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Some Aspects of Gene Therapy in Serious Diseases

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ABSTRACT

Among the most leading top 3 causes of death world wide are cancer, coronary heart disease and diabetes. Improvements in surgical, development of new treatment strategies and radio therapeutic techniques have improved outcome of many diseases. Even though, 50% of patients still die from these diseases. Advances in gene therapy have been explored as possible modality of these diseases treatments. The goal of gene therapy is to modify a gene or genetic pathway to provide therapeutic value as well as it is important to develop a method that is safe and effective for the treatment of human diseases.The objectives of this article are to provide a short overview of gene therapy for some serious diseases, discuss most recent advances in gene therapy as well as imaging techniques that are utilized to monitor patients' response to the therapies.

GENE THERAPY

A gene is the basic physical and functional unit of heredity. Gene is made up of DNA; it acts as instructions to make proteins. In humans, genes vary in size from a few hundred DNA bases to more than 2 million bases. It has been estimated that human has between 20,000 and 25,000 genes. Human DNA consists of about 3 billion bases, and more than 99 percent of those bases are the same in all individuals. The sequence of these bases determines the information and expressed the phenotypic traits of organisms. Gene therapy is the treatment of disease by repairing or reconstructing defective genetic material. This technique was designed to correct genetic disorders caused by abnormalities in a single gene, a combination of several genes, or chromosome structure. Modern gene therapy can be defined as a technique that replaces one gene with another normal or therapeutic one. Although gene therapy has been used for several decades, but so far it has not produced any clear-cut therapeutic results. Each year, an estimated 3 millions babies worldwide, have birth defects. More than 6 thousands single-gene disorders are currently known. Gene therapy is the therapeutic delivery of virus gene into a patient's cells to treat and cure diseases. DNA or RNA, which contains genetic information, is the main component of viruses, which are capable to exploit human cells. They penetrate into human cells, integrate their genetic information into the host genome, and use the cells to reproduce themselves. Most current techniques generally require millions of copies of therapeutic a gene per one cell, with the hopes that at least one copy will be successfully integrated. Nowadays, the developments of global biotechnology system to investigate, diagnose and identify genes responsible for diseases become vital issue. Therefore, the biotechnological know-how is needed to integrate current technology, to promote identifying gene responsible to diseases resistance and/or therapy [1-3]. Gene therapy is known as one of the most advanced approaches for therapeutic prospects tackling and combating genetic diseases such as cancer. In this approach, different viral and non-viral vector systems such as retrovirus, plasmid and transposon have been designed and employed. These vector systems are designed to target abnormal genes in tumor cells. Detection of the vectors containing therapeutic genes and monitoring of response to the treatment are the main issues that are commonly faced by researchers. Imaging techniques have been critical in guiding physicians in the more accurate and precise diagnosis and monitoring of cancer patients in different phases of malignancies. Imaging technique is safe and powerful tool for monitoring of the distribution of the transplanted gene, its expression over time and assessing patients who have received therapeutic gene [4,5]. When the gene therapy is a successful trial, the new gene will make a functional protein to treat and cure the disease. The first therapeutic use of gene transfer and direct insertion to human DNA was performed by French Anderson in 1990 [6].

