Genetic engineering as a method for treating human diseases

Hope for the Future

ALICJA JÓZKOWICZ
JÓZEF DULAK
Jagiellonian University, Kraków
alicia@mol.uj.edu.pl
jdulak@mol.uj.edu.pl

Dr Alicja Józkowicz studies the formation of blood vessels and tests viral vectors in gene therapy

STANISŁAW SZALA
Comprehensive Cancer Center, Gliwice
Committee on Human Genetics and Molecular Pathology
Polish Academy of Sciences
sszala@io.gliwice.pl

Prof. Józef Dulak works with medical biotechnology and vascular biology, especially the processes of angiogenesis and vasculogenesis

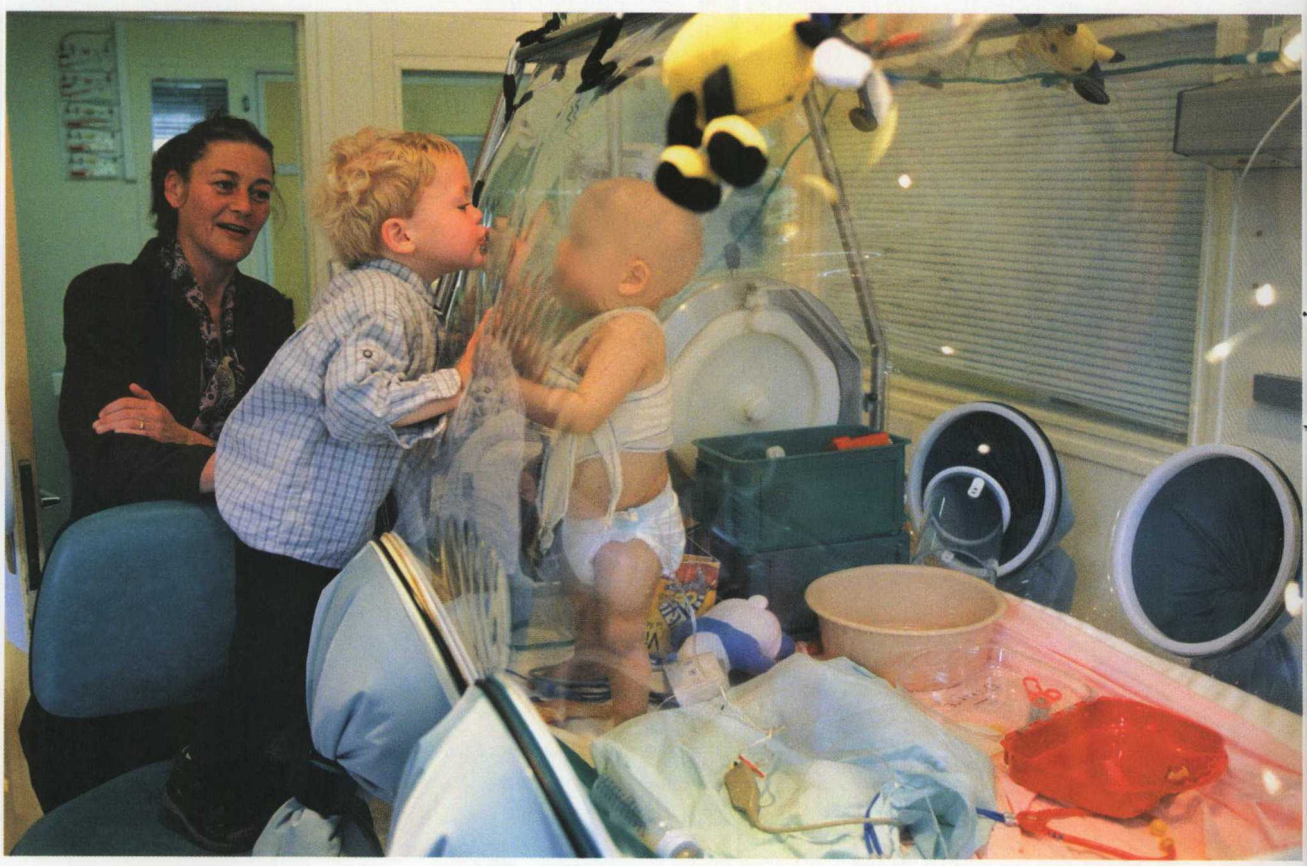
Prof. Stanisław Szala focuses on cancerous blood vessels as a target for cancer therapy

Treating a genetic disease? An easy task! Just replace the faulty gene with a correct one. Unfortunately, that idea is very difficult in practice

The development of genetic engineering has made it possible to introduce any chosen genes into mammal cells. That procedure is one form of "gene therapy," which is defined as the treatment of diseases using nucleic acids - either genes (the molecules that record protein-building blueprints), or so-called noncoding sequences of DNA or RNA. Unfortunately, gene therapy still remains mainly in the experimental realm as its use has proven significantly more difficult than initially anticipated.

