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NUCLEIC ACID BASED THERAPEUTIC DELIVERY SYSTEM: A REVIEW

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ABSTRACT

Nucleic acids have not been widely considered as an optimal material for drug delivery. The development of nucleic acid-based therapeutics has garnered tremendous interest in the past two decades as a new category of biologic. Gene therapy using nucleic acids has many clinical applications for the treatment of diseases with a genetic origin as well as for the development of innovative vaccine formulations. Targeted gene therapies have emerged as potential strategies for treatment of such diseases. These therapies depend upon rare-cutting endonucleases to cleave at specific sites in or near disease genes. **Gene expression** is the process by which information from a gene is used in the synthesis of a functional gene product. Among several talented new drug delivery systems, liposomes characterized an advance technology to deliver active molecules to the site of action, and at present, several formulations are in clinical use. This review focuses on the properties of nucleic acid based therapeutics, also focuses on potential target disease for gene therapies, gene therapies, gene expression, liposomal gene delivery system. We discuss recent progress in nucleic acid based drug delivery strategies, their potential, unique use cases, and risks that must be overcome or avoided.

KEYWORDS: Nucleic acid, Drug targeting, Gene therapy.

INTRODUCTION

Nucleic acids are biopolymers, macromolecules, essential to all known forms of life.[1] They are composed of nucleotides, which are the monomers made of three components: a 5-carbon sugar, a phosphate group and a nitrogenous base. The two main classes of nucleic acids

are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). If the sugar is ribose, the polymer is RNA; if the sugar is the ribose derivative deoxyribose, the polymer is DNA.

Nucleic acids are naturally occurring chemical compounds that serve as the primary information-carrying molecules in cells and make up the genetic material. Nucleic acids are found in abundance in all living things, where they create, encode, and then store information of every living cell of every life-form on Earth. In turn, they function to transmit and express that information inside and outside the cell nucleus to the interior operations of the cell and ultimately to the next generation of each living organism. The encoded information is contained and conveyed via the nucleic acid sequence, which provides the 'ladder-step' ordering of nucleotides within the molecules of RNA and DNA. They play an especially important role in directing protein synthesis.

Strings of nucleotides are bonded to form helical backbones—typically, one for RNA, two for DNA—and assembled into chains of base-pairs selected from the five primary, or canonical, nucleobases, which are: adenine, cytosine, guanine, thymine, and uracil. Thymine occurs only in DNA and uracil only in RNA. Using amino acids and the process known as protein synthesis,[2] the specific sequencing in DNA of these nucleobase-pairs enables storing and transmitting coded instructions as genes. In RNA, base-pair sequencing provides for manufacturing new proteins that determine the frames and parts and most chemical processes of all life forms.[3]

Nucleic acid, a highly hydrophilic and negatively charged natural biopolymer, has been relatively unnoticed as a material for DDS. Instead, nucleic acids are consistently regarded as a troublesome therapeutic cargo, requiring an advanced DDS to facilitate their delivery. Indeed, unmodified nucleic acids are hopelessly incapable of entering cells and are subject to rapid nuclease cleavage and renal/hepatic clearance. Typically, a particular intracellular localization (e.g. cytosol or nucleus) is often required prior to any mechanism of action, be it gene expression knockdown, mRNA splicing alteration, transcriptional and epigenetic regulation, and genome editing.[4] In addition, certain nucleic acid motifs can elicit a strong activation of the innate immune system even at low concentrations, e.g. certain RNA sequences (e.g. 5'-UGUGU-3') and DNA sequences containing unmethylated cytosine-phosphate-guanosine (CpG) motifs.[5,6] In fact, these motifs are being explored as potent vaccine adjuvants.[7,8] Given these limitations, nucleic acids in the past have been mainly

developed as drugs for rare diseases originating from the liver.[9] or in tissues that can be treated by local injection, such as the eye or the spinal cord.[10]

The use of nucleic acids as therapeutic agents dates back over 40 years to the initial usage of nucleoside analogues to fight disease. Nowadays, therapeutic nucleic acids (TNAs) and its precursors are applied to treat several pathologies and infections in the clinic and are readily available worldwide.[11]

Plasmids

Plasmids are high molecular weight, double stranded DNA constructs containing transgenes, which encode specific proteins. On the molecular level, plasmid DNA molecules can be considered pro-drugs that upon cellular internalization employ the DNA transcription and translation apparatus in the cell to biosynthesize the therapeutic entity, the protein.[12] The mechanism of action of plasmid DNA requires that the plasmid molecules gain access into the nucleus after entering the cytoplasm. Nuclear access or lack thereof eventually controls the efficiency of gene expression. In addition to disease treatment, plasmids can be used as DNA vaccines for genetic immunization.[13] In the early stages of development, plasmid-based gene therapy was attempted to correct inheritable disorders resulting from a single gene defect. The first federally approved human gene therapy protocol was initiated in 1990 for the treatment of adenosine deaminase deficiency.[14] Since then, more than 500 gene therapy protocols have been approved or implemented.[15] In 2002, scientists reported the successful gene-therapy-based cure for severe combined immunodeficiency (SCID).[16] In 2003, the Chinese drug regulatory agency approved the first gene therapy product for head and neck squamous carcinoma under the trade name Gendicine.[17] Currently, diseases with complex etiologies such as cancer and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease are being targeted. In addition, DNA vaccines for malaria, AIDS, and many other diseases are in development. DNA vaccines have also been used to prevent allergic response.[18]

