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Gene and Stem Cell Therapy: Alone or in Combination?

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ABSTRACT

Introduction: Both gene and stem cell therapies hold great promise in the treatment of many genetic diseases and are currently focus of interest for many investigators. While both approaches are offering great and valuable treatment options for devastating and life-threatening diseases, they hold much greater promise in combination. ***Methods:*** As there are multiple options in selecting gene transfer vehicles among the non-viral and viral vectors, there are also many options among the different transplantable cell types ranging from lineage-restricted progenitor cells to multipotent and pluripotent stem cells. Here, combination of the gene therapy and stem cell therapy is discussed. ***Results:*** Several successful gene and stem cell therapies have been reported both in animal and human trials. Combination of the gene therapy and stem cell therapy can be carried out sequentially where the cell transplantation and the *in vivo* gene therapy are accomplished one after the other; or, as it is more commonly practiced, they can be carried out as *ex vivo* gene therapy where the transplantable cells are genetically modified outside the body before being transplanted into the body. ***Conclusion:*** The combination of the stem-cell technology with gene therapy has the potential of providing both regenerative tissue and therapeutic material simultaneously; therefore, having the benefits of both technologies.

Introduction

Gene therapy consists of the introduction of genetic material into cells for a therapeutic purpose. Stem cells can be defined operationally as cells that can continuously self-renew and have the potential to generate intermediate and mature cells. Various stem cell populations could be restricted to particular developmental stages or cell types. The initial aim of the field of gene therapy was the treatment of inherited genetic diseases by providing a functional copy of the deficient gene. Stem cells on the other hand, were mostly appropriate for tissue repair. However, there are many overlapping applications of these technologies. Both gene and stem cell therapies can be applied to the treatment of genetic and acquired diseases. Bringing two methodologies together takes full advantage of their treatment capabilities.

Gene therapy

Gene therapy can be broadly defined as a transfer of genetic material into the individual's cells for therapeutic purposes. In a patient with a genetic disease, this will result in the expression of missing protein to cure their genetic defects or at least to improve their clinical status. The strategy can also be applied to prevent disease by changing the pattern of gene expression in the host cells. The transfer of the normally functioning gene into the patient's cells is carried out using non-viral or viral vectors. The process is simple in concept, but it is quite challenging in practice. Many biological obstacles must be surmounted. Non-viral vectors are considered safe, since unlike the viral vector, there is no possibility of recombination and production of virulent viruses (Oligino *et al.* 2000). However, they are much less efficient in transferring the target gene into the host cells (Salyapongse *et al.* 1999). Non-viral gene therapy can be carried out by injections of naked DNA such as plasmids, liposomes-mediated gene transfer or propulsion of DNA-coated microprojectiles (Oligino *et al.* 2000). Finally, DNA can be loaded onto a porous biomaterial scaffold (gene-activated matrix) and sent directly into the cells (Bonadio 2000; Bonadio 2002; Warren *et al.* 2002).

More commonly used technology in transferring exogenous genetic material into the targeted cells is the application of the viral vectors. Viruses are evolved through the course of evolution to enter the cells, often with great specificity to special cell types. They are efficiently able to transfer their genome into a host cell, and use the cellular machinery for DNA replication, protein synthesis and thereby production of more viral particles. In order to use these viruses as a vehicle to transfer the

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therapeutic genes into the target cells, first the endogenous disease-causing genes must be removed from viral genome. Then the therapeutic gene must be inserted into the genome. The altered genome is then ready to be packaged into the viral coat. Such replication-defective virus is now ready to take the packaged gene into the target cells where instead of producing viral toxins and causing disease, they will express the therapeutic gene and will produce the protein or enzyme which was missing before viral transduction (Robbins and Ghivizzani, 1998).

Based on the nature of the viral genome, gene therapy vectors can be divided into RNA and DNA viral vectors. The majority of RNA-based viral vectors have been derived from simple retroviruses like murine leukemia virus (MLV) or from lentiviruses, such as human immunodeficiency virus (HIV). The major advantage of the lentiviruses over the MLV, that are only able to transduce the dividing cells, is that they are able to transduce both dividing and non-dividing cells. Among the DNA viruses, used as gene therapy vectors, adenoviruses and adeno-associated viruses (AAV) are the most commonly used ones. Each category of the viral vectors has their advantages and shortcomings. Thus, the choice of the viral vector will depend on the nature of the therapy and types of the targeted cells. Our recent experiments (unpublished) demonstrate that AAV serotype rh10 has much broader distribution throughout the central nervous system (CNS), and higher transgene expression in comparison with the previously used retroviral and AAV serotypes 1, 2, and 5 (Lin *et al.* 2005; Rafi *et al.* 2005; Strazza *et al.* 2009).