Optimistic beginnings

The first successful genetic transformation of mammal cells was carried out in 1962 by the Polish scientist Wacław Szybalski, working at the University of Wisconsin, together with his wife Elizabeth. They introduced fragments of the correct DNA genome into human fibroblasts in which the metabolic salvage pathway of nucleotide synthesis had been impaired. It was then



GAMMA/BE&W

A boy with immunodeficiency living inside a sterile "bubble" - his elder colleague, thanks to successful gene therapy, is already on the other side

that Prof. Szybalski first coined the term "gene therapy," suggesting that in the future it would become possible to treat diseases by modifying the genome.

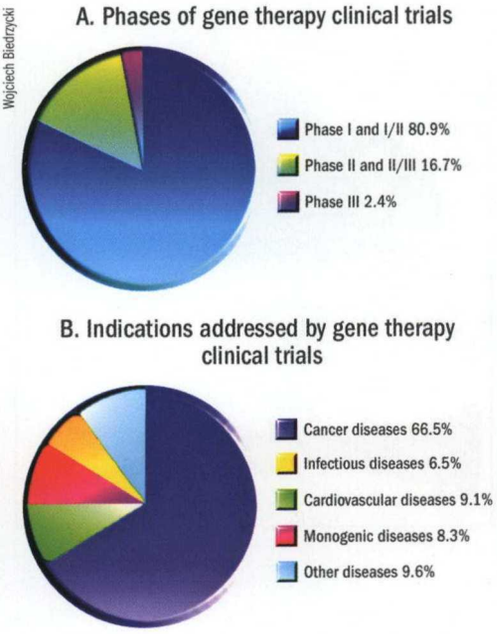
The results of animal tests justified the sense of optimism when clinical trials were next embarked upon. The first such attempt, carried out in the US in 1990, involved severe combined immunodeficiency (SCID), a disease caused by a mutation of the gene for the adenosine deaminase (ADA) enzyme. SCID sufferers have to spend their entire lives isolated from their environment, as an infection of any kind puts their life in grave danger. Leucocytes were taken from a four-year-old girl with immunodeficiency and the correct ADA gene was added to them in the laboratory; the cells thus "cured" were reintroduced into the child's body. The patient - now a young woman - is healthy and leads a normal life, although it is not clear to what extent her cure resulted from gene therapy or from the simultaneous ADA enzyme injections. Nevertheless, gene therapy alone has led to a significant improvement in subsequent tests with several other patients. Such results not only reinforced researchers' optimism, they also suggested that gene therapy would soon offer an effective treatment method for many illnesses.

Back to reality

However, introducing a gene so that it is able to function within a new cell is a very difficult task. Various methods can be attempted. To introduce a transgene (e.g. a foreign, extracellular gene) into the skeletal muscles, for instance, it suffices to inject them with a solution carrying the gene (in the form of plasmid). In experiments with rabbits in which hypoxia of the extremities had previously been induced, our introduction of the gene encoding VEGF (a protein stimulating the formation of blood vessels) triggered more active angiogenesis and a significant improvement in blood flow.

A similar approach has been tested clinically. The results of the first experiments by a team led by the late Prof. Jeffrey Isner were very promising: new vessel formation was observed and some patients' health significantly improved after such a plasmid was administered. Unfortunately, the reported effects turned out to be chiefly the outcome of the procedure itself, rather than being due to protein synthesis from the newly introduced gene (which proved to be very weak).

