11. USE OF GENETICALLY MODIFIED STEM CELLS IN EXPERIMENTAL GENE THERAPIES

To date, only nonembryonic human stem cells have been used in cell-based gene therapy studies. The inherent limitations of these stem cells, as discussed below, have prompted scientists to ponder and explore whether human embryonic stem cells might overcome the current barriers to the clinical success of cell-based gene therapies.

PRINCIPLES AND PROMISE OF GENE THERAPY

Gene therapy is a relatively recent, and still highly experimental, approach to treating human disease. While traditional drug therapies involve the administration of chemicals that have been manufactured outside the body, gene therapy takes a very different approach: directing a patient's own cells to produce and deliver a therapeutic agent. The instructions for this are contained in the therapeutic transgene (the new genetic material introduced into the patient). Gene therapy uses genetic engineering—the introduction or elimination of specific genes by using molecular biology techniques to physically manipulate genetic material—to alter or supplement the function of an abnormal gene by providing a copy of a normal gene, to directly repair such a gene, or to provide a gene that adds new functions or regulates the activity of other genes.

Clinical efforts to apply genetic engineering technology to the treatment of human diseases date to 1989. Initially, gene therapy clinical trials focused on cancer, infectious diseases, or disorders in which only a single gene is abnormal, such as cystic fibrosis. Increasingly however, efforts are being directed toward complex, chronic diseases that involve more than one gene. Prominent examples include heart disease, inadequate blood flow to the limbs, arthritis, and Alzheimer's disease.

The potential success of gene therapy technology depends not only on the delivery of the therapeutic transgene into the appropriate human target cells, but also on the ability of the gene to function properly in the cell. Both requirements pose considerable technical challenges.

Gene therapy researchers have employed two major strategies for delivering therapeutic transgenes into human recipients (see Figure 11.1. Strategies for Delivering Therapeutic Transgenes into Patients). The first is to "directly" infuse the gene into a person. Viruses that have been altered to prevent them from causing disease are often used as the vehicle for delivering the gene into certain human cell types, in much the same way as ordinary viruses infect cells. This delivery method is fairly imprecise and limited to the specific types of human cells that the viral vehicle can infect. For example, some viruses commonly used as gene-delivery vehicles can only infect cells that are actively dividing. This limits their usefulness in treating diseases of the heart or brain, because these organs are largely composed of nondividing cells. Nonviral vehicles for directly delivering genes into cells are also being explored, including the use of plain DNA and DNA wrapped in a coat of fatty molecules known as liposomes.

The second strategy involves the use of living cells to deliver therapeutic transgenes into the body. In this method, the delivery cells—often a type of stem cell, a lymphocyte, or a fibroblast—are removed from the body, and the therapeutic transgene is introduced into them via the same vehicles used in the previously described direct-gene-transfer method. While still in the laboratory, the genetically modified cells are tested and then allowed to grow and multiply and, finally, are infused back into the patient.