CANCER GENE THERAPY

Cancer of all kinds is one of the main problems in public health worldwide. All cancers arise as the result of uncontrolled cell division. Some genes (oncogenes) can induce uncontrolled cell division. In contrast, other genes (tumor suppressor genes) serve to control cell division, be used to fight cancers. Despite advances in the diagnosis and treatment of cancer, the efficiency of current treatments is still limited. Moreover, current understanding of this disease brings a number of therapies that can be useful in its treatment at least in many patients. Reports indicated that although there were too many successful stories, achievements and progress made in medical scientific research, but till now many problems are facing cancer prevention and early detection strategies. Improvements in traditional surgical and radio therapeutic techniques as well as new medications decrease the mortality rate, but unfortunately still 50% of patients still die from different kinds of cancers.With the advances in biotechnology, gene therapy has been explored as a possible modality of many diseases treatment. To date, over 600 gene therapy clinical trial protocols have been activated in USA [7-9]. The most advantage of gene therapy for cancer treatment is the specific targeting of the tumor site, hence reducing unwanted systemic toxicity of drug therapy. This advantage relies on extremely well-regulated mechanisms that control the accuracy of gene delivery and expression at the specific targeted site(s). The efforts in developing new therapeutic viral gene make molecular medicine potentially promising. However, the success of this approach very much relies on a high efficiency of gene expression at the tumor site(s) in order to achieve therapeutic benefit [10]. Gene and cell therapies have emerged as effective approaches. One of the most important challenges for cancer gene and cell therapies is correct monitoring of the modified genes. Molecular imaging has been found to be powerful tools in monitoring cancer patients who have received therapeutic gene therapies. The utilization of molecular imaging techniques showed to have several advantages, such as the ability to accurately track administered therapeutic vectors in cancer patients.In addition, imaging techniques can be used for assessing responses to treatment in cancer patients. Hence, these techniques can open a new window of opportunity for tracking, monitoring and management of cancer patient [11]. Recently, Chimeric Antigen Receptor (CAR) T cells have come to be the latest designer 'drug' for treatment of cancer. This technology has generated multiple apparent cures in the treatment of B-cell malignancies, but has had much less impact in solid tumors [12]. Nevertheless, gene therapy is still in its beginning stage but it is full of potential. But there are possible ways to improve results in the future practice.The quickest way is using combination of existing treatment modalities. This technique suggests a multimodality approach to be involved in therapeutic of cancer, integrating curative resection, including concurrent or sequential gene therapy, chemotherapy and radiotherapy [7]. Furthermore, colorectal cancer is the second most common type of malignancy.

Improvements in surgical and radiotherapeutic techniques and the increased availability of new cytotoxic drugs have improved outcome, but 50% of patients still die from recurrent or metastatic disease. Several features of its natural history render colorectal cancer a good candidate for gene therapy. Techniques include gene replacement, virus-directed enzyme–prodrug therapy, immune manipulation and virus-therapy, all of which have entered clinical trials [13].

CARDIOVASCULAR DISEASES

Coronary artery disease, heart failure, and cardiac arrhythmias are considered the major cause of morbidity and mortality in the world. The overall death rate for all cardiovascular diseases in USA is 236.1 per 100 000 persons [14,15]. Pharmacologic drugs therapy has some limitations, and need to be improved without side effects, as many patients are still left with significant morbidity despite those therapies. New treatment modality, such as the development of gene transfer vectors has given researchers the tool to target specific genes that play a role in cardiovascular diseases. Cardiovascular disease is the second most common application for gene therapy clinical trials, which most frequently employ Adenovirus Serotype 5 (Ad5) - based vectors as delivery vehicles. Although interactions of Ad5 vectors with circulating proteins and cells can limit their efficacy after systemic administration, local gene delivery strategies show great potential in the cardiovascular setting, notably in the context of vascular delivery. Studies suggested that myocardial gene transfer can improve angiogenesis with vascular endothelial growth factor or fibroblast growth factor, which induce cardiac repair and control heart rate. Successful delivery of a gene to the target cell or organ is paramount to therapeutic efficacy. A number of gene delivery methods have been developed using both viral- and non-viral-based vectors. They are easy to produce and are used extensively for treatment that does not require high-density gene transfer. The gene of interest does not need to encode a specific protein to be of therapeutic value. Viral vectors are more commonly used for cardiovascular therapeutic because they transfer genes to cardiac myocytes much more efficiently than any of the non-viral methods [16-19].

