Review / Mini-Review

Gene Silencing Method Based on DNA

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Abstract

DNA-based approaches can now be utilized as low-risk methods to change gene expression. It appears that this approach has the ability to partially replace RNA-based approaches for altering gene expression, which in the majority of cases leads to immunological responses in patients. When utilized as a technique to silence target gene expression, DNA interference (DNAi) is a single-stranded DNA created to complement the upstream region of a gene. This DNAi molecule is stabilized using a variety of chemical changes, including phosphorothioates, methylphosphonate setC, etc. Several studies of the efficient application of DNA-based methods both in eukaryotic cell lines and the therapy of various disorders, such as Duchenne muscular dystrophy, cancer, etc., have been mentioned. Understanding the DNAi process, its transfer carriers, stabilization techniques, and their limitations is crucial for advancing these applications and predicting the future of DNAi both in basic science and the treatment of disorders brought on by abnormal gene expression. The main purpose of this review is introducing benefits of using DNAi in gene silencing. this review has discussed about different applications of DNAi in drug discovery and treatment, criteria of designing DNAi, possible modifications, introducing different types of carriers and limitations of DNAi administration.

Keywords: DNAi, gene silencing, phosphorothioates, gene therapy

Introduction

Gene silencing which is known as a potential method to control some diseases, such as cancer and heteropathy, has no favorable treatment for any of them. The usage of antisense oligonucleotide techniques to treat various genetic disorders caused by mutated genes or a lack of expression of natural genes is widely used in medical, pharmaceutical, and animal science research as well as treatments. One of the powerful approaches for gene silencing that has been using since 1998, is RNA interference (RNAi) (Fire et al., 1998). RNAi depends on the formulation of double-stranded RNA (dsRNA) designed to be complementary to the transcript of the target gene. This mechanism is called the pre-translation knockout gene, and / operates through two main steps. In the first step, the enzyme Dicer cleaves long dsRNA molecules into short double-strand fragments of 21–23 bp nucleotides. Then, in the second step, siRNAs, which guide the sequence-specific cleavage of the RNA transcript of the target gene, are loaded into the effector RNA-induced silencing complex (RISC) and inhibit target genes (McManus and Sharp, 2002). Because of some limitations of the RNAi method, such as difficulty of siRNA in clinical therapy, low resistance to endogenous RNase, and the high price of this technique, some gene knockout techniques based on DNA have been proposed (Riasi et al., 2022). The DNA interference (DNAi) technique is / a novel approach that is developed to inhabit or alter gene function. Some beneficial features of these molecules are: Likely more stable in the cells in comparison with RNAi; Overall costs are lower than other oligonucleotides; would be administered in naked form. However, there is some limitations such as lack of tissue-specific action and temporal effectiveness that are considered among all antisense oligonucleotides.

Few species, such as plants, ciliates, and archaea, were the first organisms to interfere with the DNAi process (Palauqui and Balzergue, 1999., Rutherford et al., 2004. Voinnet et al., 1998). Also, the design of DNA fragments without a promotor region has been shown to induce sequence-specific gene silencing in tobacco and Adiantum (Omotezako et al., 2015. Voinnet et al., 1998). Since the high efficiency of this technique became clear, it has been used as an effective method of treatment (Tolcher et al., 2014). The importance of review articles in medicine and science is to gradually improve some intelligent

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systems for drug delivery and gene expression regulation. As far as we know, there is a small number of studies that have evaluated and reviewed the application of DNAi in gene silencing. This article investigates some gene silencing methods based on DNA, with particular references to the major interest in drug delivery. It also considers the limitations of such a technique, the proper carrier to transfer DNA, and tries to indicate some examples of how successful such a method is. We also reviewed the articles that used chemical modification to stabilize the DNA molecule and increase efficiency in inhibiting gene expression.

