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Problems with the Treatment of Rare Inherited Diseases of Humans and the Latest Therapeutic Methods

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ABSTRACT

This review briefly touches on bioengineering technologies that are causing a silent revolution in the treatment of inherited, including rare, diseases. Growing evidence suggests that rare DNA sequence variants, which are reported in greater numbers with the advances in sequencing technologies, may play an important role in the susceptibility to diseases. Along with the use of whole-genome and whole-exome sequencing data for the treatment of rare diseases, gene and cell therapies for rare diseases are gaining greater acceptance in clinical practice. There are active developments in the practical applications of the unlimited potential of various stem cell types—neural stem cells, mesenchymal stem cells, embryonic stem cells, and induced pluripotent stem cells—for the management of rare and common diseases. It should be noted that the problems with the application of mesenchymal stem cells (and other cells derived from induced pluripotent stem cells) are related to their unlimited differentiation potential. Probably, in the near future, the existing problems will be solved and stem cells will play an increasingly more important part in regenerative medicine. Clinical trials of the CRISPR/Cas9 technology are under way for the treatment of cancer and for human genome editing. Despite being at times controversial, the results of these studies are intriguing and promising. Undoubtedly, the use of modern biological technologies—genome-wide association studies (GWAS), new whole-genome and whole-exome sequencing methods, gene editing via CRISPR/Cas and therapies involving stem cells and induced pluripotent stem cells—will soon help humankind to get rid of monogenic inherited diseases, to precisely and effectively administer personalized treatment to each patient and to return them to fully functional life. Increasing specificity of delivery of genetic material to target cells is expected to make cancer treatments substantially more effective.

Keywords: Inherited disease, Genetic disorder, GWAS, Gene therapy, Stem cell, CRISPR/Cas

INTRODUCTION

There is no exact definition of rare diseases: in Europe, diseases are considered rare when they affect fewer than 5 out of 10000 people, whereas in Taiwan, a disease is regarded as rare if it affects 1 out of 10000 individuals [1] and in the US, diseases are defined as rare if they are present in fewer than 200 000 patients [2]. Since 2010, owing to the developments in sequencing technologies, the numbers of detectable genes and mutations have been growing by leaps and bound. The number of known genes associated with rare diseases is increasing too and reached 3573 as of May 2017 [3]. According to some estimates, by the end of 2017, approximately 8000 rare diseases had been documented worldwide [1] and 80% of them are genetically inherited uncurable pathologies afflicting a patient for life, thus shortening the lifespan and worsening quality of life. Moreover, 75% of rare diseases occur in children and 30% of these patients die within the first 5 years of life [4]. Given that the early diagnosis of diseases ensures the greatest reduction in mortality, the applicability of single-nucleotide polymorphism (SNP) markers of orphan diseases to the period of prenatal development represents a clear advantage for diagnosis as compared with the traditional methods based on signs and symptoms of a disease or its biochemical markers suggestive of disease development before clinical manifestations in the patient.

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The clinical search for SNP markers of orphan diseases is the most labor-intensive and expensive approach because of their low incidence [5]. For this reason, the known rare SNP markers have been discovered mostly by chance [6]. The greatest success has been achieved on SNP markers in protein-coding regions of genes because of permanent damage to the structure of the proteins encoded by these genes; this damage is easy to detect due to the absence or deficiency of the protein's function [6]. Meanwhile, regulatory SNPs are still the least studied SNP type because of variations in their clinical manifestation from cell to cell, from tissue to tissue, from patient to patient and among subpopulations, whereas their obvious biomedical advantage is the possibility of pharmacological correction of the clinical manifestations because the protein-coding part of the gene is intact. SNPs in regulatory regions may contribute to the development of complex diseases by changing, for example, 1) the binding affinity of transcription factors; 2) activity of enhances; 3) post-translational modifications of histones; and 4) interactions of enhancers with promoters. Emerging evidence indicates that rare variants of DNA sequences, which are documented in increasing numbers with the development of sequencing technologies, may play a more important role in the susceptibility to diseases than "common" variants can [7].

Rare diseases represent a serious economic burden regardless of a country's size and demographics. The reason is primarily the increasing healthcare expenditures [1] due to costly treatment of "rare" patients. Accordingly, we can conclude that research into specific genes of rare diseases is crucial for their diagnosis, and the importance of such studies is not diminished by comprehensive genome research.

A diagnosis is affected by various factors including the selection of patients during the setup of experimental case-control groups, the age bracket of the patients and genetic screening. A disease may be caused by somatic mutations, mutations in mitochondrial genes, and more complicated genetic aberrations. Besides, with Mendelian genetic disorders, there may be difficulties with the diagnosis when a genetically pathological variant is not yet defined as pathogenic. This may be because rare SNPs are filtered out and are not identified in GWASs or they are difficult to detect by the existing bioinformatic tools. It may also be impossible to make a diagnosis because a given DNA sequence variant is not defined as contributing to the disease or is not among the known genes associated with the disease.