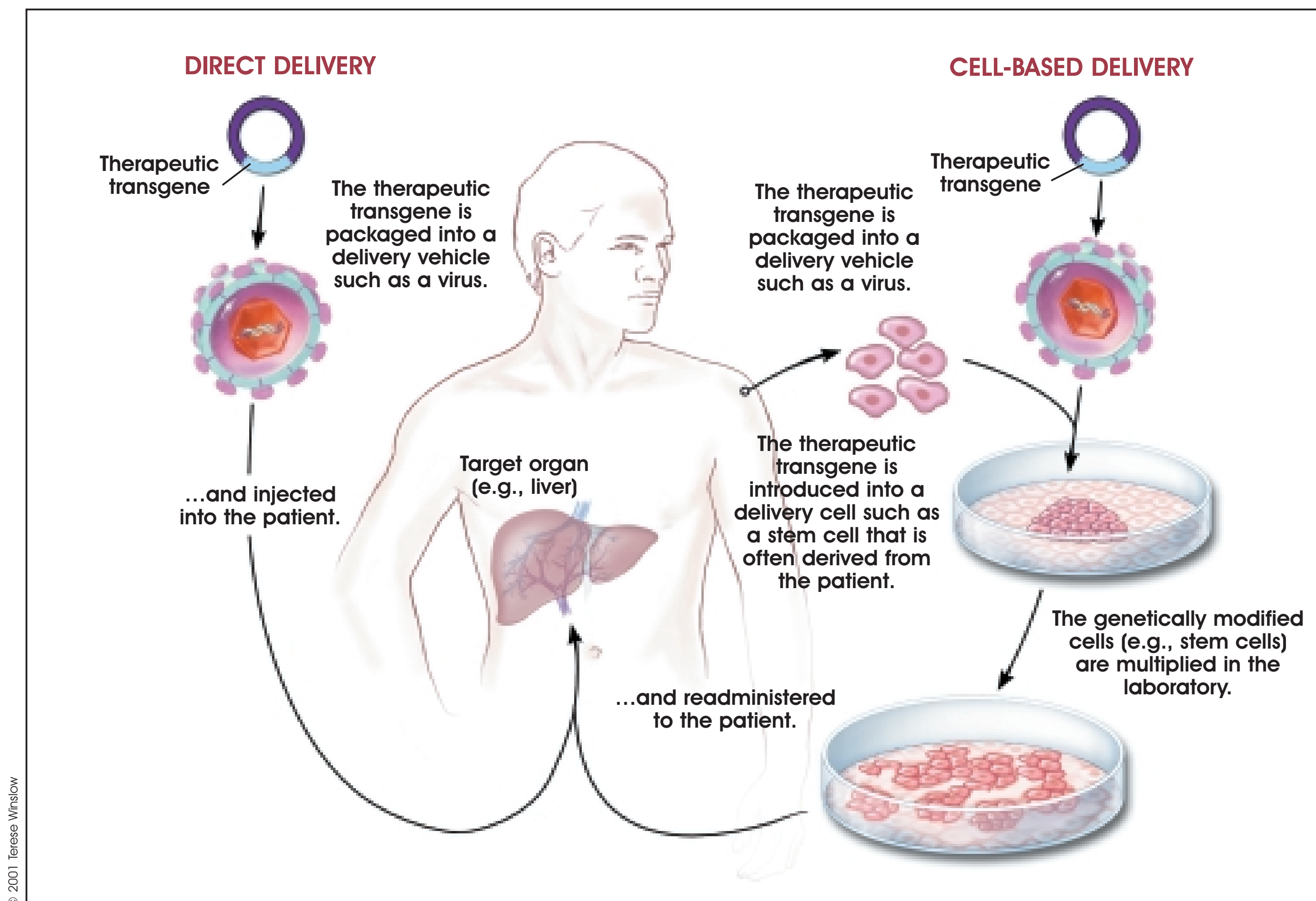

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Figure 11.1. Strategies for Delivering Therapeutic Transgenes into Patients.

Gene therapy using genetically modified cells offers several unique advantages over direct gene transfer into the body and over cell therapy, which involves administration of cells that have not been genetically modified. First, the addition of the therapeutic transgene to the delivery cells takes place outside the patient, which allows researchers an important measure of control because they can select and work only with those cells that both contain the transgene and produce the therapeutic agent in sufficient quantity. Second, investigators can genetically engineer, or "program," the cells' level and rate of production of the therapeutic agent. Cells can be programmed to steadily churn out a given amount of the therapeutic product. In some cases, it is desirable to program the cells to make large amounts of the therapeutic agent so that the chances that sufficient quantities are secreted and reach the diseased tissue in the patient are high. In other cases, it may be desirable to program the cells to produce the therapeutic agent in a regulated fashion. In this case, the therapeutic transgene would be active only in response to certain signals, such as drugs administered to the patient to turn the therapeutic transgene on and off.

