Sixteen Gene Therapy Drugs

Subjects: Genetics & Heredity

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Gene therapy has become a rapidly growing field with significant advancements. This innovative therapeutic approach is revolutionizing the treatment of various diseases. Gene therapy drugs have revolutionized the field of medicine by providing a targeted approach to treating genetic disorders.

Keywords: human gene therapy ; gene therapy drugs ; viral vectors ; non-viral vectors

1. Small Interfering RNA (siRNA)

siRNA is a synthetic molecule used to knock down the expression of any gene with a complementary sequence. The molecule works by targeting specific mRNA and cleaving it, preventing it from being translated into protein. Additionally, siRNA can also be used to regulate protein-coding genes and transposons, as well as functioning as an antiviral defense mechanism.

The size of siRNA ranges from 20 to 25 base pairs. One of the key advantages of siRNA is its high specificity due to its 100% complementarity to the target mRNA. This makes it an attractive drug candidate for diseases caused by specific gene mutations. Additionally, siRNA has been shown to be effective in delivering drugs to the brain, a feat that is notoriously difficult to achieve.

However, siRNA therapy also has some limitations. One major concern is off-target effects, which means that the siRNA may unintentionally target genes with similar sequences to the intended target. Another potential issue is innate immunity, which can cause an immune response and limit the effectiveness of the therapy [1][2][3][4][5][6][7].

2. MicroRNAs (miRNAs)

miRNAs are short non-coding RNA molecules that regulate gene expression at the post-transcriptional level. They play a significant role in a wide range of cellular processes, including differentiation, apoptosis, and development. In plants, miRNAs and their target mRNA are almost perfectly complementary, making them highly effective. They are involved in developmental timing, tissue growth, and left–right asymmetry in the nervous system. In animals, miRNAs comprise only approximately 1% of all genes, but they play an essential role in regulation, including mRNA degradation, translational repression, and the regulation of protein-coding genes.

MicroRNAs (miRNAs) offer a key advantage in gene therapy due to their small size and manipulability. Moreover, around 12 miRNAs have been identified for suppressing endogenous CFTR mRNA expression in the Caco-2 cell line. CFTR, responsible for the monogenic autosomal recessive cystic fibrosis (CF), impacts 1 in 3500 global live births. Maria V. Esposito et al. [8] examined 706 CF carriers, revealing undiagnosed CFTR-RD among a subset. Genetic testing scanning analysis aids in CFTR-RD identification, offering potential for tailored follow-up and therapies to enhance outcomes.

However, functional duplexes in animals can be more variable in structure than in plants, with only short complementary sequence stretches that may contain gaps and mismatches. Specific rules for functional miRNA–target pairing that capture all known functional targets have not been developed to date [3][8][9][10][11].

3. PIWI-Interacting RNAs (piRNAs)

piRNAs are small non-coding RNA molecules that interact with PIWI proteins to repress transposable elements in the genome.

piRNAs are known to have diverse functions such as transcriptional or post-transcriptional repression of transposons and multigenerational epigenetic phenomena in worms. In addition to transposon silencing, pre-pachytene piRNAs also have

roles in the formation of the nuage, a perinuclear structure in germ cells.

These RNAs are larger than other small RNAs, typically ranging from 26 to 32 nucleotides in length.

While the exact mechanisms of piRNA biogenesis remain unclear, current models suggest that they are processed from long, single-stranded RNA precursors in a Dicer-independent manner. Studies on piRNAs are still ongoing to understand the full range of their functions and mechanisms of action [7][12].

4. Short Hairpin RNA (shRNA)

shRNA is an artificial RNA molecule used for gene silencing via RNA interference. This type of drug contains a hairpin turn that tightly binds to its target gene, leading to suppression of its expression.

shRNAs range in size from 19 to 29 base pairs and have the advantage of being relatively resistant to degradation and turnover, providing long-lasting gene silencing effects.

However, to use shRNA, an expression vector is required, which may cause side effects when used as a medicine.

Despite these limitations, shRNA is considered an effective tool for gene therapy due to its specific targeting ability and long-term effects [13][14][15][16][17].

5. Antisense Oligonucleotides (ASOs)

ASOs are a type of drug that have gained significant attention in recent years due to their potential in gene therapy. ASOs are single strands of DNA or RNA that are complementary to a specific sequence of mRNA. They work by binding to the targeted RNA and blocking the translation of certain proteins, thereby modulating gene expression. ASOs are relatively small in size, ranging from 18 to 30 base pairs, which enables them to easily penetrate cell membranes and target both nuclear- and cytoplasmic-located long non-coding RNAs (lncRNAs).

Despite their potential therapeutic benefits, ASOs have several limitations that need to be addressed. One major concern is off-target effects, where ASOs bind to unintended RNA sequences and cause unwanted biological effects. Additionally, ASOs may have insufficient biological activity, limiting their efficacy in gene therapy.