GWAS (genome-wide association study) technologies—involving high-throughput genotyping and sequencing, accelerating whole-genome mapping for large groups of people—have made a major contribution to the main achievements in the field of genetic research. The use of GWAS results accelerate a diagnosis but explain only some genetic variation related to "common" SNPs and increases the percentage of false positive and false negative variants. A GWAS cannot register an association of rare SNPs with a low risk of a disease: this can be done by the "manual" method with direct experimental validation. Blanco-Gómez et al. [8] have stated that during analysis involving a GWAS, a substantial proportion of "missed or lost" genetic variants are not explained and neither are a substantial proportion of the resultant disease risks. There is a need for a specific and detailed analysis of each genetic variant to successfully detect a candidate SNP marker, to evaluate its influence on the susceptibility to a disease and to determine functional involvement.

In another study [9], it is pointed out that during detection of rare variants of genes associated with autism, mutations located in regulatory regions are difficult to identify and require annotations involving methods of direct sequencing in large groups of patients and healthy people to make a more accurate diagnosis. To ensure reliable discovery of rare variants and of their involvement in a disease, a large sample size is necessary, which is often impossible in the case of rare diseases affecting a tiny fraction of the population [10]. Lately, gene sequencing panels became available that enable testing of genes associated with a cancerous process and allow for choosing of patients with a mutation, for which a certain treatment strategy will be optimal. Researchers have stated that one of the disadvantages of this diagnostic test is high cost [11].

New possibilities have emerged with the application of new technologies of whole-genome and whole-exome sequencing, although the practical use of exome sequencing results is limited to protein-coding regions of the genome. The algorithms involved in this analysis make probabilistic estimates of a variant's pathogenicity until this variant is discovered in independent patients with identical clinical characteristics according to identical functional tests. Nonetheless, the use of both technologies can be successful for detection of rare variants. In addition to the results of whole-genome and whole-exome sequencing, physicians are starting to use gene therapy and cell therapy as well as the new CRISPR/Cas technology for the treatment of rare diseases.

Gene therapy, i.e., introduction of a genetic material into target cells is employed for correction of a DNA sequence responsible for a genetic disorder. Successful gene therapy requires correct and effective delivery of the new genetic information into target cells and this genetic material or cell type should be present in a sufficiently large amount and persist and replicate in the recipient, in order to maintain a desired therapeutic effect. The transmission of genes should overcome complex cellular and tissue barriers for delivery of the new genetic information into a target cell, in order to stimulate the expression of the delivered molecule without disruption of the main regulatory mechanisms. Gene therapy is used for treating many diseases, including cancer [12] and

monogenic [13], cardiovascular and neurodegenerative diseases [14]. Among the modern methods of gene delivery, there are viral vector systems for gene transduction [15], physical methods, including direct microinjections [16] and chemical methods involving nano-carriers (lipids, calcium phosphate and cationic polymers) [17]. As of late 2013, 1800 clinical trials of gene therapy were being conducted across the globe.

The first gene transfer with the first clear-cut results was carried out in 1995 on the Scandinavian Peninsula. The results indicated that the effective gene transfer into the human brain can be achieved via direct delivery of a gene in vivo [18]. In 2003, China became the first country to apply gene therapy to clinical treatment of cancer [19]. In 2004, healthcare group Ark received the first commercial certificate in the EU for the manufacture of gene therapy agents based on an adenoviral vector carrying the herpes simplex virus gene of thymidine kinase, intended for treating malignant brain tumors [20]. Originally developed for cancer treatment, gene therapy has expanded its applications from cancer to monogenic and rare diseases. Encouraging results have been obtained in clinical trials of gene therapy products for the treatment of thalassemia [21], Wiskott-Aldrich syndrome [22] and other diseases. Gene therapy has a great potential for the destruction of cancer cells without any damage to normal tissues. For this purpose, some investigators have developed various systems of delivery of chemotherapeutic agents into tumor cells. At present, much attention is focused on mesenchymal stem cells (MSCs) as carriers for gene delivery. The intrinsic characteristics of MSCs make them an especially attractive agent of cell therapy. They have low immunogenicity, thus overcoming the problem of immune rejection [23].