Application of DNAi for drug discovery and treatment

Antisense oligonucleotide (AONs) therapeutic approaches as a potential therapy have been used over the past decades for treating rare genetic diseases, cancer, and viral infections (Touznik et al., 2014). AONs are short synthetic single-stranded DNA or RNA of 15–35 nucleotides in length that selectively bind to specific regions in the pre-mRNA through Watson-Crick hybridization (Lee et al., 2013). Antisense therapies aim to modulate splicing through the use of AONs. There are various mechanisms by which AONs can target pre-mRNA, depending on their design and chemical modifications. For example, targeted RNA can be cleaved through RNAse H-mediated mechanisms or interference RNA. Nowadays, intelligent systems of drug delivery, with the aim of improving therapeutic indicators, increasing drug concentration in tumor cells, and minimizing drug side effects through reducing exposure to natural tissues are being promoted. Therefore, considering some different aspects of such a gene knockout method based on DNA, several new perspectives will be created to use such a method more effectively and safely in treatment (Purwaningsih and Schubert, 2010). Cancer is one of the chronic, non-communicable diseases that threaten the health of people and their minds and can affect the economy and social situation. B-cell lymphoma 2 belongs to the family of antiapoptotic proteins that control cell death and is encoded in humans by the BCL2 gene (Tsujimoto et al., 1984). BCL-2 is located on the outer membrane of mitochondria and plays a key role in the action of pro-apoptotic proteins and cellular survival. Overexpression of the BCL-2 gene leads to hematopoietic and lymphatic cancers (Davids and Letai, 2012., Yonetani et al., 2001). BCL-2 silencing is a desirable goal for therapeutic improvement. Accordingly, one study has been conducted by Rodrigueza et al. in 2014 that evaluated inhibition of the BCL2 gene by DNAi *in vitro* as well as *in vivo*. The oligonucleotide sequence designed to target 5′ region upstream of the BCL2 gene, was tested for antiproliferative activity against breast and melanoma cell lines. The preclinical results of that study indicated that PNT2258, a specific DNAi that can suppress the BCL2 gene, with a dose of up to 150 mg/m2 had an effective activity to suppress solid tumors. Furthermore, in vitro studies demonstrated some progress toward increasing the effectiveness of DNAi-based therapeutics. A study on down-regulation of BCL-2 expression in DLCL2 cell lines carried out by Saravani et al. in 2018, due to the high expression of the BCL2 gene in non-Hodgkin lymphoma cancer, this study evaluated the effect of antisense oligonucleotide-based drugs on inhibiting the expression of this gene. thus, for manufacturing DNAi containing pegylated nanoliposomes, three formulations with 5, 10, and 15% PEG with defined ratios of phospholipid and cholesterol were prepared. The results showed that using PEG10 nanoliposomes could efficiently increase DNAi preservation and release (Saravani et al., 2018). In this regard, Tolcher et al. have published an article with the aim of BCL2 suppression in patients with advanced solid tumors. In this study, the 5-untranscribed regulatory region of the BCL2 gene was selected to inhibit the gene by using the DNAi method. The sequence of 24 nucleotides single-strand DNA encapsulated in the aqueous core within the liposomal nanoparticle, named PNT2258, was given to 22 patients intravenously through sixty 21-day cycles at varying doses of 1 to 150 mg/m2. It was found that a dose of 150 mg/m2 of PNT2258 had no clinically unmanageable grade 3 or 4 toxicities, and higher doses could be suggested in clinical programs. Though no biopsy was performed in patients to determine the expression of the BCL2 gene, fortunate results were provided for further research (Table 1) (Tolcher et al., 2014).

Application of Exon-skipping for drug discovery and treatment

Another method is antisense-mediated exon skipping. Exon skipping employs AONs to modulate splicing by hiding specific splicing sites within exons or introns from the splicing machinery. Therefore, this will exclude the target exon from mRNA, leading to exon skipping, restoration of the