WHY STEM CELLS ARE USED IN SOME CELL-BASED GENE THERAPIES

To date, about 40 percent of the more than 450 gene therapy clinical trials conducted in the United States have been cell-based. Of these, approximately 30 percent have used human stem cells—specifically, blood-forming, or hematopoietic, stem cells—as the means for delivering transgenes into patients.

Several of the early gene therapy studies using these stem cells were carried out not for therapeutic purposes per se, but to track the cells' fate after they were infused back into the patient. The studies aimed

to determine where the stem cells ended up and whether they were indeed producing the desired gene product, and if so, in what quantities and for what length of time. Of the stem cell-based gene therapy trials that have had a therapeutic goal, approximately one-third have focused on cancers (e.g., ovarian, brain, breast, myeloma, leukemia, and lymphoma), one-third on human immunodeficiency virus disease (HIV-1), and one-third on so-called single-gene diseases (e.g., Gaucher's disease, severe combined immune deficiency (SCID), Fanconi anemia, Fabry disease, and leukocyte adherence deficiency).

But why use stem cells for this method of gene therapy, and why hematopoietic stem cells in particular? The major reason for using stem cells in cell-based gene therapies is that they are a self-renewing population of cells and thus may reduce or eliminate the need for repeated administrations of the gene therapy.

Since the advent of gene therapy research, hematopoietic stem cells have been a delivery cell of choice for several reasons. First, although small in number, they are readily removed from the body via the circulating blood or bone marrow of adults or the umbilical cord blood of newborn infants. In addition, they are easily identified and manipulated in the laboratory and can be returned to patients relatively easily by injection.

The ability of hematopoietic stem cells to give rise to many different types of blood cells means that once the engineered stem cells differentiate, the therapeutic transgene will reside in cells such as T and B lymphocytes, natural killer cells, monocytes, macrophages, granulocytes, eosinophils, basophils, and megakaryocytes. The clinical applications of hematopoietic stem cell-based gene therapies are thus also diverse, extending to organ transplantation, blood and bone marrow disorders, and immune system disorders.

In addition, hematopoietic stem cells "home," or migrate, to a number of different spots in the body—primarily the bone marrow, but also the liver, spleen, and lymph nodes. These may be strategic locations for localized delivery of therapeutic agents for disorders unrelated to the blood system, such as liver diseases and metabolic disorders such as Gaucher's disease.

The only type of human stem cell used in gene therapy trials so far is the hematopoietic stem cell. However, several other types of stem cells are being studied as gene-delivery-vehicle candidates. They include muscle-forming stem cells known as myoblasts, bone-forming stem cells called osteoblasts, and neural stem cells.

Myoblasts appear to be good candidates for use in gene therapy because of an unusual and advantageous biological property: when injected into muscle, they fuse with nearby muscle fibers and become an integral part of the muscle tissue. Moreover, since muscle tissue is generally well supplied with nerves and blood, the therapeutic agents produced by the transgene are also accessible to nerves and the circulatory system. Thus, myoblasts may not only be useful for treating muscle disorders such as muscular dystrophy, but also possibly nonmuscle disorders such as neurodegenerative diseases, inherited hormone deficiencies, hemophilia, and cancers.

Several promising animal studies of myoblast-mediated gene therapy have been reported [17]. For instance, this approach was successful in correcting liver and spleen abnormalities associated with a lysosomal storage disease in mice. Investigators have also achieved stable production of the human clotting factor IX deficient in hemophilia at therapeutic concentrations in mice for at least eight months. Myoblasts engineered to secrete erythropoietin (a hormone that stimulates red blood cell production) were successful in reversing a type of anemia associated with end-stage renal disease in a mouse model of renal failure.