ASOs have shown promising results in clinical trials for treating genetic disorders, such as spinal muscular atrophy and Huntington’s disease, and several ASOs have been approved by the FDA [18][19][20][21][22][23][24].

6. Oligodeoxynucleotides (ODNs)

ODNs are synthetic DNA molecules that have shown potential as a gene therapy tool. ODNs work through two main mechanisms: the antisense strategy and the antigene strategy (also known as the decoy strategy). In the antisense strategy, ODNs bind to the targeted mRNA and block protein synthesis, while the antigene strategy involves the use of ODNs to bind to specific transcription factors and inhibit their activity, thus preventing the expression of downstream genes.

One of the benefits of using ODNs is the simplicity of their synthesis and manipulation, as well as the tissue specificity of their target transcription factors. This specificity enables precise targeting of specific genes or cell types, reducing the risk of off-target effects.

However, ODNs also have several limitations that need to be addressed. One major concern is their high rate of degradation by endocytosis or nucleases, which limits their stability and effectiveness. Additionally, their short lifetime may reduce the duration of therapeutic effects.

Despite these limitations, ODNs have shown potential in preclinical and clinical trials for treating various diseases, such as cancer and autoimmune disorders [25].

7. Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR)/CRISPR-Associated Protein 9 (Cas9)

In recent years, CRISPR/Cas9 has been making waves in the field of gene therapy. This protein, formerly known as Cas5, Csn1, or Csx12, plays a critical role in the defense of certain bacteria against DNA viruses and plasmids. Its primary

function is to cut DNA, which allows for the alteration of a cell's genome.

One of the biggest advantages of CRISPR/Cas9 is its ease of design. It can be delivered via plasmid or viral vectors, which makes it accessible to many researchers. However, there are some downsides to consider. For example, off-target editing is common without an additional homologous sequence, which can be a challenge.

Another drawback is the requirement for a PAM or Protospacer Adjacent Motif, a short sequence downstream of the target DNA sequence. While this motif is necessary for CRISPR/Cas9 to work, it can also limit the range of targets available for editing [26][27][28][29][30].

8. Plasmid DNA (pDNA)

One of the most promising vectors for gene therapy is pDNA. These small, extrachromosomal DNA molecules replicate independently and are physically separated from chromosomal DNA within a cell. pDNA gene therapy has been shown to be particularly effective in the treatment of cardiovascular diseases because it allows for targeted transfer to cardiac or skeletal muscle.

One of the major advantages of using pDNA as a vector is its versatility in size. This allows for a wide range of genes to be delivered using this method. Additionally, plasmids can be engineered to include a variety of promoters and enhancers to increase gene expression in the target tissue.

However, like all gene therapy vectors, pDNA has its drawbacks. One of the biggest concerns is its potential for immunogenicity, which can cause an immune response and limit the effectiveness of the therapy. Therefore, careful consideration must be given to the design and delivery of pDNA to minimize this risk [31][32][33][34][35][36][37][38][39][40].

9. Messenger Ribonucleic Acid (mRNA)

mRNA is a promising drug for gene therapy. It is a single-stranded molecule of RNA that corresponds to the genetic sequence of a gene and is read by a ribosome in the process of protein synthesis. By delivering corrected mRNA into cells, they can receive the right blueprint for creating healthy proteins, which can help treat a variety of genetic disorders.

One of the major advantages of mRNA as a drug is its ease of manipulation. It can be rapidly produced and modified to fit specific needs. Additionally, it offers transient expression, meaning protein production is not permanent and can be turned off if necessary. Moreover, mRNA is adaptive and can be converted without mutagenesis.

However, there are some downsides to using mRNA for gene therapy. One of the major drawbacks is its immunogenicity, meaning the body's immune system may recognize it as foreign and attack it, leading to negative side effects. Additionally, it can be challenging to control the concentration of reporter mRNA, which can lead to unintended effects [41][42][43].

10. Meganucleases

Meganucleases are a promising tool for targeted gene editing, characterized by a large recognition site and high specificity. They can be thought of as molecular DNA scissors, capable of replacing, eliminating, or modifying specific sequences in a highly precise manner.

The small size of meganucleases allows for their use with many viral vectors, and they can tolerate some mismatches, resulting in low off-target editing.

However, the design and reengineering of meganucleases for new specificities can be extremely challenging.

Despite these obstacles, recent research has demonstrated the potential of meganucleases to induce homologous recombination in yeast and mammalian cells, highlighting their potential as a tool for precise gene editing in various applications [44][45][46][47][48].

11. Zinc Finger Nucleases (ZFNs)

ZFNs are artificial endonucleases that have been developed for targeted gene editing. They are composed of a designed ZFP and the cleavage domain of the FokI restriction enzyme. The ZFP is engineered to recognize and bind to specific DNA sequences, while the FokI domain cleaves the DNA at the target site.

