since the conjugation position or degree of the different components on the backbone cannot be precisely controlled, such conjugates are called DPC. The advantages of this technology lie in its controllable and changeable structure, which can realize the selective delivery of different targets. At the same time, a variety of design functions can be integrated and flexibly adjusted via DPC, which is expected to play a unique role in the delivery of siRNA drugs.

GalNAc-siRNA

GalNAc-siRNA is a stable conjugate formed by the covalent conjugation of GalNAc to the 3' end of the sense sequence of siRNA. GalNAc can bind to the highly expressed sialic acid receptors in the liver and guide the siRNA bound to itself into liver cells, which has good application potential in treating liver-targeted diseases. GalNAc has become a prominent delivery carrier and has been applied to a variety of drugs.

Clinical indications for siRNA drugs

siRNA drugs are most widely used in oncology and rare diseases. Currently, several siRNA drugs for rare diseases have been approved for marketing, such as:

Patisiran (Hereditary trans thyroxine amyloidosis, ATTR)

Exondys51 (Duchenne muscular dystrophy, DMD)

Givosiran (Acute intermittent porphyria, AIP)

Inclisiran (Hyperlipidemia)

Tivanisiran (Dry eye syndrome, DES)

QPI-1007 (Optic atrophy)

SYL040012 (Glaucoma)

QPI-1002 (Severe kidney disease)

Conclusion

With the continuous advancement of science and technology, it is believed that the development of the siRNA drug delivery system will step to a new stage in the near future, realizing targeted delivery for a variety of diseases and better improving human health.