DIABETES

A total of 415 million people had been estimated to have diabetes worldwide during 2015. Furthermore, from 2012 to 2015, approximately 1.5 to 5.0 million deaths each year resulted from diabetes [20,21]. It is well known that diabetes is a group of metabolic disorder. If not treated, diabetes can cause many complications which may lead to death. The cause of diabetes is genetic defect of β-cell function and consequently, defect in insulin processing and action. Prevention and treatment involve insulin injections, to lower blood sugar levels, besidesthere are many different classes of anti-diabetic medications in the market [22-24]. Most diabetes treatments work by giving the body the insulin it needs to break down blood sugar. In recent years, scientists have taken steps by attempting to stimulate or replace the cells in the pancreas responsible for producing insulin in the first place, researcher hoping to use gene therapya potentially one-time and long lasting treatment. Insulin gene therapy is approach that involves the introduction of a foreign gene into any cell type in the body, allowing it to produce insulin. The gene(s) introduced could be the insulin gene itself, perhaps under control of a tissue-specific promoter, allowing for expression in a select non–β-cell type, or a gene encoding a factor that in turn activates the insulin gene. Furthermore, new technique of implantation of β-cells (cell-replacement therapy) that involve the creation or expansion of insulin-producing cells in vitro followed by their implantation in the patient. The number of people suffering from diabetes is growing at curvilinear rate. One approach, which might be useful to treat diabetes, is gene therapy. The first gene therapy approach to diabetes was put forward shortly after the cloning of the insulin gene. Another gene therapy approach aims at genetically manipulating β-cells so that they produce a local β-cell protection factor. In individuals where autoimmune destruction of β- cells has begun, but not reached the end stage, it would make sense to rescue the remaining β-cells by such a gene therapy approach. Assuming that it is possible to target a vector to the β-cell *in vivo*, the resulting β-cell production of a local survival factor would not only save the β-cells, it would also leave the immune system in general unaffected as the transgenic production is localized to the islets. Recently, Doiron has developed a treatment consisting of three molecules (Syner-III): glucokinase, PTP1B and Pdx-1. These are transcription factors that regulate genes, when infused into the body to help stimulate the formation of new beta cells. Those molecules are administered via a gene therapy procedure, as they stuffed into a modified virus and injected directly into the pancreas in a one-time treatment. Furthermore, research showed a group of compounds called aminopyrazines could be packed into a pill and lead to more beta cells, and more insulin, in mice. However, results have succeeded in producing beta cells in mice, but failed to reproduce insulin in humans [25-27]. Recently researchers have suggested that a new form of gene therapy may provide future help for people with diabetes. To treat diabetes, gene therapy investigators are currently studying approaches to efficiently transfer the insulin gene into other cells such as the liver, stomach, or intestines. Alternatively, cell therapy approaches for this disease are focused on developing the most efficient methods for the isolation of pancreas β-cells or appropriate stem cells, appropriate location for cell transplant, and improvement of their survival upon infusion. Gene and cell therapy scientists are developing methods to reprogram some of the other cells of the pancreas to secrete insulin. It was reported that a successfully inserted human insulin gene into the genome of liver cells using cell cultures *in vitro*, which has resulted in producing insulin [28].

NEW MOVE TOWARD BETTER HORIZON OF GENE THERAPY

Gene therapy is as one of the most advanced approaches for therapeutic prospects of tackling diseases. In this approach, different viral and non-viral vector systems such as retrovirus, plasmid and transposon have been employed. These vector systems are designed to target different mutant (abnormal) genes in various tissues and cells such as tumor cells [29]. Selection of suitable delivery system is one of the crucial aspects in gene therapy that determines the efficiency of gene therapy. The past two decades have witnessed extensive efforts for finding safe and efficient vectors to overcome the limitations of viral vectors. The utilization of DNA transposon-based vectors for gene therapy has emerged as a promising non-viral alternative. DNA 'cut-and-paste' is one of the main mechanisms of genome engineering by transposon elements. PiggyBac (PB) is known as a highly efficient DNA transposon. It has been shown that PB can be functional in various species including mammalian systems. This vector can overcome some limitations of other vectors in cancer gene therapy. Some advantages of PB include the capacity for integration into the genome and providing a stable expression, without a significant reduction in their transposition activity and display non-overlapping targeting preferences [30]. A part from gene therapy, Mesenchymal Stem Cells (MSCs), are multipotent stromal cells that can differentiate into a