Stem cell therapy

Stem cells are primitive cells with the capacity to self-renew and the ability to differentiate into different cell types. The most primitive stem cells are embryonic stem (ES) cells that are totipotent and can normally form all kinds of cells and tissues of an organism and therefore may have broad applications as they are able to differentiate into all cells that arise from the 3 germ layers. These cells are produced in the inner cell mass (ICM) of the 5-day-old blastocyst in mammal's development. Lower down the road, are the pluripotent stem cells that can turn into (or) differentiate into any cell within a germ layer and multipotent or adult stem cells localized in most adult tissues. These cells can produce a limited range of cell lineages; therefore, they are called multipotent stem cells. Finally, progenitors are the unipotent stem cells that are committed to generate only one specific cell type. The differentiation ability of stem cells to various other tissue types is referred to as plasticity that range from totipotency to pluripotency to multipotency to unipotency. Because of their capacity of unlimited expansion and their plasticity, stem cells are also widely used in regenerative medicine. Autologous stem cells are obtained from one's own body for surgical procedures. Most adult stem cells are multipotent and are generally referred to by their tissue origin such as mesenchymal stem cell, adipose-derived stem cell, or neural stem cells (NSCs).

Recently, pioneering work has revealed that terminally differentiated somatic cells can be reprogrammed to generate induced pluripotent stem (iPS) cells via overexpression of a defined set of transcription factors (Takahashi *et al.* 2007). These iPS cells are morphologically and phenotypically similar to ES cells and thus offer exciting possibilities in stem cell research and regenerative medicine. Their potential application in stem cell therapy has to be explored. The most widely used set of reprogramming factors, Oct4, Sox2, Klf4 and c-Myc, in producing iPS cells, was identified initially by screening 24 pre-selected factors in mouse embryonic fibroblasts (Takahashi and Yamanaka 2006). Cocktail of these four transcription factors was shown to work for different types of somatic cells and for different species, including rhesus monkey (Liu *et al.* 2008) and human cells (Takahashi *et al.* 2007). The method of producing such cells is striking in that it can convert somatic cells directly into pluripotent cells. However, the feasibility of using iPS cells as therapeutic tools has to be established.

Methodology

It is likely that most gene transfer strategy to stem cells will be performed in *ex vivo*, with subsequent delivery of the genetically modified stem cells or differentiated cells to the body. Although, a persistent expression of the transferred genes is required to correct genetic diseases but in some applications, such as tissue repair after an injury, transient expression of the transgene is preferable (Strulovici *et al.* 2007).

Although many hereditary disorders can be targeted by gene therapy alone, the combination of gene therapy and stem cell therapy may have additional efficacy, where the modified cells would be able to migrate and would act as factories to produce the transgene product. These cells can be delivered to an appropriate location, differentiate to the correct cell type to have the desired impact. *Ex vivo* gene therapy offers several unique advantages over the direct gene transfer into the body. Since the addition of the therapeutic transgene to the delivery cells takes place outside the patient's body, it allows researchers to select and work only with those cells that contain the transgene and produce sufficient quantity of the therapeutic agent. The other advantage of this combined strategy is that it allows the investigators to engineer an expression-controllable cell line where the pro-

duction of the therapeutic agents can be turned on or off at different time points. Even the intensity of the expression can be adjusted at a desired level. In this case, the therapeutic transgene would be active or partially active only in response to certain signals, such as drugs administered to the patient to turn the therapeutic transgene on and off. Hematopoietic stem cells (HSCs) and several other types of stem cells including mesenchymal stem cells (MSCs), neural stem cells (NSCs), muscle-forming stem cells known as myoblasts and osteoblasts are being extensively studied as gene-delivery candidates.

