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Potential Medical Applications with Current Biotechnology

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INTRODUCTION

The medical industry is today's biggest customer for biotechnology. This industry includes everything from physicians in hospitals to manufacturers of every kind of equipment, diagnostic techniques, drugs, vaccines, and other biochemicals. This text reviews new knowledge for molecular and cellular biology, fundamental techniques, historical accounts, and applications in medicine. Medical biotechnology's big promise is to subvert genetic destiny and cure the previously incurable; to rig the lottery and make everyone a winner.

As we know a length of DNA makes one individual different from another. One person may be born to live a long life into healthy old age while another suffers a crippling disability leading to an early grave. According to this view of things, our state of health is already circumscribed by genes months before we even began to draw breath, primed by an inheritance that will eventually give us one disease or another. The present chapter introduces more advanced biotechnology applied in the medical field, including the diagnosis and treatment of disease. The GWAS can locate the risk factors of DNA associated with disease. On one hand, disease related DNA can be corrected in the future using Cas9 technology; on the other hand, Cas9 can be studied in the pathogenesis of disease and play an important role in cell and tissue engineering. Some diseases

can be treated with drugs and surgery while others cannot use these therapies because of the loss of organ function; thus, we need regenerative medicine. Regenerative medicine is a new medical treatment modality for modern clinical applications with a significant impact on the development of medical theory, treatment, and rehabilitation.

GENOME WIDE ASSOCIATION STUDY (GWAS)

The study of human genetic variation and its relationship with disease has opened up a new era of individualized treatment where each person's genetic code is parsed and used to guide clinical practice. Common genetic variation and its relationship with complex diseases also make it possible to individualize treatment. The central goal of human genetics is to identify genetic risk factors for common, complex diseases such as type II diabetes, cardiovascular disease, and cancers as well as for rare inherited diseases such as cystic fibrosis, muscular dystrophy, Huntington's disease, and sickle cell anemia. In diseases with an environment component, genetic testing offers information that may change how a person lives their life. There are many different technologies, study designs, and analytical tools available to identify these genetic risk factors. We will first examine the genome wide association study (GWAS) that measure and analyzes DNA sequence variations from across the human genome in order to identify genetic risk factors for diseases common to the population. GWAS plays a central role in the human genetics revolution. Identification of single nucleotide polymorphisms (SNPs) associated with phenotypic traits or a particular disease is the purpose of GWAS (Figure 1)[1]. The ultimate goal of GWAS is to make predictions about who is at risk and identify disease susceptibility for the purpose of developing new prevention and treatment methods.

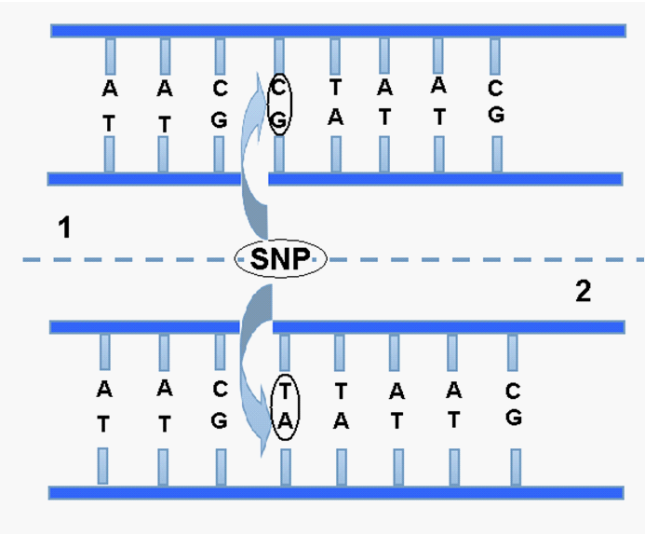

Figure 1: Genome wide association studies (GWAS) identify genetic associations by comparing common single nucleotide polymorphisms (SNP: a single DNA base which changes in the genome between paired human chromosomes).

SNP primarily refers to DNA sequence polymorphisms caused by a single nucleotide change in the genome level. It is one of the most common genetic variations in the human genome, accounting for more than 90% of all known genomic polymorphisms.SNP is widespread in the human genome, averaging one per every 500 ~ 1000 base pairs with a total that could reach 3 million or more.

SNP polymorphisms refer only to a single base variation, which can be made from a single base transition or transversion. They can also be caused by base insertions or deletions. Typically, SNP does not include the next two cases. The incidence rate of transitions is always significantly higher than several other variants because the CpG nucleotide cytosine residues are the most easily mutated of the human genome. This is mostly due to methylation, which can remove an amino group to spontaneously form a thymine residue. Any base can change in genomic DNA, so it is possible that SNPs in the gene sequence can have functional consequences, resulting in changes in amino acids, mRNA transcript stability, and transcription factor binding affinity. These changes have significance in the study of genetic diseases outside of the non-coding sequences.