Oligonucleotides

Oligonucleotides are short single-stranded segments of DNA that upon cellular internalization can selectively inhibit the expression of a single protein. For antisense applications, oligonucleotides interact and form a duplex with the mRNA or the pre-mRNA and inhibit their translation or processing, consequently inhibiting protein biosynthesis. For antigen applications, oligonucleotides must enter the cell nucleus; form a triplex with the

double- stranded genomic DNA, and inhibits the translation as well as the transcription process of the protein. On the molecular level, numerous mechanisms have been proposed to explain the basis of oligonucleotide action.[19] For therapeutic purposes, oligonucleotides can be used to selectively block the expression of proteins that are implicated in diseases. With successful antisense inhibition of proteins in animal models, the first antisense drug, fomivirsen sodium (Vitravene, Isis Pharmaceuticals, Carlsbad, CA) was approved for the treatment of cytomegalovirus retinitis in AIDS patients in 1998.[20] Antisense oligonucleotides such as MG98 and ISIS 5132, designed to inhibit the biosynthesis of DNA methyltransferase and c-raf kinase, respectively, are in human clinical trials for cancer.[21] Synthetic antisense DNA oligonucleotides and oligonucleotide analogs, which inhibit the replication of several infectious agents such as hepatitis C virus, human cytomegalovirus, human immunodeficiency virus and papilloma virus, have also been designed.

Aptamers

DNA - Aptamers are double-stranded nucleic acid segments that can directly interact with proteins. Aptamers interfere with the molecular functions of disease-implicated proteins or those that participate in the transcription or translation processes. Aptamers are preferred over antibodies in protein inhibition owing to their specificity, non-immunogenicity, and stability of pharmaceutical formulation. DNA-aptamers that have demonstrated promise in intervention of pathogenic protein biosynthesis are HIV-1 integrase enzyme.[22]

DNAzymes

DNAzymes are analogs of ribozymes with greater biological stability. The RNA backbone chemistry is replaced by the DNA motifs that confer improved biological stability. DNAzyme directed against the vascular endothelial growth factor receptor 2 was confirmed to be capable of tumor suppression by blocking angiogenesis upon intratumoral injections in mice.[23]

Gene therapy

Human gene therapy seeks to modify or manipulate the expression of a gene or to alter the biological properties of living cells for therapeutic use.

Gene therapy is a technique that modifies a person’s genes to treat or cure disease. Gene therapies can work by several mechanisms:

- Replacing a disease-causing gene with a healthy copy of the gene

- Inactivating a disease-causing gene that is not functioning properly
- Introducing a new or modified gene into the body to help treat a disease

  Gene therapy products are being studied to treat diseases including cancer, genetic diseases, and infectious diseases.[24]

There are a variety of types of gene therapy products, including:

- **Plasmid DNA:** Circular DNA molecules can be genetically engineered to carry therapeutic genes into human cells.
- **Viral vectors:** Viruses have a natural ability to deliver genetic material into cells, and therefore some gene therapy products are derived from viruses. Once viruses have been modified to remove their ability to cause infectious disease, these modified viruses can be used as vectors (vehicles) to carry therapeutic genes into human cells.
- **Bacterial vectors:** Bacteria can be modified to prevent them from causing infectious disease and then used as vectors (vehicles) to carry therapeutic genes into human tissues.
- **Human gene editing technology:** The goals of gene editing are to disrupt harmful genes or to repair mutated genes.
- **Patient-derived cellular gene therapy products:** Cells are removed from the patient, genetically modified (often using a viral vector) and then returned to the patient.[25]

Gene therapy products are biological products regulated by the FDA's Center for Biologics Evaluation and Research (CBER). Clinical studies in humans require the submission of an investigational new drug application (IND) prior to initiating clinical studies in the United States. Marketing a gene therapy product requires submission and approval of a biologics license application (BLA).[26]

Gene targeting

Gene targeting (also, replacement strategy based on homologous recombination) is a genetic technique that uses homologous recombination to modify an endogenous gene. The method can be used to delete a gene, remove axons. add a gene and modify individual base pairs (introduce point mutations). Gene targeting can be permanent or conditional. Conditions can be a specific time during development/ life of the organism or limitation to a specific tissue, for example. Gene targeting requires the creation of a specific vector for each gene of interest. However, it can be used for any gene, regardless of transcriptional activity or gene size.[27]

Gene targeted therapy

Targeted therapy or molecularly targeted therapy is one of the major modalities of medical treatment (pharmacotherapy) for cancer others being hormonal therapy and cytotoxic chemotherapy. As a form of molecular medicine, targeted therapy blocks the growth of cancer cells by interfering with specific targeted molecules needed for carcinogenesis and tumor growth, rather than by simply interfering with all rapidly dividing cells (e.g. with traditional chemotherapy).