One advantage of ZFNs is that they can tolerate some mismatches, which reduces off-target editing. However, G-rich regions of DNA can be challenging for ZFNs, and their design can be difficult. Multiplexing, or targeting multiple genes at once, is also a challenge with ZFNs [49][50].

12. Transcription Activator-like Effector Nucleases (TALENs)

TALENs are a promising gene therapy tool that can be used to cut specific sequences of DNA. TALENs are restriction enzymes that have been engineered to bind and cut DNA in a highly specific manner.

Their size ranges from 32 to 40 base pairs, and they are capable of tolerating some mismatches, resulting in low off-target editing. Additionally, TALENs have moderate design requirements.

However, TALENs do have some limitations. Specifically, they require a 5' T for each TALEN and are challenging to multiplex. Furthermore, their large size makes it difficult to utilize viral vectors, and repetitive sequences can lead to unwanted recombination [51][52][53][54][55].

13. DNA Aptamers

DNA aptamers are short sequences of artificial DNA that can bind specific target molecules with extremely high affinity based on their structural conformations. These aptamers are becoming increasingly popular in various biosensing and therapeutic applications due to their stability and the ease of their generation and synthesis. They are also known for having almost no immunogenicity and for their efficient penetration, lower batch variation, easy modification, cost-effectiveness, and short production times.

DNA aptamers have been compared with other biorecognition elements, such as antibody scFv and antibody Fab' fragments. They have also been used to select molecules that bind to specific targets, such as gluten, cocaine, and malachite green. In addition, their secondary structural requirements have been investigated through thermodynamic and mutation studies. The 2.8 A crystal structure of the malachite green aptamer and the structural investigations of RNA and DNA aptamers in solution have been described in detail in previous studies [56][57][58][59][60][61][62][63][64][65].

14. Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL/APO2L)

TRAIL is a member of the tumor necrosis factor family. Its main function is to induce apoptosis, or programmed cell death, in cancerous cells by binding to the death receptor 4 (DR4) or DR5. Unlike other treatments, TRAIL has the advantage of selectively targeting tumor cells while avoiding side effects in normal tissues. This makes TRAIL a promising therapy for cancer treatment.

One of the most significant benefits of TRAIL is its ability to efficiently kill tumor cells. By activating the apoptotic pathway in cancer cells, TRAIL induces their death, halting their growth and spread. However, some tumor cells are resistant to TRAIL, which limits its effectiveness. The existence of TRAIL-resistant tumor cells remains a challenge that needs to be overcome to maximize the benefits of TRAIL therapy.

Despite the limitations, TRAIL is a promising therapy for cancer treatment due to its selective toxicity towards cancer cells. Researchers are working to overcome the problem of TRAIL resistance by combining it with other treatments such as chemotherapy or radiation therapy. These combinations have shown promising results in clinical trials and may improve the effectiveness of TRAIL therapy in the future.

In conclusion, TRAIL has emerged as a promising therapy for cancer treatment due to its ability to selectively target tumor cells. While the existence of TRAIL-resistant tumor cells limits its effectiveness, ongoing research aims to overcome this challenge and improve the therapeutic potential of TRAIL [66][67].

15. Phosphorodiamidate Morpholino Oligomers (PMOs)

PMOs are short, single-stranded DNA analogs built upon a backbone of morpholine rings connected by phosphorodiamidate linkages. These uncharged nucleic acid analogs are designed to bind to complementary sequences of target mRNA through Watson–Crick base pairing, which results in the blocking of protein translation through steric blockade.

PMO oligomers range in size from 6 to 22 base pairs and have been shown to be resistant to a variety of enzymes present in biologic fluids, making them ideal for in vivo applications.

The resistance of PMO to nucleases and other enzymes is a major advantage for their use in gene therapy. By preventing degradation, they can efficiently target and inhibit translation of specific mRNA molecules. PMOs have been used in clinical trials to treat various diseases such as Duchenne muscular dystrophy and spinal muscular atrophy, demonstrating their therapeutic potential [68][69].

16. Naked DNA

Naked DNA, which is simply DNA without any associated proteins, has been widely investigated as a gene transfer tool for several tissues including skin, thymus, cardiac muscle, skeletal muscle, and liver cells. This method involves direct injection of DNA into the target tissue, allowing for the transfer of a gene with a size range of 2–19 kb.

Naked DNA-based gene transfer is a safe and straightforward approach, but its application is limited to certain areas such as DNA vaccination. In skeletal muscle, long-term expression has been observed after injection for more than 19 months.

Despite these advantages, the efficiency of naked DNA for gene delivery is low, with less than 1% of total myofibers showing transgenic expression following a single injection. However, repeated injections can improve the overall transfection efficiency, making naked DNA a viable option for certain gene therapy applications [70].

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