reading frame, and finally, producing a semi-functional protein instead of a non-functional protein (Aartsma-Rus et al., 2005. Spitali and Aartsma-Rus, 2012). Exon-skipping was first demonstrated by Dominski and Kole in 1993. They used AONs (2-omethylribooligonucleotides) to restore correct splicing of mutated human ß-globin pre-mRNAs in various ß-thalassemia disorders (Dominski and Kole, 1993). Since then, exon-skipping therapeutic strategies have been exploited in a wide range of genetic diseases, including lung cancer (Taylor et al., 1999), Usher's syndrome (Slijkerman et al., 2016), allergic diseases (Arthur and Cruse, 2018) and multiple neuromuscular disorders, including Duchenne muscular dystrophy (DMD) (Wilton et al., 2007), limb-girdle muscular dystrophy 2B (Aartsma-Rus et al., 2010) and spinal muscular atrophy (SMA) (Rigo et al., 2012). DMD is a fatal, rare, X-linked recessive, and progressive muscle wasting disorder that is often caused by out-of-frame intragenic deletions of one or more exons in the dystrophin gene, leading to premature termination of the transcript and the consequent loss of the dystrophin protein (Aartsma-Rus et al., 2010. Muntoni et al., 2003. Touznik et al., 2014). A milder version of the disease, Becker muscular dystrophy (BMD), is also caused by mutations in the dystrophin gene that arise from in-frame mutations and produce a truncated but functional dystrophin protein (Van Den Bergen et al., 2014). Corticosteroids are currently used to treat DMD, which help maintain muscle strength and delay disease progression. These drugs, despite their significant efficacy, have also had several adverse effects, such as vulnerability to infection, weight gain, cataracts, and weakened bones (Dzierlega and Yokota, 2020., Manzur et al., 2008). The majority of patients (~70%) carry large out-of-frame deletions in the dystrophin gene. Exon-skipping has emerged as a promising therapeutic approach for DMD (Bladen et al., 2015). The aim of exon-skipping therapy in DMD is to restore the reading frame with excluding out-of-frame exons from the DMD pre-mRNA using AONs to bypass exons flanking deletions, resulting in the production of a truncated but functional protein instead of a non-functional protein (Touznik et al., 2014).

To date, four antisense-mediated exon skipping drugs have been approved by the Food and Drug Administration (FDA) for treating DMD: Eteplirsen (EXONDYS 51) is used to induce exon 51 skipping in the dystrophin gene (Lim et al., 2017); Golodirsen (VYONDYS 53) is an exon skipping drug that targets exon 53 (Anwar and Yokota, 2020); Viltolarsen (VILTEPSO) targets exon 53 for skipping (Roshmi and Yokota, 2019); and Casimersen (AMONDYS 45) is designed to skip exon 45 (Shirley, 2021).

SMA is the second most common lethal, rare autosomal recessive neuromuscular disease after cystic fibrosis in children. This disorder is caused by a mutation in the survival motor neuron 1 gene (SMN1), which results in skeletal muscle weakness and atrophy. The survival motor neuron (SMN) gene is composed of an inverted duplication consisting of telomeric survival motor neuron 1 (*SMN1*) and centromeric survival motor neuron gene (SMN2) (Melki et al., 1990). SMN2 is an almost identical gene to SMN1, which is different by only 5 nucleotides. SMN2 contains a silent c.840C>T transition in exon 7, which leads to disruption of the exonic splicing enhancer, exclusion of exon 7, and ultimately a production of a non-functional SMN protein (Koed Doktor et al., 2011). About 10% of the SMN2 mRNA transcript enable to escape the exon 7 exclusion and produce a full-length SMN2 transcript that generates a functional SMN protein (Pao et al., 2014), so SMN2 partially moderates the severity of SMA (Touznik et al., 2014). SMA is caused by a homozygous deletion of SMN1 exon 7 in approximately 96% of patients (Lefebvre et al., 1995). Recently, modulation of pre-mRNA SMN2 splicing using AONs has provided a promising therapeutic approach for the treatment of SMA (Sumner and Crawford, 2018). The aim of this strategy is to enhance functional SMN protein by promoting exon 7 inclusion with AONs (Finke, 2016). Nusinersen (SPINRAZA), a modified 2′-omethoxyethyl, is the first AONs drug for patients with SMA that was approved by the FDA in 2016 (Sumner and Crawford, 2018). It is designed to promote the inclusion of exon 7 by binding to the intron-splicing silencer region of SMN2 pre-mRNA and thereby increasing the production of full length SMN protein (Singh et al., 2006).

Design of DNAi

According to criteria described by Rodrigueza and his colleagues, efficient design of DNAi is: i) having the proper length of 20–34 bases; ii) using at least one GC pair; iii) having at least 40% C and G content; and iiii) having no more than five consecutive bases of the same sequence. In 2004, Kawai-Toyooka et al. reported the first DNAi

designed to complement the AcPHOT2 gene in Fern adiantum.

