GENE THERAPY

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S u m m a r y. Gene therapy is a new method of treatment of genetic diseases involving a variety of effects on the patient's genetic material. The work on genetic therapy gives hope for effective therapies of many diseases that are currently considered incurable. The delivery of genetic material to the cell may take place using two methods: direct method – it consists in infecting into the tissues genetic material in the form of a solution of plasmid DNA in physiological saline. This procedure, carried out only *In vivo*, may cause slight inflammatory reactions at the injection site, the advantage is the ease of performing the procedure and the low cost of producing plasmids. Indirect methods can be divided into three groups: biochemical, biological and physical methods. In gene therapy, the target for antisense nucleotides seems to be genes related to the neoplastic process, especially those that are overexpressed and should be silenced during treatment.

K e y w o r d s: gene therapy, antisense nucleotides DNA vaccine

GENE THERAPY AND ITS METHODS

The term "gene therapy" was introduced by scientists Waclaw and Elizabeth Szybalski. In 1962, they carried out the first genetic transformation introducing fragments of genomic DNA into human bone marrow cells. The first use of the gene therapy for therapeutic purposes in man took place in the early 90s of the 20th century, it involved the introduction of a correct ADA-gene to the lymphocytes of a 4-year old girl suffering from a severe immunodeficiency caused by a mutation in the adenosine deaminase gene (ADA). Currently, many clinical trials of gene therapy are carried out worldwide in the United States, Great Britain, Switzerland and Germany. Despite many studies and experiments, this field is still an experimental

part of medicine and most of the methods are still at the stage of laboratory research [2].

Gene therapy is a new method of treatment of genetic diseases involving a variety of effects on the patient's genetic material. Damaged or malfunctioning patient genes can be theoretically replaced by the correct genes, introduced by the appropriate methods into the cell. In other disease entities, new genes could counteract the further development of pathological changes, protein as products of the expression of these genes would restore proper functions in cells. The sum up repair genetic defect you can get through:

- Introducing a correct copy of the mutant gene into the cell and activating its expression
- Introducing additional copies of genes to the cell in order to enhance their functions
- Using of non-coding nucleic acid sequences to inhibit the undesired expression of specific genes [5].

TECHNIQUES AND THE APPLICATION OF CLINICAL GENE THERAPY

The methods of gene therapy and their applications can (according to Podolska,2008) be divided into:

1. Complementation of genetic defect. In cells containing genetic defects, a proper duplicate of the gene is created, thanks to which a protein is formed, the previous deficiency or impaired function of which caused the emergence of the disease. Application – treatment of cystic fibrosis, sickle cells anemia, hemophilia (monogenic recessive diseases).
2. Repair of mutations. The strategy is based on the regeneration of the genetic defect, i.e. on replacing the impaired part with the proper sequence. Generally, oligonucleotides or ribozymes are used to obtain a therapeutic effect. Application -Huntington's chorea.
3. Inhibition of the activity of the modified gene. The technique consists in inactivation of genes contributing to the appearance of the disease. It uses siRNA technique, rybozymes or antisense-oligonucleotides. Application – dominant monogenic, cancerous and infectious diseases.
4. Death of cells. The cDNA of genes encoding the proteins that are responsible for the degradation of impaired cells are transfected into the appropriate cells. They are used, among others toxic proteins, pro-apoptotic factors or strengthening the immune system. Application - cancer and infectious diseases.
5. Adding new phenotypic properties to the cells. The treatment is based on the use of genes with cDNA recognizing proangiogenic factors that generate new blood vessels. Therapy supported by DNA vaccines is also meant to strengthen the immunization of the system. Application - proangiogenic therapy in states of cardiac ischemia, antiangiogenic therapy in cancer.

The main strategies of modern gene therapy are based on the following techniques:

- inhibition of the mutant gene expression (antisense oligonucleotides, ribozymes, siRNA),
- correcting a genetic defect by introducing a correct copy of the gene,
- correcting the mutation by introducing the correct nucleotide sequence,
- elimination of abnormal cells (pro-apoptotic factors, toxic proteins),
- new phenotypic traits are given to cells that present up to now undesirable traits [9].

Most of the experimental work and laboratory tests concern the application of gene therapy in the treatment of cancer. In addition to these diseases, research is also conducted on the treatment of cardiovascular diseases, infectious diseases, hereditary monogenic diseases and other. The work on genetic therapy gives hope for effective therapies of many diseases that are currently considered incurable. The major challenge in addition to cancerous changes are congenital genetic diseases. There are ongoing attempts to improve the carriers of genetic material to cells, and it is also important to maintain the long-term expression of therapeutic genes, implanted into cells with undesirable genome traits and exhibiting phenotypic defects [7].

The development of genetic engineering is very dynamic, confirms the belief that in the next few years gene therapy will become a safe and very effective method of treatment of most diseases [5, 6].

METHODS OF INTRODUCING NUCLEIC ACID INTO THE INTERIOR OF A CELL

There are a number of methods for transfection of eukaryotic cells to introduce genes. *Ex vivo* manipulations are that the cells are taken from the patient, the desired genes are introduced and then the cells are delivered back to the patient's body, mainly blood cell and the bone marrow. In some cases, additional cell growth *In vitro* is needed [13].

In vivo methods involve the introduction of genes directly into the body *via* an appropriate vector to evoke transfection in the cells of the body and appropriate gene expression [3].