Another animal study of myoblast-mediated gene transfer involved a mouse model of familial amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease), a fatal disorder characterized by progressive degeneration of the brain and spinal cord nerves that control muscle activity. Investigators injected myoblasts containing the transgene for a human nerve growth factor into the muscles of the ALS mice before the onset of disease symptoms and motor neuron degeneration. The transgene remained active in the muscle for up to 12 weeks, and, most importantly, the gene therapy successfully delayed the onset of disease symptoms, slowed muscle atrophy, and delayed the deterioration of motor skills [16].

In a series of experiments in rodents, a team of investigators has been testing neural stem cells as vehicles for cell-based gene therapy for brain tumors known as gliomas. Gliomas are virtually impossible to treat because the tumor cells readily invade the surrounding tissue and migrate extensively into the normal brain. The researchers genetically modified human neural stem cells to produce a protein—cytosine deaminase—that converts a nontoxic precursor drug into an active form that kills cancer cells. The engineered neural stem cells were then injected into the brains of mice with human-derived gliomas. Within two weeks of the gene therapy and systemic treatment with the precursor drug, the tumors had shrunk by 80 percent. The animal studies also revealed that neural stem cells were able to quickly and accurately "find" glioma cells, regardless of whether the stem cells were implanted directly into the tumors, implanted far from the tumors (but still within the brain), or injected into circulating blood outside the brain [1].

Another cell-based gene therapy system under investigation involves the use of osteoblasts, or bone-forming stem cells. In a recent preliminary study examining a gene therapy approach to bone repair and regeneration, researchers genetically engineered osteoblasts to produce a bone growth factor. The osteoblasts were added to a biodegradable matrix that could act as a "scaffold" for new bone formation. Within a month after the cell-impregnated scaffold was implanted into mice, new bone formation was detectable. Although this work is in the very early stages, it offers hope of an effective alternative to conventional bone-grafting techniques [14].

HOW EMBRYONIC STEM CELLS MIGHT PLAY A ROLE IN GENE THERAPY RESEARCH

With one notable exception, no therapeutic effects have been achieved in gene therapy trials to date. The first successful gene therapy occurred in a recent French study in which a therapeutic transgene for correcting X-linked severe combined immune deficiency was introduced into the bone marrow cells of children, resulting in improved function of their immune systems and correction of the disease [5]. This encouraging success aside, the generally disappointing results are due, in part, to the inherent limitations of adult and cord blood stem cells. In principle at least, the use of human embryonic stem cells might overcome some of these limitations, but further research will be needed to determine whether embryonic stem cells are better suited to meet the needs of gene therapy applications than are adult stem cells.

One important feature of the optimal cell for delivering a therapeutic transgene would be its ability to retain the therapeutic transgene even as it proliferates or differentiates into specialized cells. Most of the cell-based gene therapies attempted so far have used viral vehicles to introduce the transgene into the hematopoietic stem cell. One way to accomplish this is to insert the therapeutic transgene into the one of the chromosomes of the stem cell. Retroviruses are able to do this, and for this reason, they are often used as the vehicle for infecting the stem cell and introducing the therapeutic transgene into the chromosomal DNA. However, mouse retroviruses are only efficient at infecting cells that are actively dividing. Unfortunately, hematopoietic stem cells are quiescent and seldom divide. The percentage of stem cells that actually receive the therapeutic transgene has usually been too low to attain a therapeutic effect. Because of this problem, investigators have been exploring the use of viral vehicles that can infect nondividing cells, such as lentiviruses (e.g., HIV) or adeno-associated viruses. This approach has not been entirely successful, however, because of problems relating to the fact that the cells themselves are not in an active state [13, 19].

One approach to improving the introduction of transgenes into hematopoietic stem cells has been to stimulate the cells to divide so that the viral vehicles can infect them and insert the therapeutic transgene. Inder Verma of the Salk Institute has noted, however, that this manipulation can change other important properties of the hematopoietic stem cells, such as plasticity, self-renewal, and the ability to survive and grow when introduced into the patient [23]. This possibility might be overcome with the use of embryonic stem cells if they require less manipulation. And in fact, some preliminary data suggest that retroviral vectors may work more efficiently with embryonic stem cells than with the more mature adult stem cells. For example, researchers have noted that retroviral vectors introduce transgenes into human fetal cord blood stem cells more efficiently than into cord blood stem cells from newborns, and that the fetal cord blood stem cells also had a higher proliferative

capacity (i.e., they underwent more subsequent cell divisions). This suggests that fetal cord blood stem cells might be useful in cell-based in utero gene therapy to correct hematopoietic disorders before birth [15, 21].