Table 1. Summary of studies using the method based on DNAi to inhibit gene expression

Target gene	Description	Delivery method	Reference
Myostatin gene	29bp, single-stranded, Inhibition of gene in C2C12 cell	Transfected to cell by PEI-g-PEG	(payande et al., 2019)
BCL-2 gene	24bp, single-stranded, Inhibition of gene in patients with advanced solid tumors and cell	encapsulated in a variety of liposome compositions	(Rodrigueza et al., 2014)
BCL-2 gene	24 bp, single-stranded, Inhibition of gene in DLCL2 Cell	pegylated nanoliposome with different percent of PEG 2000	(Saravani et al., 2018)
BCL-2 gene	24 bp, single-stranded, unmodified, Inhibition of gene in patients with advanced solid tumors	encapsulated in the aqueous core within the liposomal nanoparticle	(Tolcher et al., 2014)
MYC gene	Downregulation of gene in cell lines	-	(Psaras et al., 2021)
BCL-2 gene	24 bp, single-stranded, Inhibition of BCL-2 gene in patients	-	(Harb et al., 2021)
BCL-2 and CDK4 gene	13-16 bp, single-stranded, Inhibition of gene in DLCL2 cell	-	(Ebrahim et al., 2017)
BCL-2 gene	24 bp, single-stranded, Inhibition of gene in rats	Modified by epigenic modification (Bicelin) and injected	(Sheikhnejad et al., 2021)
-	54bp, single-stranded, inhibition of HIV replication	-	(Moelling et al.,2006)
Myostatin gene	29bp, single-stranded, Inhibition of gene in C2C12 cell	Modified by phosphorothioate modification and injected intraperitoneally	(Riasi et al., 2023)

The aim of this study was to efficiently deliver dsDNA fragments homologous to an endogenous gene, which may cause a knockout phenotype of a corresponding gene in Adiantum (Kawai-Toyooka et al., 2004). The results indicated that, by delivering dsDNA fragments homologous to endogenous genes in gametophytic cells, we can induce sequence-specific gene silencing. By using such a method, a maximum gene knockout efficiency of >90% has been achieved for transformed plants. Please add an explanation regarding other researchers who used DNAi design.

Modification

Chemical variants of DNA and RNA backbones were originally developed as an artificial means to stabilize oligonucleotides against nuclease degradation. Also, it can be used in such studies based on antisense pharmacology, structure-function, and biosynthesis education *in vitro*, where their resistance against nuclease enzymes and enhanced cellular uptake are imperative (Thaler et al.,1996). The first generation of modified oligonucleotides by replacing the backbone phosphodiester bond with phosphorothioates (Matsukura et al., 1987), methylphosphonates (Miller et al., 1979), phosphoroamidates (Letsinger et al.,1988), phosphotriesters (Miller et al., 1971), or phosphorodithioates (Marshall and Caruthers, 1993) has been improved. It has been proven that such oligonucleotide modifications display greatly improved resistance to nucleases and better bioavailability (Uhlmann and Peyman, 1990. Varma, 1993). In this class, one of the oxygen atoms is replaced with sulfur in the phosphate group. The resulting compound is negatively charged and much more resistant to nucleases (Cohen and Cohen, 1989). Also, it seems that the replacement of sulfur bands confers several properties onto antisense oligonucleotides, which play a critical role in the use of systemic drugs (Eckstein, 2000). The second generation of antisense oligonucleotide backbones, in which two sugar units are connected by a linkage that does not contain a phosphorus atom, appeared

and was replaced by amino acids. In some cases, the full modification of the backbone by such subunits might have some disadvantages, such as solubility and aggregation problems. In contrast, these natural modifications improve the cellular uptake and resistance of modified oligonucleotides towards nucleases (De Mesmaeker et al.,1995). And the third generation belongs to modifications at the sugar moiety by introducing a 6-aminohexyl group. Such modification strategies are essential for two main reasons: first, the stability of the 6-aminohexylmodified oligonucleotides against the catalytic degradation effect of ribozymes is largely improved, and second, the electrostatic repulsion between the zwitterionic antisense cords is reduced. The flexible alkyl group between the oxygen of the ribose and the terminal amino function acts as a spacer, enabling an optimal arrangement of the amino group for a linkage to the complementary DNA or RNA strands (Figure 1 A and B) (Urban and Noe, 2003). Table 2 illustrates a list of studies contain applying modified DNA-based techniques for gene silencing.