Vector constructs, both viral and non-viral, have found numerous applications as delivery agents. The use of viral vectors has given rise to the problem of patients' safety. The main risks of gene therapy have been and still are related to non-specific integration of a vector into regulatory or transcriptionally active regions of a gene, thereby possibly leading to mutagenesis and carcinogenesis. To prevent these problems in practical applications of vectors, researchers started to employ targeted incision of the genome by means of custom-made sequence-specific nucleases as well as insertion of a transgene into a predetermined genome site [24]. On the other hand, the development of good biological vectors that have low toxicity and high effectiveness is still the most prominent problem in the field of gene therapy.

One of the main drawbacks of non-viral vectors, which include cationic liposomes, polymers and nano-carriers [25], is the risk of an immune response [26], their low efficiency of transfection, and substantial toxicity (e.g., cytotoxicity, cellular necrosis, or erythrocyte aggregation). Many research groups are conducting new studies for increasing gene transfection efficiency and decreasing toxicity of cationic nano-carriers based on lipids (mostly by structural modification of lipids) [27] and are making some progress. The majorities of cationic polymers slowly degrades under physiological conditions and are slowly released from endosomes, thereby resulting in cytotoxicity and low transfection efficiency [28,29]. Because of the toxicity, low transfection efficiency and many other problems associated with gene delivery, the potential practical application of non-viral vectors in vivo is being delayed, but the prospect of widespread use of non-viral vectors in gene therapy still holds much promise [30,31].

It should be noted that treatment of humans by gene therapy has turned out to be more complicated than expected; however, the promising "genome editing" is more widely being used in human cells and in a number of model organisms, thus opening up opportunities for the development of new experimental and therapeutic methods for the management of diseases, including rare ones. It is worth mentioning that the use of gene therapy from the ethical standpoint is more acceptable for lethal diseases than, for example, mental or mild physical disorders. Besides, treatment with gene therapy products will be expensive. Considering that management of rare diseases is expensive too, a question arises: will gene therapy agents be accessible to all those who need them or only to those who can afford them? Lately, new gene therapy products entered the market, and it is likely that soon, gene therapy will gain well-deserved recognition in the areas of clinical practice where this approach is necessary.

Cell therapy takes advantage of the regenerative potential of stem cells, e.g. for the treatment of severe diseases and rehabilitation of patients after trauma. Stem cell therapy holds a big therapeutic potential for degenerative, autoimmune, and genetic disorders and for elucidation of their etiology and pathogenesis.

It is known that functional liver disorders are some of the main problems in health care worldwide. Therefore, transplantation of the liver, in contrast to that of other organs, has long reached the high level of efficiency and is successfully applied in clinical practice. Nonetheless, because the number of human liver donors is limited, transplantation of hepatocytes from the liver started to gain traction and so did transplantation of hepatocytes derived from human induced pluripotent stem cells. Impressive results have been obtained on model strains of animals. For instance, in an immunodeficient strain of mice, stem cell derived hepatocytes took hold, proliferated and showed all the functional abilities of isolated primary human hepatocytes [32]. From human stem cells, researchers have derived myotubes and motor neurons for the assessment of severity of such diseases as amyotrophic lateral sclerosis, spinal muscle atrophy and other neurodegenerative diseases or conditions after trauma, because there is no phenotypic model of a neuro-muscular interface of humans for the

development of the corresponding treatment [33]. There are two studies revealing a successful transplant of epithelial pigment cells (derived from human embryonic stem cells) to two patients with age-related macular degeneration (yellow spot disease) and two patients with Stargardt macular dystrophy [34].

Many studies have evaluated the therapeutic potential of various types of stem cells: NSCs, MSCs, embryonic stem cells and human induced pluripotent stem cells. Their results are intriguing but also controversial [35]. Most of clinical trials are aimed at assessing the safety of stem cells and determining the optimal dose and maximal tolerated dose. Mostly unknown mechanisms are being investigated, via which various types of stem cells exert a therapeutic effect. Although preclinical studies on animals yield promising results, the medical community is highly skeptical, because many studies on the use of stem cells in humans have so far not produced stable benefits for patients [36]. For successful clinical application of cell therapy, it is necessary to solve several major problems, for example, to determine the optimal cell type for the treatment of specific clinical cases, the dose of injected cells, the route and timing of administration, and the role of the microenvironment [37]. In one study [38], it was demonstrated that in myocardial infarction, because of the microenvironment of the damaged myocardium, the transplanted stem cells manifest a low survival rate; such situations strongly limit their therapeutic potential.

MSCs, as mentioned above, are employed as carriers in gene therapy. Their low intrinsic immunogenicity and resolution of the problem of immune rejection make MSCs quite attractive for these purposes [39]. MSCs can differentiate into many cell types of mesodermal origin [40], neuroectodermal origin (neurons, astrocytes and oligodendrocytes) and endodermal origin (hepatocytes) [41]. In addition to the wide spectrum of differentiation potentials, MSCs have diverse immunomodulatory properties. The severe complications seen in some patients treated with MSCs can be explained by either suppression or promotion of inflammation by these cells, depending on their environment [42].