Wojciech Biedrzycki

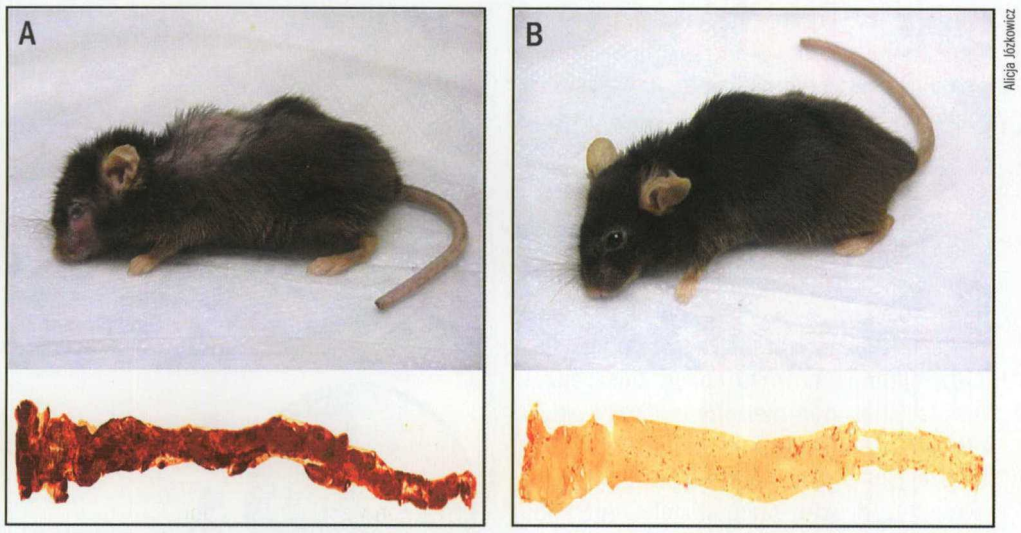


Most of the gene therapies used in the world remain on the level of clinical trials in various stages (A). At present such methods are most frequently being tested in the treatment of cancer (B). Source: www.wiley.co.uk/ /genmed/clinical

Boosting expression

Achieving a high level of gene expression required a different approach, which is called the "viral vector" method. A vector is a kind of vehicle for carrying genes, and a viral vector is a specially-altered virus where genes required for the life of the virus have been replaced with a certain gene we wish to introduce into cells (called a transgene). This makes the virus unable to reproduce, yet retains its capacity to introduce genes into cells. These days adenoviral vectors (i.e. vectors based on adenoviruses, a certain class of viruses) are most frequently used in view of their effectiveness, although they do have a serious drawback: triggering a strong immune system response. That poses a threat to the health and even life of the recipient, and the cells which accept the virus become damaged by the immune system in a specific way. A strong reaction to adenoviral vectors led to the death of one patient in 1999, after which clinical trials using them were suspended for a certain time.

At present adenoviruses are chiefly used in cancer treatment tests, since the increased immune system activity can in this case even make therapy more effective. One adenovirus was used to create Gendicine, the world's first carrier approved for treat-



Alicja Józkowicz

Both of these mice were born diseased, with a damaged apoE gene leading to serious arthrosclerosis. The mouse on the right was treated with a gutless adenoviral vector containing cDNA for the correct apoE gene, while the control mouse on the left indeed suffers from strong arthrosclerosis. The carotid arteries below, stained red, illustrate the atherosclerotic plaques

ing tumors of the neck and head in China. Combined with radiotherapy, it prolongs patients' lives significantly.

"Eviscerated" viruses

It turns out that the best way to solve this program is to remove all coding DNA sequences from the adenovirus genome, creating what is called a "gutless" virus. Gutless viruses have enabled the team led by Prof. Lawrence Chan in Houston (including one of the present authors, Alicja Jozkowicz) to completely block the development of atherosclerosis in mice deprived of the gene apoE, without any simultaneous side effects. The effect was sustained throughout the animal's life, i.e. for more than 2.5 years. However, gutless vectors have their drawbacks as well: their capsid protein may trigger an immune response, and their difficult production method impedes their use in clinical trials.

Greater hopes can be pinned on easier-to-obtain AAV viruses, which likewise have all their viral genes extracted. AAV viruses have been used to obtain a stable improvement in the symptoms of hemophilia and muscular dystrophy not only in mice but also in larger animals (dogs and rhesus monkeys). Promising results have also been obtained in clinical trials, e.g. in treating Parkinson's disease. Several months ago one woman suffering from rheumatoid arthritis was reported to have died after having been given an AAV vector carrying a therapeutic gene. However, the cause turned out to lie not in the vector but in the side effects of another medication.

In view of the above difficulties, another strategy is also being tested: here cells are genetically modified *in vitro* and subsequently introduced into the body. Retroviral vectors, carriers which build a transgene into the genone, are suitable for such use, and this is presently one of the most frequently employed strategies in gene therapy clinical trials. The use of retroviruses nevertheless entails a certain risk, long considered theoretical: we are unable to control the position of the genome where the gene becomes introduced. It might end up in the vicinity of what are called oncogenes, i.e. genes whose mutation leads to cancer. The odds of this happening are of course minimal, yet they have unfortunately proven to be real: one group of boys treated with modified blood cells showed significant improvement but three patients unfortunately developed leukemia within 2-3 years, leading to the death of one of them. That dramatic side effect was likely the outcome of the vector's integration in the vicinity of the oncogene LMO2, triggering uncontrolled cell division. Clinical trials in France have been suspended until safer carriers are developed.

