Possibilities and perspectives of gene therapy

Gene therapy refers to the introduction, removal or modification of a defective gene in cells for the purpose of treating genetically inherited diseases. Editing a gene can lead, for example, to the creation of a certain drug or a functional enzyme, etc. In the early days of treatment, the therapy proved particularly effective in immunodeficient conditions. The first patient was a four-year-old girl with a rare immune disorder (SCID). Her treatment consisted of genetically modifying her own dysfunctional white blood cells, adding a gene to ensure their proper function. The result was only a partial improvement, but it was still a success. It happened on September 14, 1990.

Use of gene therapy

The most common principle of gene therapy is to replace a functional gene with a mutated gene. It is estimated that the method has the greatest potential for use in monogenic diseases – cystic fibrosis, hemophilia, Duchenne muscular dystrophy, etc. However, its use is also considered in the treatment of cancer.

Gene therapy terms of use

- Knowledge of the exact sequence of the gene under investigation.
- Knowledge of the cause and pathological process of the disease (insufficient amount of product, creation of a pathologically acting product of a mutated gene, etc.).
- Selection of a suitable vector - carrier (retroviruses, adenoviruses).
- Patient consent.

Methods of gene insertion into human chromosomes

Ex vivo techniques

The cells of the affected area are surgically removed. We insert a healthy form of the aa gene into the obtained tissue and culture it in a suitable environment. After a sufficient number of cells have multiplied, the patient goes to surgery again, during which the tissue is applied to the original site of excision.
A very often taken tissue for culture is a bone marrow sample. The latter, as a producer of blood cells, then ensures the distribution of genetically modified cells through the blood throughout the body. The advantage of easier distribution is often overshadowed by the disadvantages of this technique – the procedure is very painful for the patient and must take place in two stages (removal of bone marrow and subsequent reimplantation), since the **cultivation** itself requires many hours.

In vivo techniques

This method does not require surgery or anesthesia. The modified gene is applied directly to the cells of the body. However, viruses must be used as carriers of information.

2 main groups of viruses are used:

- **Simple retroviruses**
  - Their advantage is the complete suppression of viral DNA . Only genetically modified DNA information is transmitted. Their results are long-lasting and patients are not attacked by viruses.
  - The disadvantage is that it only affects newly formed daughter cells . They have no effect on already existing defective cells.
- **Adenoviruses**
  - It works faster than retroviruses, but the duration of the effect is shorter - within weeks. However, the patient's immune system has a greater tendency to interfere with these viruses and create a reaction. Therefore, patients suffer from cold and runny nose symptoms. However, cases of more serious interactions are also known (see below).

To achieve the same result, several times less amount of adenovirus solution (within milliliters) is needed than with retroviruses (within liters).

Types of gene therapy

Somatic gene therapy

- it repairs the patient's genes without passing on the repaired genes to future generations

Germline gene therapy

- it would change the genes already in the germ cells (sperm, egg) and the gene change would be transferable to the next generations. This type of therapy is not yet allowed.

Gene therapy strategies

Direct gene therapy

- error correction, i.e. DNA sequence changes that are responsible for malignant transformation. E.g. removing a mutation in a proto-oncogene or introducing missing tumor suppressor genes
- not all cells can be repaired because the genotype is multifactorial in nature

Indirect gene therapy

- the introduction of new genetic information into a cell (tumor or other type) that leads to the destruction of tumor cells. Ex. introduction of DNA sequences that code for e.g. stimulation of the antitumor immune response or change tumor angiogenesis or activate an inactive antimetabolite molecule to its cytotoxic activity
- strict ethical criteria, strict selection of patients, the safety of the procedure for both the patient and the nursing staff is considered, the effectiveness of the therapy

Custom gene manipulation

It takes place ex vivo (outside the whole organism). There are various techniques for introducing a gene into isolated cells:

1. Physical methods
   - enable the direct introduction of nucleic acid into the target cells (microinjection, microprojectiles or electroporation – introduction of a weak electric current to the cell suspension in the presence of the gene to be incorporated into the cell)
   - efficiency < 1%
2. Chemical methods
   - they use e.g. calcium phosphate, liposomes for the incorporation of genes into cells (they improve passage through the cell membrane)
3. Biological methods
   - used viruses as DNA vectors , no. DNA viruses (papovaviruses, adenoviruses, herpes simplex virus) and retroviruses
   - transmission efficiency of the required sequence – almost 100%
   - other vectors: plasmids – contain multiplied copies of the selected gene, are injected into the bloodstream or directly into the area of tumor growth
   - monoclonal antibodies
   - *in vitro* multiplied tumor infiltrating lymphocytes (TIL)

After iv administration, TILs selectively infiltrate the postoperative remnants of the tumor from which they were isolated. Ex vivo, e.g. the gene for TNF (tumor necrosis factor) can be integrated into the TIL genome. The TNF gene (it is part of the TIL genome) is transcribed and the synthesized protein is secreted directly into the tumor tissue, which is eliminated by the necrotic process. Genetically controlled enhancement of immunobiological activity.

Ex. retrovirus: It is first genetically modified in vitro. The sequences coding for the viral proteins are removed, leaving only the sequences controlling their expression (LTR). This is followed by excision of the sequences encoding the production of the product chosen for the respective gene therapy. These recombinant retroviruses have the ability to infect cells and incorporate exogenous genes into their genome, but cannot replicate. An example of this type of gene therapy is the treatment of malignant glioma (a tumor with high mitotic activity) - it does not metastasize and is surrounded by nerve tissue that does not replicate.

Ex. Herpes simplex virus (HSV): Its gene for the enzyme thymidine kinase has been integrated into the genome of the target cell, and it converts an otherwise inactive drug form (prodrug) into an active substance with a cytostatic effect.

Treatment approaches

If the cause of the disease is insufficient production of a certain protein encoded by a defective gene, we choose to apply the modified gene anywhere in the human genome. However, if the pathological agent is a product resulting from the transcription of a mutated gene, it is necessary to specifically eliminate this gene from translation.

Current usage

Currently, however, gene therapy is still used rather rarely. This is due to ongoing concerns about its safety. This is still the manipulation of human DNA, which can also cause **ethical problems**.

In the past, there have been several cases of serious disability in gene therapy patients. Several deaths were also recorded. It was probably (although the causes are being investigated for a long time) a massive immune reaction of the organism to the repeated application of adenoviruses.

Today, gene therapy is used more for very **serious diseases** that cannot be treated in any other way, and which usually end fatally. A significant disadvantage of the method is its financial and technical complexity. Last but not least, there are also concerns about the effect of therapy on **tumor growth**. Introduced viral vectors can interfere with other regions of DNA and alter, for example, tumor suppressor genes or proto-oncogenes.

Links

Related Articles

- stem cells
- cloning
- mutation

External links

- Genová terapie (http://www.genetika-biologie.cz/genova-terapie)
- Krize genové terapie (http://www.gate2biotech.cz/krize-genove-terapie/)
- ORACLE GENE Schol - Gene Therapy (http://library.thinkquest.org/28599/gene_therapy.htm)
- Gene therapy, přehledový článek (NEJM, 1.8.2019) (https://www.nejm.org/doi/full/10.1056/NEJMra1706910)

References

- ALBERTS, Bruce, et al. *Základy buněčné biologie.* 2. edition. 2005. ISBN 80-902906-2-0.