In some cases—such as a treatment of a chronic disease—achieving continued production of the therapeutic transgene over the life of the patient will be very important. Generally, however, gene therapies using hematopoietic stem cells have encountered a phenomenon known as "gene silencing," where, over time, the therapeutic transgene gets "turned off" due to cellular mechanisms that alter the structure of the area of the chromosome where the therapeutic gene has been inserted [6, 7, 11, 22, 24]. Whether the use of embryonic stem cells in gene therapy could overcome this problem is unknown, although preliminary evidence suggests that this phenomenon may occur in these cells as well [8, 18].

Persistence of the cell containing the therapeutic transgene is equally important for ensuring continued availability of the therapeutic agent. Verma noted that the optimal cells for cell-mediated gene transfer would be cells that will persist for "the rest of the patient's life; they can proliferate and they would make the missing protein constantly and forever" [23]. Persistence, or longevity, of the cells can come about in two ways: a long life span for an individual cell, or a self-renewal process whereby a short-lived cell undergoes successive cell divisions while maintaining the therapeutic transgene. Ideally, then, the genetically modified cell for use in cell-based gene therapy should be able to self-renew (in a controlled manner so tumors are not formed) so that the therapeutic agent is available on a long-term basis. This is one of the reasons why stem cells are used, but adult stem cells seem to be much more limited in the number of times they can divide compared with embryonic stem cells. The difference between the ability of adult and embryonic stem cells to self-renew has been documented in the mouse, where embryonic stems cells were shown to have a much higher proliferative capacity than do adult hematopoietic stem cells [25].

Researchers are beginning to understand the biological basis of the difference in proliferative capacity between adult and embryonic stem cells. Persistence of cells and the ability to undergo successive cell divisions are in part, at least, a function of the length of structures at the tips of chromosomes called telomeres. Telomere length is, in turn, maintained by an enzyme known as telomerase. Low levels of telomerase activity result in short telomeres and, thus, fewer rounds of cell division—in other words, shorter longevity. Higher levels of telomerase activity result in longer telomeres, more possible cell divisions, and overall longer persistence. Mouse embryonic stem cells have been found to have longer telomeres and higher levels of telomerase activity compared with adult stem cells and other more specialized cells in the body. As mouse embryonic stem cells give rise to hematopoietic stem cells, telomerase activity levels drop, suggesting a decrease in the self-renewing potential of the hematopoietic stem cells [3, 4]. (For more detailed information regarding telomeres and telomerase, see Figure C.2. Telomeres and Telomerase.)

Human embryonic stem cells have also been shown to maintain pluripotency (the ability to give rise to other, more specialized cell types) and the ability to proliferate for long periods in cell culture in the laboratory [2]. Adult stem cells appear capable of only a limited number of cell divisions, which would prevent long-term expression of the therapeutic gene needed to correct chronic diseases. "Embryonic stem cells can be maintained in culture, whereas that is nearly impossible with cord blood stem cells," says Robert Hawley of the American Red Cross Jerome H. Holland Laboratory for Biomedical Sciences, who is developing gene therapy vectors for insertion into human hematopoietic cells [12]. "So with embryonic stem cells, you have the possibility of long-term maintenance and expansion of cell lines, which has not been possible with hematopoietic stem cells."