Hematopoietic stem cells

Among the different accessible stem cell, HSCs are most desirable cells in stem cell therapy domain for several reasons. First, although small in number, they are readily removable from the circulating blood in adults or the umbilical cord blood of newborn infants. In addition, they can be easily isolated and manipulated in laboratory before returning to patient's body. The HSCs not only can give rise to different types of blood cells but they can also reside in several remote spots such as liver, spleen, and lymph nodes. This is important especially when the goal is to deliver the therapeutic materials in these locations. Microglia differentiated from the genetically modified HSCs can also settle in CNS to deliver protein or RNA from the transgene that they carry. This is the logic of using bone marrow transplantation (BMT) in the treatment of some neurodegenerative disorders (Priller *et al.* 2001).

Mesenchymal stem cells

MSCs are considered to be a promising platform for cell and gene therapy for a variety of diseases. Their ability to self-renew at a high proliferation rate makes these cells ideal targets for retrovirus-mediated transgene delivery (Deans and Moseley 2000). Genetically modified MSCs have been subject of investigations in rodent models of neurodegenerative disorders, such as Parkinson's disease (Dezawa *et al.* 2005), and lysosomal storage disorders (Martino *et al.* 2002; Jin and Schuchman 2003; Sakurai *et al.* 2004). Although they are capable of differentiating along multiple lineages, and have significant expansion capability, at least in *in vitro* condition, the expanded MSCs have so far failed to provide long-lasting therapeutic effects. Therefore, additional investigations are needed to explore their *in vivo* performance.

Neural stem cells

The differentiation potential of NSCs, and their capacity to be genetically modified for the purpose of cellular transplantation, has been encouraging topic in the treatment of several neurodegenerative disorders. NSCs have the ability of long-distance migration and cellular integration after transplantation (Jandial *et al.* 2008). These properties can be exploited for delivery and expression of therapeutic genes. It has been shown that the NSCs can be isolated from CNS, proliferated in cultured media, genetically modified and re-implanted back into the brain, where its progeny could integrate perfectly, differentiate into both neuronal and glial cells, and import foreign genes into the CNS (Behrstock *et al.* 2006). Neural cells with the properties of stem cells have been isolated from the embryonic, neonatal, and adult rodent and human CNS using several different *in vitro* expansion methods. When NSCs are maintained in specific culture conditions, they can form floating clusters called neurospheres. The ability of these cells to form "spheres" can suggest that the cells are actively proliferating, which is an essential requirement for the stem cell. The differentiation potential of the cells is confirmed by removing the growth factors from the media and plating them on an adhesive substrate such as fibronectin, laminin and including serum in the media. Transplantation of the genetically modified NSCs has also been shown effective in the treatment of brain cancer cells. The animal studies reveal that NSCs are able to quickly and accurately "find" glioma cells, regardless of whether the stem cells were implanted directly into the tumors, or far from the tumors, or even injected into circulating blood outside the brain (Aboody *et al.* 2000).

Myoblasts

Among the candidate targets for gene therapy are the muscle stem cells or myoblasts. Muscles are highly vascularized making them very attractive target for systemic delivery of the transgene products (Ozawa *et al.* 2000). Genetically modified myoblasts can be used to produce locally acting or circulating therapeutic proteins therefore, preventing or treating an array of other diseases such as cancer, heart failure, and kidney disease by using the muscle as a factory for producing the desired protein. 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 non-muscle disorders such as neurodegenerative diseases.

Osteoblasts

Delivery of gene products to osteoblasts or bone forming stem cells represents a novel method to manipulate biological pathways responsible for osteogenesis (Laurencin *et al.* 2001). Such methods may promote osteogenesis, thus accelerating fracture healing. In recent studies, using adenoviruses or other viral vectors, genetically engineered osteoblasts were used to produce a bone growth factor. Adenovirus-mediated gene therapy of osseous tissues provides significant advantages over other modes of gene therapy since adenovirus transduction efficiency is very high, and the virus is unable to integrate into the

host genome, therefore, avoids potential problems associated with insertional mutagenesis. The latter point is particularly important for regenerative therapies because the viral genome is not replicated with host cells and will eventually be lost by dilution after multiple cell divisions.

Limitations of gene and cell therapy

Gene therapy is not without serious risks; the safety of the practice is the major concern. Before gene therapy becomes a routine and successful treatment option, some potential adverse issues should be dealt with. These include possibility of immune and inflammatory responses from the host, toxicity from over expression of the transgene or lack of enough expression and risk of insertional mutagenesis in the case of the integrating viral vectors. In addition, there is always the fear that the viral vector, once inside the patient, may recover its ability to cause disease.