Retroviral vectors are also being tested in cancer treatment - here cancer cells are modified by introducing cytokine genes into them, so that they trigger an immune system response. Such research has been conducted for many years by a team led by Prof. Andrzej Mackiewicz in Poznań. Promising results have also recently been reported in treating metastasizing melanoma using lymphocytes carrying receptor

genes recognizing melanoma's characteristic MART-1 protein. This approach assumes that the modified lymphocytes will generate receptors on their surface recognizing that protein and thus destroy cells which contain it. Of a group of patients so treated, two have evidenced a significant improvement and the disappearance of metastasis.

However, gene therapy is not always targeted at introducing genes to thereby cause the production of a specific protein. Increasing numbers of tests are now focusing on how to inhibit undesirable gene activity, using noncoding fragments of DNA or RNA introduced into cells via vectors. At present, the greatest hopes are being pinned on interfering RNA molecules (siRNA) and on RNA aptamers (molecules which bind to the VEGF protein). One such application lies in treating patients with age-related macular degeneration, the most frequent cause of blindness in individuals over 65 years old. Clinical trials and doctors' observations indicate that aptamers applied as a medicine can significantly slow the loss of sight.

Over-enchantment

In the early 1990s it might have seemed that gene therapy would soon come into widespread use. Such overblown hopes ultimately had to end in disappointment. The first optimistic reports were interpreted quite uncritically: the media carried sensational stories of "breakthroughs" achieved in treating successive diseases, even though animal experiments had only been carried out or clinical trials had just been initiated. Society obviously judges gene therapy trials by a different standard than trials involving traditional medicines, perceiving the former as some unauthorized attempt at "playing god" or overstepping limits. Each time a side effect is noted, it attracts broad coverage from the media. While such side effects certainly cannot be ignored, it is also worth bearing in mind that the side effects of conventional drugs are estimated to cause more than 15,000 deaths per year in the US alone. Unfortunately there is no effective therapy that involves zero risk; what is crucial is the magnitude of that risk with respect to the benefits.

Hopes and challenges

We now have a real hope that gene therapy can and will be harnessed in cancer treatment - where the activity of nucleic acids may be shorter and triggering an immune response may even be desirable. A vast challenge still lies ahead in finding treatments for congenital genetic diseases, on the other hand, since we continue to lack a safe vector to facilitate long-term transgene expression. One increasingly promising line of research involves hybrid vectors combining the advantages of various types of carriers (e.g. gutless and AAV), and vectors that allow genes to be introduced at precisely pinpointed genome locations (based on transposons). Another idea involves using transgenes whose activity can be regulated (e.g. through a higher level of cholesterol) or which are only active within a specific tissue.

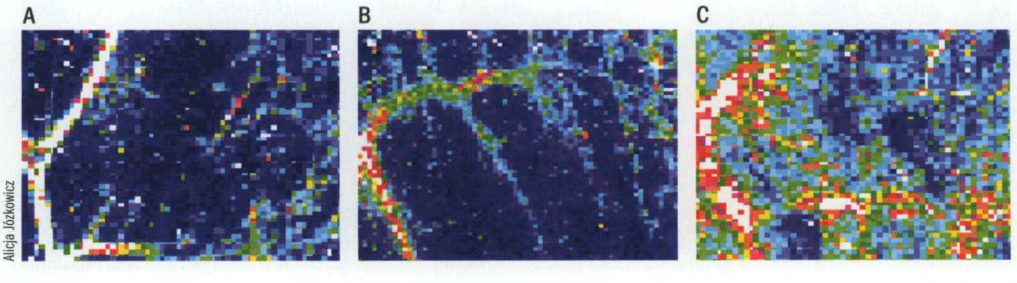
Even though it has so far encountered more failures than successes, we firmly believe that gene therapy remains a beacon of hope for the future medical field and will ultimately bring assistance to patients suffering from untreatable diseases. ■

Further reading:

Szala S. (2003). *Terapia genowa* [*Gene Therapy*]. Warsaw: Wydawnictwo Naukowe PWN.

Van Rooij E., Olson E.N. (2007). MicroRNAs: powerful new regulators of heart disease and provocative therapeutic targets. *J Clin Invest, 117*, 2369-2376.

Jazwa A., Józkowicz A., Dulak J. (2007). New vectors and strategies for cardiovascular gene therapy. *Current Gene Therapy, 7*, 7-23.



Alicja Józkowicz

Gene therapy techniques are tested in animals. A plasmid containing cDNA for human vascular endothelial growth factor (VEGF) alters the flow of blood in a muscle with insufficient oxygen supply. A - flow immediately after induction of ischemia, B - two weeks after administering control gene, C - two weeks after administering the VEGF gene. Brighter coloring indicates greater blood flow