Among the various types of modification, two types of phosphorothioates and methylphosphonates are more commonly used for DNA. Some studies have shown the biological effects of phosphorothioate oligonucleotides *in vivo*. For example, in 1996 Thaler et al. tested four artificial backbone chemistries for DNA and RNA (Thaler et al., 1996) phosphorothioate DNA, phosphorothioate RNA, 2'-0-methyl RNA, and methylphosphonate DNA. The results of comparing such modifications proved that only phosphorothioate DNA supported genetic information transfer *in vivo*. Another group utilized phosphorothioate oligonucleotides to induce gene expression in mice and demonstrated that such modifications have a long-lasting effect (Dean and McKay, 1994). The result indicated a significant effect of this type of modification of gene inhibition in mice. In 1992, to determine the inactivation of the expression of Tax and NF-KB proteins, Kitajima et al. performed a study. In this investigation, they used 3'-terminal phosphorothioate (PS)-modified antisense oligodeoxynucleotides (ODNs) in cell lines transformation and successful reducing tumor growth. On the other hand, oligonucleotides seem useful as radiopharmaceuticals for nuclear medicine imaging studies. It is mentioned in many sources that single- or double-stranded oligonucleotides are utilized to block transcription or silencing of genes within the cell nucleus and inhibit translation of messenger RNA in the cytoplasm (Dewanjee, 1993., Uhlmann and Peyman, 1990). The Modification of DNA with a phosphorothioate backbone has various applications in treatment (Graham et al., 1998).

Delivery systems for oligonucleotides

The main limitations of oligonucleotide (ONs)-based therapeutics are difficulties in their delivery to target tissues and rapid digestion by exo- and endonucleases *in vivo*. Therefore, oligonucleotides must be structurally modified to increase their resistance to nuclease degradation while preserving their target specificity (Buck et al., 2002). To achieve this goal, various technologies, such as conjugates and nanoparticles (NPs), are used as delivery systems. Carriers used for delivery should be stable, non-toxic, and improve the absorption, distribution, metabolism, and excretion (ADME) properties (Kontturi et al., 2019). The conjugation of chemical molecules such as lipids, polymers, and peptides to oligonucleotides is an exciting approach for improving cellular uptake, stability, and resistance to nucleases (Lei Mon et al., 2020. Odeh et al., 2019).

Lipids

Conjugation of oligonucleotides with lipids such as cholesterol and cholic acid improves delivery by enhancing plasma half-life and tissue accumulation (Prakash et al., 2019). Cationic liposomes are one of the most widely used oligonucleotide delivery systems. Oligonucleotides are anionic and easily encapsulated into cationic liposomes by electrostatic interactions (Wang et al., 2015). The main components of these carriers are cationic lipids that have a hydrophobic chain and a cationic head, in fact, negatively charged oligonucleotides interact with the cationic head. In addition to the cationic head function, the alkyl chain also contributes to the endosomal escape of the liposome. However, the use of cationic liposomes for *in vivo* applications is limited because too much positive charge might be toxic, activate the innate immune system, and have poor pharmacokinetic properties (Knudsen et al., 2015). Because neutral or anionic liposomes interact less with complement components and serum proteins, they are less toxic and have superior pharmacokinetic characteristics. However, cellular absorption and intracellular delivery of entrapped ONs with neutral liposomes are typically subpar because non-cationic lipids do not interact with cellular membranes as effectively as cationic ones.