The patient's immune system response can be another significant challenge in gene therapy. Most cells have specific proteins on their surface that allow the immune system to recognize them as either "self" or "nonself." These proteins are known as major histocompatibility proteins, or MHC proteins. If adult stem cells for use in gene therapy cannot be isolated from the patient, donor cells can be used. But because of the differences in MHC proteins among individuals, the donor stem cells may be recognized as nonself by the patient's immune system and be rejected.

John Gearhart of Johns Hopkins University and Peter Rathjen at the University of Adelaide speculate that embryonic stem cells may be useful for avoiding such immune reactions [10, 20]. For instance, it may be possible to establish an extensive "bank" of

embryonic stem cell lines, each with a different set of MHC genes. Then, an embryonic stem cell that is immunologically compatible for a patient could be selected, genetically modified, and triggered to develop into the appropriate type of adult stem cell that could be administered to the patient. By genetically modifying the MHC genes of an embryonic stem cell, it may also be possible to create a "universal" cell that would be compatible with all patients. Another approach might be to "customize" embryonic stem cells such that cells derived from them have a patient's specific MHC proteins on their surface and then to genetically modify them for use in gene therapy. Such approaches are hypothetical at this point, however, and research is needed to assess their feasibility.

Ironically, the very qualities that make embryonic stem cells potential candidates for gene therapy (i.e., pluripotency and unlimited proliferative capacity) also raise safety concerns. In particular, undifferentiated embryonic stem cells can give rise to teratomas, tumors composed of a number of different tissue types (see Chapter 10. Assessing Human Stem Cell Safety). It may thus be preferable to use a differentiated derivative of genetically modified embryonic stem cells that can still give rise to a limited number of cell types (akin to an adult stem cell). Cautions Esmail Zanjani of the University of Nevada, "We could differentiate embryonic stem cells into, say, liver cells, and then use them, but I don't see how we can take embryonic stem cells per se and put genes into them to use therapeutically" [26].

Further research is needed to determine whether the differentiated stem cells retain the advantages, such as longer life span, of the embryonic stem cells from which they were derived. Because of the difficulty in isolating and purifying many of the types of adult stem cells, embryonic stem cells may still be better targets for gene transfer. The versatile embryonic stem cell could be genetically modified, and then, in theory, it could be induced to give rise to all varieties of adult stem cells. Also, since the genetically modified stem cells can be easily expanded, large, pure populations of the differentiated cells could be produced and saved. Even if the differentiated cells were not as long-lived as the embryonic stem cells, there would still be sufficient genetically modified cells to give to the patient whenever the need arises again.

Achieving clinical success with cell-based gene therapy will require new knowledge and advances in several key areas, including the design of viral and nonviral vehicles for introducing transgenes into cells, the ability to direct where in a cell the transgene is introduced, the ability to direct the genetically modified stem cells or the secreted therapeutic agent to diseased tissues, optimization and regulation of the production of the therapeutic agent within the stem cell, and management of immune reactions to the gene therapy process. The ability of embryonic stem cells to generate a wide variety of specialized cell types and being able to maintain them in the laboratory would make embryonic stem cells a promising model for exploring critical questions in many of these areas.

"There are possibilities of long-term maintenance and expansion of embryonic stem cells and of differentiation along specific lineages that have not been possible with hematopoietic stem cells," Zanjani says. "And if they [embryonic stem cells] could be used [in the laboratory] as a model for differentiation, you could evaluate … vectors for gene delivery and get an idea of how genes are translated in patients." Cynthia Dunbar, a gene therapy researcher at the National Institutes of Health, similarly notes that embryonic stem cells could be useful not only in screening new viral and nonviral vectors designed to introduce therapeutic transgenes into cells, but especially for testing levels of production of the therapeutic agent after the embryonic stem cells differentiate in culture [9]. Explains Dunbar, "These behaviors are hard to predict for human cells based on animal studies … so this would be a very useful laboratory tool." Indeed, the major contribution of embryonic stem cells to gene therapy may be to advance the general scientific knowledge needed to overcome many of the current technical hurdles to successful therapeutic gene transfer.

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