The International HapMap Project was designed to identify variation across the genome and to characterize correlations among variants. This project used a variety of sequencing techniques to discover and catalog SNPs, including 11 human populations such as individuals from the Centre d'Etude du Polymorphisme, Humain collected in Utah, USA with ancestry from northern and western Europe (CEU); Han Chinese in Beijing, China (CHB); Japanese in Tokyo, Japan (JPT); Yoruba in Ibadan, Nigeria (YRI); African ancestry in the southwestern USA (ASW); Chinese in metropolitan Denver, Colorado, USA (CHD); Gujarati Indians in Houston, Texas, USA (GIH); Luhya in Webuye, Kenya (LWK); Maasai in Kinyawa, Kenya (MKK); Mexican ancestry in Los Angeles, California, USA (MXL); and samples collected in Tuscany, Italy (TSI) with genotypes for 1.6 million SNPs [2].

The important factor in performing GWAS is to select "tag SNPs". These steps involve [3]: (i) defining the case phenotype in adequate detail; (ii) checking the heritability of the disease in question; (iii) considering whether a population-based study is the appropriate design for the research question; (iv) the appropriate selection of controls; (v) sample size calculations and (vi) giving due consideration to whether it is a de novo or replication study.

The Potential Application with GWAS

We focus here on the application of GWAS. One of the early successes of GWAS was the use of single-nucleotide polymorphisms to identify the complement factor H gene. This revealed a common coding variant that increases the risk factor for age-related macular degeneration (AMD). This common variant likely explains approximately 43% of AMD in older adults[4]. Many other diseases like type II diabetes, psoriasis, some autoimmune diseases, Alzheimer's disease, and asthma were studied with GWAS to find candidate genes risk factors associated with these diseases. Another successful application of GWAS has been in the area of pharmacology. The goal

of pharmacogenetics is to identify DNA sequence variations that have relationships with drug metabolism and efficacy as well as side effects. For example, warfarin is a blood-thinning drug that helps prevent blood clots in patients. Determining the appropriate dose for each patient is important and is believed to correlate to polymorphisms in vitamin K epoxide reductase complex 1 (VKORC1) and the cytochrome P450 2C9 (CYP2C9) genes[5]. This can lead to genetic tests for warfarin dosing that can be used in a clinical setting. This type of genetic test has given rise to a new field called personalized medicine that aims to tailor health care to individual patients based on their genetic background and other biological features. The widespread availability of low-cost technology for measuring an individual's genetic background has been harnessed by businesses that are now marketing genetic testing directly to the consumer.

There are many genetic risk factors for some common human diseases that we need gene therapy to correct. Gene therapy makes use of nucleic acid polymers as a drug to treat disease, which can enter the patient's cells to express proteins, interfere with the expression of protein, or even to correct genetic mutations. The most common gene therapy includes the use of DNA to encode a functional gene to replace the mutated gene. In gene therapy, the nucleic acid molecule is packaged within a "vector", which is used to transfer the molecule into cells within the body. Gene therapy was first conceptualized in 1972, but clinical successes have bolstered new optimism in the promise of gene therapy. These include successful treatment of patients with the retinal disease Leber's congenital amaurosis, X-linked SCID, ADA-SCID, adrenoleukodystrophy, chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), multiple myeloma, haemophilia, and Parkinson's disease. These clinical successes have led to a renewed interest in gene therapy, with several articles in scientific and popular publications calling for continued development in the field. CAS9, a potential gene, cell, and organism treatment will soon be in clinical therapy.

CRISPR/CAS9

Since the discovery of double helix in the DNA, researchers and clinicians have been considering the possibility that the cell and organism's genome specific sites can change.