MSCs can differentiate into endothelial cells and create a capillary network [43]. For this reason, by expanding the new generation of blood vessels, MSCs tend to promote metastases. Injected MSCs migrate to secondary tumor sites and produce proangiogenic factors (e.g. vascular endothelial growth factor, basic fibroblast growth factor, TGF-β, platelet-derived growth factor and angiopoietin 1), performing an important function in angiogenesis (whose regulation is still poorly understood [44]), thereby leading to neovascularization.

NSCs, capable of differentiating into neurons, astrocytes and oligodendrocytes in the nervous system, are a promising cell type for treating central nervous system injuries. One of the aims of an NSC transplant is to replace or replenish lost or nonfunctional neurons of the central nervous system. In addition, NSCs can stimulate regeneration of nerve tissue by secreting neurotrophic factors [45].

Although there are some documented successes in the treatment of central nervous system diseases by means of NSCs, some unsolved problems remain. For instance, the mechanism of precise regulation of NSCs after transplantation is unclear, and therefore there are complications after the transplantation. At present, most studies on the transplantation of stem cells are at the stage of animal trials, because this method currently is too risky for clinical practice and the risks include differentiation into unintended lineages and malignant transformation. These major safety issues should be solved or minimized before clinical use of a population of differentiated cells derived from induced pluripotent stem cells.

Thus, the problems with practical application of MSCs and other stem cells obtained via differentiation of induced pluripotent stem cells are related to their unlimited differentiation potential [45]. In the near future, the existing problems will probably be solved and stem cells will become more and more useful for regenerative medicine. It is worth noting that the researchers working on stem cells solve problems associated not only with the treatment of genetic disorders and creation of human tissues and biomaterials from stem cells [46] but also with ethical problems linked to the possibility of human cloning and creation of human–animal chimeras.

The CRISPR/Cas system: the emergence of this system (created by nature to defend bacteria from bacteriophages) in genetic experiments has caused a furor in genetic engineering technologies based on zinc finger nucleases and resulted in a novel tool for genome editing. Discovered in bacteria as part of their adaptive immune system, the CRISPR system has rapidly gained popularity as a method for editing genomes of various species, including humans [47]. The latest versions of this technology allow for precise sequence-specific incision of DNA [48] for reversal of mutations. Clinical trials of CRISPR/Cas9 now include cancer patients. The applications are mostly limited to diseases in which a knockout or knockdown of a defective gene is needed. In 2016, the first clinical trial of CRISPR was conducted by the University of Pennsylvania for cancer immunotherapy by means of T lymphocytes modified by CRISPR [49]. In China, a study is under way that is aimed at knocking out the PD-1 gene in the T lymphocytes of patients' with non-small cell lung cancer [50]. In another work [51], there are data on genome editing using the CRISPR system as a potential therapeutic modality against dystrophic cardiomyopathy.

Another major direction in the field of CRISPR/Cas9 applications is models of brain tumor initiation and progression in laboratory animals for elucidation of the

pathogenesis and for the development of novel treatment methods. It has been found that a rat model of glioma can be created by implantation of cultured glioma cells from a patient [52]. Medulloblastoma has been regarded as the most prevalent pediatric brain cancer with a bad prognosis in vivo; this tumor is often modeled by transplantation of chemically modified human medulloblastoma cells [53]. The advantage of genetically modified murine models is their resemblance of human glioblastoma because the histological characteristics of the tumor in a transgenic mouse are similar to those of the human tumor. Nevertheless, the big drawback of such models is that their creation takes a long time, and it is difficult to distinguish a primary mutation from a secondary one. Nonetheless, because animal models of brain cancer are in short supply, it is important to create such models and to study the relevant molecular mechanisms for the discovery of effective therapeutic strategies for humans [54-57]. While the clinical trials of CRISPR/Cas9 for the treatment of human inherited diseases are still at the rudimentary stage, already a number of relevant scientific problems have been identified and some impressive results have been reported, which deserve a separate review article. Undoubtedly, this revolutionary technology will find broad applications in the treatment of human inherited diseases.

CONCLUSION

The advances of biomedicine are a priority in many countries. For example, in Germany, scientists have obtained successful results on cancer treatment with modified cells from the patients themselves. It has transpired that metabolic reorganization in cancer represents a big gap in knowledge at present. Right now, researchers' and clinicians' efforts are directed at reducing off-target cytotoxicity to improve the safety of cancer treatments for humans [58]. Overall, improvements in the delivery specificity of therapeutic agents will substantially increase the effectiveness of antitumor therapies.

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CONFLICTS OF INTEREST

The authors declare that they do not have conflicts of interest.

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