Table 2. Summary of studies using modified DNAi to suppress gene expression in treatment

Modification type	Aim	References
Phosphorothioate backbone	Suppressed factor-beta 2 in pancreatic cancer in cell and mice	(Sheikhnejad et al., 2021)
Phosphorothioate backbone	Suppressed gene modulated in the central nervous system in mice	(Lei Mon et al., 2020)
Locked nucleic acid	Inhibition of tumor growth by RNA polymerase II gene in mice	(Fluiter et al., 2002)
Locked nucleic acid	Knockdown of PKC-a in human lung carcinoma in cell	(Hansen et al., 2003)
Locked nucleic acid	Inhibition of Human Telomerase in cell	(Elayadi et al., 2002)
2'-O-methyl oligonucleotides and Locked nucleic acid	Block inhibition of HIV-1 in cell	(Arzumanov et al., 2003)
Locked nucleic acid	inhibition of HIV-1 genome dimerization and inhibit virus replication in cell	(Elmén et al., 2004)
2′F-ANA gapmer phosphorothioate oligos	Inhibition Bcl-2 gene expression in cell	(Souleimanian et al., 2012)
Phosphorothioate backbone	Inhibition MSTN gene in mice	(Riasi et al., 2022)

Ionizable lipids are currently the most ideal lipid formulations (Blenke et al., 2018). When the oligonucleotides are loaded into liposomes, these lipids are able to carry positive charges, however, after administration but before delivery to the cell, they are able to lose their charges (Wang et al., 2015).

In our previous study, Lipofectamine 3000 (Sigma-Aldrich, Germany) was used to increase the efficacy of transfection, ultimately maximizing the efficiency of genetic modifications and simplifying the downstream processes (payande et al., 2019. payande et al., 2019).

Polymers

Conjugation of polyethylene glycol (PEG) has mainly been used for therapeutic proteins, but recently it has also been used for oligonucleotides, for example the PEG RNA aptamer pegaptanib that selectively targets vascular endothelial growth factor (VEGF). PEG is a highly hydrophilic, non-charged, and flexible polymer with end group functionalization. PEG coatings on drug conjugates protect them with a hydration shell that prevents biomacromolecules from binding to the drug. Also, PEGylation enhances blood circulation time by increasing oligonucleotide stability and reducing renal clearance (Ng et al., 2016). PEI-g-PEG (Sigma-Aldrich, Germany) was able to effectively transforme the DNAi molecule into the C2C12 cell (payande et al., 2019).

Peptides

Cell-penetrating peptides (CPPs) are another group of compounds used as carriers to deliver oligonucleotides (Lehto et al., 2016). CPPs commonly consist of 5–30 amino acids. They are often positively charged due to the presence of basic amino acids such as lysine and arginine in their structure (Szabó et al., 2010). CPP can be directly conjugated to oligonucleotides, thus increasing the delivery of oligonucleotides across biological barriers and cell membranes. Also, the usage of nanoparticles can potentially enhance the activity of such complexes. CPP/ON nanoparticles are formed by hydrophobic and electrostatic interactions between anionic ONs and cationic CPPs. Diverse types of CPPs have shown significant potential for oligonucleotide delivery in a nanoparticle format, including PepFects (PFs) derivatives and MPG (Lehto et al., 2016).