Early methods made use of oligonucleotides or small molecule recognition of DNA bases, such as bleomycin, psoralen, peptide nucleic acids (PNAs), and polyamide combined with DNA. Another strategy that relied on nucleic acid base pairing was the use of self-splicing introns to change sequences at the DNA or RNA level. These approaches did not lead to robust results until zinc finger–mediated DNA binding occurred. Zinc finger nuclease (ZFN) is a protein that is a chimeric fusion structure between a Cys2-His2 zinc-finger protein (ZFP) and the cleavage domain of FokI endonuclease. It uses the structure of the zinc finger domain to recognize the specific DNA sequence for the accurate positioning of targets. At the same time, using the hydrolysis activity of the DNA nucleic acid enzyme makes the double chain break in the target DNA. After the genome was interrupted by ZFN, another foreign donor DNA (donor DNA) can repair the gap effectively by homologous recombination and finally replaces the donor DNA sequences to the parts of the

genome of the cell. Although ZFNs are effective genome editing reagents for some experiments, they were not widely adopted because of the difficulty in designing and validating such proteins for a specific DNA locus of interest. Thus, the transcription activator–like (TAL) effector occurred, which are found naturally in bacteria that infect plants and enable the rapid creation of FokI coupled versions that could be used similarly to ZFNs for site-directed genome editing. Each TALE contains a repeat area located in the central, which consists of 33-35 amino acids for a variable number of repeat units responsible for the identification of specific DNA sequences. Each repeat sequence is basically the same, in addition to two variable amino acids called repeat variable diresidues (RVD). TALE identifies the nucleotide of DNA by RVD. Attaching a nuclease to TALE generates TAL effector nucleases (TALENs). Such TALENs were easier than ZFNs to produce and validate, generating widespread excitement about the possibility of facile genome editing that would be fast and inexpensive. However, difficulties associated with protein design, synthesis, and validation remains a barrier to widespread adoption of these engineered nucleases for routine use.

So, now we focus on the clustered, regularly interspaced, short palindromicrepeats/CRISPR-associated proteins (CRISPR/Cas) systems. The simple two-component CRISPR/Cas system, using Watson-Crick base pairing by guide RNA to identify target DNA sequences, is a versatile technology that has already stimulated innovative applications in biology.

In many bacteria and most Archaea, CRISPR forms peculiar genetic loci, which provide acquired immunity to eliminate viruses and plasmids by Cas proteins. They recognize target sites within the invader genome (known as protospacers) via base-pairing complementarity and then to cleave DNA within the protospacer sequences. There are three types (I, II, and III) based on the sequence and structure of the Cas protein. Types I and III need multiple Cas subunits; however, the type II is simple, with Cas9 protein and guide RNA (gRNA) as the core. Cas9 is a large multi domain protein with two nuclease domains, a RuvC-like nuclease domain near the amino terminus, and an HNH (or McrA-like) nuclease domain in the middle, which play a role in on crRNA maturity and cleaves double-stranded DNA (dsDNA) in a sequence-specific manner.

First, pre-CRISPR RNA (pre-crRNA) is transcribed. At the same time, its repetitive sequence complementary trans-activated crRNA (Trans-activating crRNA, tracrRNA) is transcribed and inspires Cas9 and double-stranded RNA nucleic acid enzyme specificity RNaseIII on processing pre-crRNA. After the mature crRNA, tracrRNA, and Cas9 form a complex and bind to the crRNA complementary sequence, the double-stranded DNA is unlocked to form an R-loop. CrRNA is hybridized with the complementary strand while another chain must be kept free. At last, the HNH of Cas9 shears crRNA complementary DNA chains while RuvC shears the non-complementary strand, causing a double-stranded DNA break (DSB)[6](Figure 2).

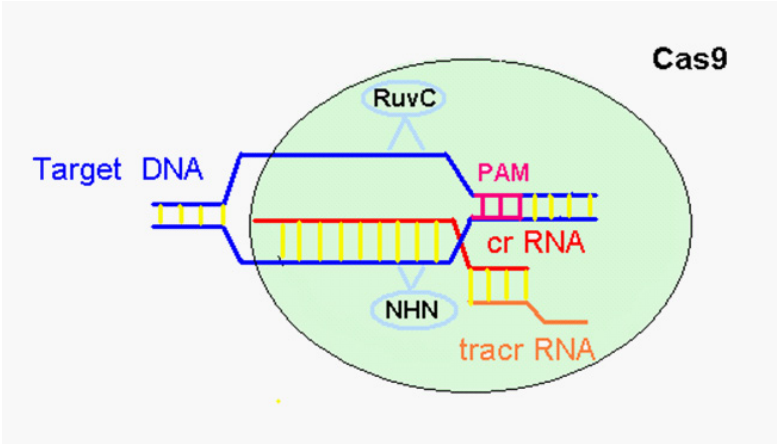

Figure 2: Cas9 with the antirepeat-repeat RNA (tracrRNA:crRNA) duplexes, R-loop formation, and target DNA cleavage.