The delivery of genetic material to the cell may take place using two methods: direct method – it consists in infecting into the tissues genetic material in the form of a solution of plasmid DNA in physiological saline. This procedure, carried out only *In vivo*, may cause slight inflammatory reactions at the injection site, the advantage is the ease of performing the procedure and the low cost of producing plasmids. Indirect methods can be divided into three groups: biochemical, biological and physical methods. Biochemical methods use chemicals that become carriers along with nucleic acids. Such complexes pass into the cell through fusions from cytoplasmic membrane or as a result of phagocytosis. The role of carriers can be cast: DEAE – dextran, liposomes, amine polymers and calcium phosphate in the form of phosphorus – calcium precipitates. The physical methods include mainly electroporation, during which the cells are subjected to a short term electrical impulse with a high, predetermined voltage. As a result of this action, a cellular pore is formed in the membrane, through which the genetic material can be introduced into the interior of the cell [1].

Electroporation is a high efficiency method, but it can sometimes lead to cell damage. It is used *In vitro* but also *In vivo*, especially when introducing DNA into skeletal muscles and skin [1, 8].

Biological methods include the use of so-called vectors or molecules or organisms capable of transferring biological material, mainly DNA, from one cell to another. The vector carries the desired nucleotide sequences to the recipient's cell, should therefore be stable, preferably not to provoke an immune response, to protect the therapeutic gene from destruction and to be able to overcome the blood – tumor barrier. The most frequently used vectors and viruses (adenoviruses, retroviruses) [16].

GENE THERAPY USING ANTISENSE NUCLEOTIDES

Antisense nucleotides are 20-30 ribonucleotide chains or deoxyribonucleotides, the sequences of which are complementary or otherwise antisense to the mRNA fragment or also the DNA of the gene or its promoter [10].

The creation of such a combination inhibits gene expression by:

- Blocking the initiation and elongation of transcription and the attachment of transcription factors
- Inhibition of the formation of modifications at the 3 and 5 mRNA ends (7-methylguanidine and poly A chain.)
- Inhibiting post- transcriptional processing of the mRNA and clearing introns (splicing)
- Blocking the readout of genetic information by ribosomes
- Stimulating the enzymatic activity of RNase H, digestive DNA/RNA complexes.

Modification of elements of the antisense oligonucleotide structure makes them impervious to the enzymatic activity of endonucleases. The most effective in this respect is the change in the phosphate group structure of the nucleotide, consisting in the conversion of one atom of oxygen to a sulfur atom or a methyl group. In this way, methylphosphonate or thiophosphate analogs of DNA are formed [14].

The RNase enzyme recognizes and digests complexes formed form the oligonucleotide phosphorothioate and complementary RNA fragments, thus preventing later translation and biosynthesis of the protein. Gene expression can also be inhibited at the transcription stage, as a result of hybridization of phosphorothioate nucleotides with complementary DNA segments, and the triple forms are formed, the so- called DNA triplexes [15].

In gene therapy, the target for antisense nucleotides seems to be genes related to the neoplastic process, especially those that are overexpressed and should be silenced during treatment. It is possible that the combined use of the compounds and traditional chemotherapeutics will result in the best effects of treatment. In the case of the activity of genes that are not sensitive to conventional anticancer agents, the use of antisense nucleotides takes on special significance [10].

Exemplary gene groups of high importance in the pathology of tumors to which ASO nucleo-

tides are currently designed are *BCL-2*, *BCL-X* genes and the C protein kinase gene (PKC) [14].

DNA VACCINES

The phenomenon of immunization of the body through the application of the exogenous DNA is from a clinical perspective a very modern form of the immunotherapy. DNA vaccines are usually circular, double-stranded plasmids, which act as vectors introducing exogenous genetic material into the cells of the immunized recipient. They contain genes in their DNA that code for different pathogens, against which we want to develop immunity in a given organism. Recombinant plasmids are propagated in cells of specific species of bacteria, after which they are isolated and injected intradermal or intramuscular with the individual in the vaccine [8].

The effectiveness of vaccination according to many authors depends on the route of administration, the prevailing reviews of the gloss and the intradermal administration of the preparation, due to better antigen presentation in this tissue, is more effective [4].

Stages of DNA vaccine activation [11, 12]:

- injecting the DNA intradermal or alternatively the route to muscle
- transcription
- biosynthesis of proteins as a result of expression of an implanted *transgene*
- the synthesized protein plays the role of a vaccine antigen
- presentation of antigen on the surface of MHC molecules class I of the tissue compatibility system
- presentation of the MHC I-associated antigen and cytotoxic T lymphocytes (CTL)
- activation of T lymphocytes presenting the CD-8 surface antigen
- destruction of cells containing on their surface a protein pathogen antigen
- development of resistance to this antigen, propagation of immune memory cells.

Examples of the use of DNA vaccines [13, 18]:

- the production of antibody; production of mono-and polyclonal serum
- cancer diseases; clinical trials vaccine against of B lymphoma with DNA coding the pathological immunoglobulin of this tumor
- vaccine containing DNA carcinoembryonic antigen expressed in colon and breast cancer, as well as in small cell lung cancer
- allergies; inhibition of the production of specific IgE against beta-galactosidase, treatment of respiratory tract sensitivities against household dust mite allergens
- autoimmune diseases, prevention of autoimmune encephalitis and spinal cord inflammation by immunization with DNA encoding the T-lymphocytes receptor variable region gene
- prophylactic vaccination, mainly on animal model;
- positive effect of protection against infections: parasitic – malaria, bacterial - *Mycoplasma, Mycobacterium, Leishmania and* viral - influenza, measles, rabies, HSV-1, HSV-2, and others [17].

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