variety of cell types, including: osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells) and adipocytes (fat cells). MSCs have been found in organisms (*in-vivo*) and have been studied as well in tissue culture (*in-vitro*), which will be a vital issue in the future [31]. There are promising results whereby MSCs can be used as cancer gene therapeutic biological vehicles in cell therapy have several advantages, including immune-silence, tumor tropism, easy and rapid isolation [32]. Molecular imaging is a type of medical imaging that provides detailed pictures of what is happening inside the body at the molecular and cellular level and to follow up the consequences gene therapy. It allows scientists to see how the body is functioning and to measure its chemical and biological processes. Furthermore, it is able to identify disease in its earliest stages and determine a patient's response to gene therapy. Imaging techniques can guide physicians in the more accurate and precise diagnosis and monitoring of cancer patients in different phases of malignancies. Imaging techniques are non-invasive and powerful tools for monitoring of the distribution of transgenic expression over time and assessing patients who have received therapeutic genes. Future technological progress in imaging techniques will help not only to develop safe and efficient gene therapy protocols for clinical application but also enable physicians to monitor therapeutic effects in cancer patients who have received therapeutic genes [4]. Recently, researchers from the National Institutes of Health and the University of Chicago improved the speed, resolution and light efficiency of an optical microscope by switching from a conventional glass cover slip to a reflective, mirrored cover slip and applying new computer algorithms to process the resulting data (High Resolution Optical Imaging, HROI). This technique is just like a lot "looking into a mirror" [33]. Finally, human genetic variation both within and among populations should be considered when applying stem cells and gene therapy as well as molecular imaging. There may be multiple variants (allele's polymorphisms) of any given gene in human population.The frequencies of alleles at a single locus vary among populations and within different generations within same population due to the evolutionary forces of natural selection, mutation, migration and genetic drift [34]. Furthermore, humans' populations showed diverse genetic make-up. Genes involved in the development of hair follicles, pigmentation, metabolic pathways, drug metabolism, susceptibility, disease resistance and immune system were found to have higher levels of population differentiation. Disease-related genes demonstrate excessive SNPs with high levels of population differentiation. In general, population differentiation under evolution is mostly influenced by demographic history, adaptation to a local environment, and selection [35]. Nevertheless, variation in alleles/genes expression is extensive among tissues, individuals, strains, populations and species. The interactions among these sources of variation are relevant for physiological studies such as disease or toxic stress; for example, it is common for pathologies such as cancer, heart failure and metabolic disease to be associated with changes in tissue-specific gene expression or changes in metabolic gene expression. Half of the genes (48%) were differentially expressed among individuals within a population-tissue group and 76% were differentially expressed among tissues. Result indicated that many tissue-specific differences in gene expression are unique to one population and thus are likely to contribute to fundamental differences between tissue types [36].

CONCLUSION

According to the above issues, it is concluded that the following considerations should be taken if gene or stem cells transplantation being successful process. As patients (known as the recipients of gene therapy) have multiple variants of any given gene, leading to polymorphism. No one gene or one stem cell can fit all patients. Moreover, disease-related genes demonstrate excessive Single Nucleotide Polymorphism (SNPs) with high levels of population differentiation. The gene expression also may vary extensively among tissues, individuals and populations. Research should be focused to identify compatibility of donor's gene (viral or non-viral vector systems) and that transplanted one into recipient patient related to immune system and function, as well as to consider variations in genes expression among the various tissues, differences among individuals and geographic populations.

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