Types of drug targeted therapy mechanisms

Most targeted therapies are either small-molecule drugs or monoclonal antibodies.

Small-molecule drugs are small enough to enter cells easily, so they are used for targets that are inside cells.

- Imatinib (Gleevec, also known as STI–571) is approved for chronic myelogenous leukemia, gastrointestinal stromal tumor and some other types of cancer. Early clinical trials indicate that imatinib may be effective in treatment of dermatofibrosarcoma protuberans.
- Gefitinib (Iressa, also known as ZD1839), targets the epidermal growth factor receptor (EGFR) tyrosine kinase and is approved in the U.S. for non small cell lung cancer.
- Erlotinib (marketed as Tarceva). Erlotinib inhibits epidermal growth factor receptor,[50] and works through a similar mechanism as gefitinib. Erlotinib has been shown to increase survival in metastatic non small cell lung cancer, when used as second line therapy. Because of this finding, erlotinib has replaced gefitinib in this setting. Sorafenib (Nexavar).[51]
- Zoptarelin doxorubicin (AN-152), doxorubicin linked to [D-Lys (6)] - LHRH, Phase II results for ovarian cancer.[52]
- Braf inhibitors (vemurafenib, dabrafenib, LGX818) used to treat metastatic melanoma that harbors BRAF V600E mutation
- MEK inhibitors (trametinib, MEK162) are used in experiments, often in combination with BRAF inhibitors to treat melanoma
- CDK inhibitors, e.g. PD-0332991, LEE011 in clinical trials.[28]

Monoclonal antibodies

Monoclonal antibodies, also known as therapeutic antibodies, are proteins produced in the lab. These proteins are designed to attach to specific targets found on cancer cells. Some monoclonal antibodies mark cancer cells so that they will be better seen and destroyed by the immune system. Other monoclonal antibodies directly stop cancer cells from growing or cause them to self-destruct. Still others carry toxins to cancer cells.

Several are in development and a few have been licensed by the FDA and the European Commission. Examples of licensed monoclonal antibodies include:

- Pembrolizumab (Keytruda) binds to PD-1 proteins found on T cells. Pembrolizumab blocks PD-1 and help the immune system kill cancer cells. It is used to treat melanoma, Hodgkin's lymphoma, non-small cell lung carcinoma and several other types of cancer.
- Rituximab targets CD20 found on B cells. It is used in non Hodgkin lymphoma.
- Trastuzumab targets the Her2/neu (also known as ErbB2) receptor expressed in some types of breast cancer.
- Alemtuzumab
- Cetuximab target the epidermal growth factor receptor (EGFR). It is approved for use in the treatment of metastatic colorectal cancer and squamous cell carcinoma of the head and neck.
- Panitumumab also targets the EGFR. It is approved for the use in the treatment of metastatic colorectal cancer.
- Bevacizumab targets circulating VEGF ligand. It is approved for use in the treatment of colon cancer, breast cancer, non-small cell lung cancer, and is investigational in the treatment of sarcoma. Its use for the treatment of brain tumors has been recommended.[29]

Gene expression

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, but in non-protein coding genes such as transfer RNA (tRNA) or small nuclear RNA (snRNA) genes, the product is a functional RNA.

Transcription

Synthesis of an RNA that is complementary to one of the strands of DNA.

Transcription factors

- Transcription factors to initiate the process. RNA polymerase I is responsible for transcription of ribosomal RNA (rRNA) genes.
- RNA polymerase II (Pol II) transcribes all protein-coding genes but also some non-coding RNAs (e.g., snRNAs, snoRNAs or long non-coding RNAs).
- Pol II includes a C-terminal domain (CTD) that is rich in serine residues. When these residues are phosphorylated, the CTD binds to various protein factors that promote transcript maturation and modification.
- RNA polymerase III transcribes 5S rRNA, transfer RNA (tRNA) genes, and some small non-coding RNAs (e.g., 7SK). Transcription ends when the polymerase encounters a sequence called the terminator.

Translation

Ribosomes read a messenger RNA and make protein according to its instruction.

The basic process of protein production is addition of one amino acid at a time to the end of a protein. This operation is performed by a ribosome. A ribosome is made up of two subunits, a small 40S subunit and a large 60S subunit. these subunits come together before translation of mRNA into a protein to provide a location for translation to be carried out and a polypeptide to be produced. The choice of amino acid type to add is determined by an mRNA molecule.[30]

CONCLUSION

To summarize, the role that nucleic acids play in therapeutics has been redefined in the last decade. Originally a substance known for information storage and therapeutic potential, nucleic acids have emerged as a game changer in drug delivery in many aspects, spanning traditional delivery approaches, targeted delivery, and smart logic gated devices.DNA amphiphiles can form self-deliverable nanoparticles that simultaneously transport payloads with opposing physical properties into cells without a complex co-carrier. Aptamers impart antibody-level targeting capabilities to associated drugs using an all-nucleic acid composition.It is foreseeable that nucleic acids will continue to play an important role in the design of modern DDSs.The main approaches for therapeutic targeting based on nucleic acids include the use of proteins, gene editing, and cell therapy through NPs.

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