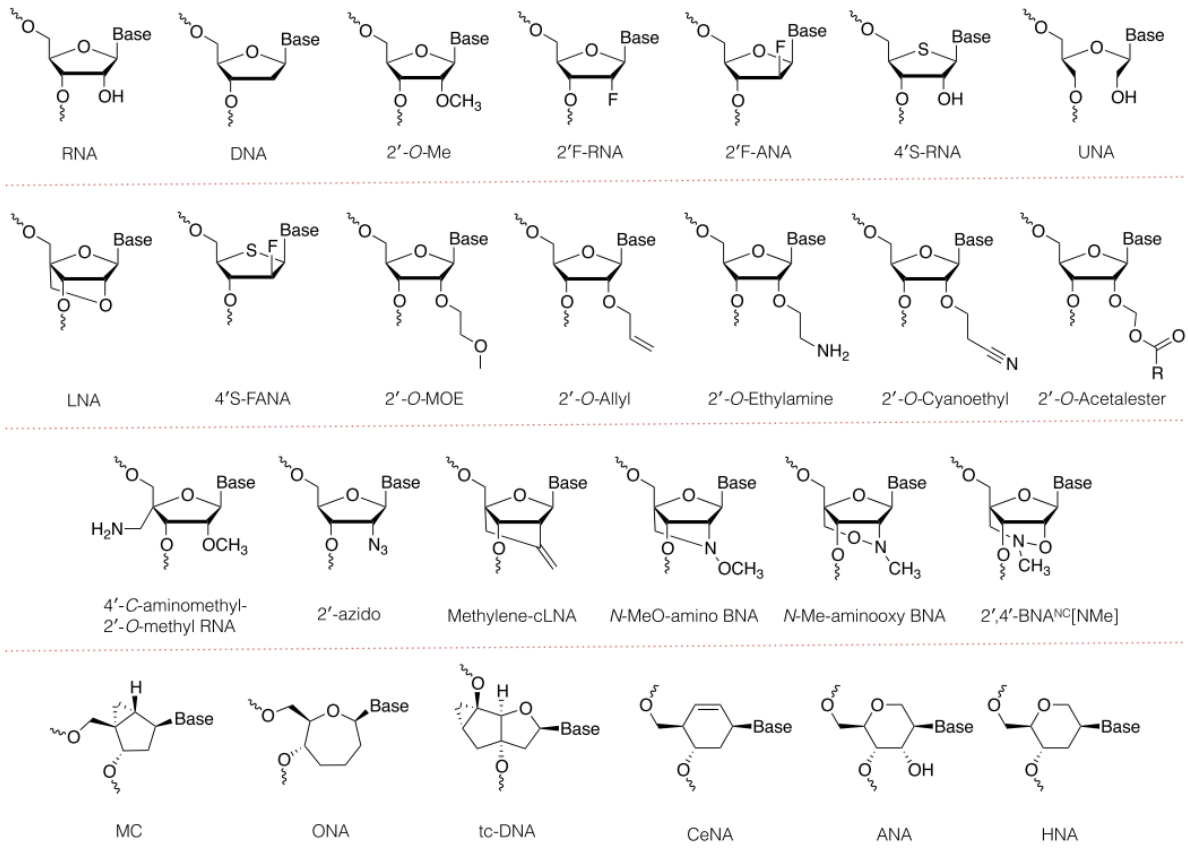

Figure 1. Several chemical modifications were applied in the design of oligonucleotides to improve nuclease resistance, biological activity, duplex thermal stability, and cellular uptake (A) Phosphodiester backbone, and (B) Sugar backbone (Deleavey and Damha, 2012)

Limitations of DNAi

Despite some advantages of the DNAi method, there are severe limitations regarding down-regulation of gene expression *in vivo,* such as efficient delivery of DNA to the target tissue, low half-life of DNA in the circulatory system, DNase sensitivity, toxicity, and immunogenicity, and stable gene silencing (Riasi and Javadmanesh, 2020). Additionally, the use of small antisense oligonucleotides as a form of therapy, despite serious limitations, can provide a greater range of target genes compared to some approaches based on proteins. Usually, targets for traditional small molecular drugs are enzymes (e.g., proteases, kinases, topoisomerases, and polymerases) and some receptors for small molecular weight ligands (e.g., neurotransmitters, eicosanoids, steroids, and retinoids). Advances in structural biology and screening technologies have increased the number of genes available for small-molecule drugs, but success in using these approaches is still limited (Węsierska-Gądek et al., 2002). Therefore, a large number of target genes that are considered important for drug purposes are not exploited. On the other hand, another limitation of antisense oligonucleotides is their pharmacokinetics, which means that they do not distribute equally to all cell types within tissue (Bennett and Swayze, 2010). It seems that because of such limitations, drugs based on the antisense oligonucleotide cannot cross the blood-brain barrier; thus, they are not an operative treatment for neurologic diseases. Apart from these issues, the delivery of DNA by nuclease is the main challenge *in vitro* and *in vivo*. Recently, some studies have focused on using oligonucleotides *in vivo.*

Conclusion

Oligonucleotides, such as DNAi, have demonstrated great therapeutic potential for a treatment of various diseases, including cancer, infectious diseases, neurological diseases, and genetic diseases. Despite numerous obstacles, there have been recent advancements in the utilization of

oligonucleotides as effective drugs in clinical applications. The problems with oligonucleotides' stability in plasma are successfully improved by a variety of chemical modifications. In addition, some delivery methods based on nanoparticles have been used to encapsulate oligonucleotides and increase their accumulation in the target tissue. Therefore, in the near future there would be a higher confidence to use DNAi as an efficient gene silencing molecule with higher precision and lower side effects.

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