Immune response

Humans are well protected against the invasion of introduced genetic or therapeutic materials. This has been an evolutionary adaptation designed to maintain our genetic stability. Anytime a foreign object is introduced into human tissues, the immune system is designed to attack the invader. The risk of stimulating the immune system in a way that reduces gene therapy effectiveness is always a potential risk (Gerecht-Nir and Itskovitz-Eldor 2004).

Control over gene expression

For gene transfer to be effective and safe, the expression of transgenes must be controlled so that neither too little nor too much of the gene product is generated. Loss of transgene expression few weeks after gene therapy, called gene silencing, is a common and not fully understood phenomenon. Although, temporary and self-limiting gene expression could be useful in some treatment strategies such as the treatment of musculoskeletal injuries, in which only transient high levels of growth factors are needed to promote a healing response, it is not ideal in most of gene therapy objectives. Many research projects are currently focusing on the development of specific inducible promoters that regulate the messenger RNA transcription. Such inducible promoters could help to control the expression of the transferred gene; they could modulate implanted genes as well as turn them on and off (Ramezani *et al.* 2003). Although these systems are very attractive, they remain under extensive investigation in many laboratories and are not yet ready for clinical trials.

Insertional mutagenesis

Retroviral and lentiviral gene transfer vectors randomly integrate into the genome of cells that they infect, along with the genetic cargo they carry (Baum *et al.* 2003; Ferguson *et al.* 2005; Hendrie and Russell 2005; Sinn *et al.* 2005). The expression of the transgene is therefore likely to last for the life-time of the cell. This is a powerful feature of these vectors, especially for rapidly and regularly dividing cell types where each daughter cell receives a copy of the genetic payload at cell division.

However, when techniques that rely on the random integration of exogenous DNA into the human genome are used for gene transfer, the risk of insertional mutagenesis remains a major issue. Some insertions may disrupt genes or perturb their transcription, altering the biological properties of the transduced cell. This may occur when insertion activates a proto-oncogene and triggers cell transformation, as has been reported in some gene therapy applications (Yu *et al.* 2007). Strategies that facilitate the targeted and site-specific integration of the transgene in the host genome would be of a significant importance in safe practice of gene therapy. An ideal site for transgene integration will allow robust and stable transgene expression across different cell types, and no disruption of regulatory or coding sequences.

Conclusion and future directions

Gene therapy and stem cell research have become areas of great importance. Stem cells are of great benefit to cell-based gene therapy because they are self-renewing and thus might reduce or eliminate the necessity for repeated administrations of the therapeutic cells. The overall goal of gene therapy is to cure diseases caused by malfunctioning genes. It does so by substituting the function of a normal gene for the defective gene. Until now, the most commonly used procedure in human gene therapy clinical trials is the insertion of a normal copy of the target gene in a nonspecific location into the host genomic DNA. Gene therapy was originally conceived as a means of correcting only hereditary disorders (Friedmann 1996). However, more recent work is applying the same gene transfer technologies to situations where there is no underlying genetic defect, but, instead, a need to produce sustained amounts of a biologically active molecule.

After three decades since gene therapy was firstly proposed, the transfer of genes into higher organisms remains an enormous technical challenge. While the viral vectors remain the most popular vehicle of choice, because of their efficient transduction, some critical limitations should be addressed. These shortcomings include their relatively small capacity in carrying large transgenes, lack of expression control, random genome insertion, transcriptional silencing and positional effects (Challita and Kohn 1994). Additional hurdles to the successful therapeutic application of the genetically mod-

ified stem cells include whether the cells themselves, or their transgene product, will be toxic or immunogenic in the recipient. A recipient cell who has never been exposed to the protein product before, may demonstrate an immune reaction against it, limiting the effectiveness of the therapy.

Generating a safe and versatile viral vector with stable and regulated gene expression is the focus of many laboratories at this time to address the current shortcomings. Therefore, major advances in vector development can be expected in the near future (Robbins and Ghivizzani 1998). While gene therapy may represent the only treatment option for severe disorders such as cancer, and many monogenic inherited disorders, the risk of side effects may be unacceptable in elective reconstructive surgery.

In conclusion, combination of gene and stem cell therapy has the potential to be much more powerful technology, but certain obstacles must be overcome before the combination can be effectively applied.

Ethical issues

None to be declared.

Conflict of interests

No conflict of interests to be declared.

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