The CRISPR/Cas9 splice site is the NGG loci with the characteristic 5 '- GG - N18 - NGG - 3' of the PAM (Protospacer Adjacent Motif) area that is located in the downstream crRNA complementary sequence. The PAM is an essential targeting component that also serves as a self versus non-self recognition system to prevent the CRISPR locus itself from being targeted. Many type II systems have differing PAM requirements, which can limit their ease of targeting. Type II systems may differ in the details of pre-crRNA production and crRNA-tracrRNA processing. Through artificial design of these two kinds of RNA, which can transform into the sgRNA (short guide RNA), enough to guide Cas9 cut any site of DNA.

Compared with ZFN and TALEN, CRISPR/Cas is easier to operate, more efficient, easier to attain homozygous mutants, and can bring multiple mutations in different sites at the same time. The RNA guided enzyme Cas9 is transforming biology by providing a genome engineering tool. Ease of use and efficiency has led to rapid adoption by laboratories around the world.

The Potential Application with CRISPR/CAS9

Given the enormous utility Cas9 for regulating and modifying complex biological system, might the Cas9 system prove equally useful as the basis for new therapies? We imagine a few ways in which Cas9 – mediated technology can be used as treatment interventions.

Firstly, CRISPR-Cas9 represents an efficient tool to edit the genomes of human cells. These studies involve a variety of different types of cells (including cancer cells and induced pluripotent stem cells), different genetic loci (including those genetic loci that had been modified by ZFNs or TALENs, such as CCR5, AAVS1, etc.), and various modifications (including gene knockout, homologous recombination, fixed-point integration, deleting, more genes knocked out at the same time, etc.). With custom guide RNA (gRNA) in human cells, scientists obtained targeting rates for the endogenous AAVS1 locus of 10 to 25% in 293T cells, 13 to 8% in K562 cells, and 2

to 4% in induced pluripotent stem cells. They show that this process not only relies on CRISPR components, but is also sequence-specific. Introducing multiple gRNAs at the same time can effect multiplex editing target locii [7]. In future, combined with induced pluripotent stem cells (iPS) technology, cas9 can facilitate the iPS cells developing into normal tissues and organs for use in patients.

Secondly, it can reproduce cancer and other diseases models in vivo that better reflect the complexity of human disease. By delivering combinations of small guide RNAs (sgRNAs) and Cas9 with a lentiviral vector, up to five genes were modified in a single mouse hematopoietic stem cell (HSC). This generated models of acute myeloid leukemia (AML) with cooperating mutations in genes encoding epigenetic modifiers, transcription factors, and mediators of cytokine signaling, recapitulating the combinations of mutations observed in patients [8]. An improved method to generate liver cancer in mice facilitated by CRISPR-Cas9 was also recently reported [9]. CRISPR-Cas9 thus provides a technology for studying the development and progression of cancers or other diseases.

Thirdly, it can also analyze the gene functions in mammalian cells. This approach was also used to identify genes essential for cell viability in cancer and pluripotent stem cells [10]. RNA interference (RNAi) to reduce gene expression has been the predominant method thus far but its utility is limited by the inherent incompleteness of gene knockouts and off-target effects. In a melanoma model, they screened for genes whose loss is involved in resistance to vemurafenib, a therapeutic that inhibits mutant protein kinase BRAF[10]. The use of CRISPR-Cas9 for genome-wide studies will enable large-scale screening for drug targets.

Some studies underscore the potential for this technology to be used for human gene therapy to treat genetic disorders. One approach is to correct the genetic mutations responsible for inherited disorders. For example, mice with a dominant mutation in Crygc gene that causes cataracts. Coinjection into zygotes of Cas9 mRNA and a single-guide RNA (sgRNA) targeting the mutant allele can rescue the phenotype [11]. Another approach is to integrate CRISPR/Cas9 with human stem cells to treat human genetic diseases. Using the CRISPR/Cas9 genome editing system to correct the cystic fibrosis (CF) transmembrane conductor receptor (CFTR) locus by homologous recombination in cultured intestinal stem cells of CF patients, this disease can be corrected[12].

With the development of the CRISPR-Cas9 technology, we have targeted genome modifications in animal models such as fruit flies, zebrafish, nematodes, salamanders, frogs, mouse, rat, pigs, and monkeys. These animal models are better suited to pharmacological studies and the understanding of the pathogenesis of human diseases.

Although the missing problems are a big obstacle in the clinical application of CRISPR/Cas9 system, it will remain widely used in biology and medicine with a profound influence on our lives.

MESENCHYMAL STEM CELLS (MSC) AND INDUCED PLURIPOTENT STEM CELLS (IPS) IN REGENERATIVE MEDICINE

Regenerative medicine is a branch of translational research for tissue engineering and molecular biology that focuses on the replacement or regeneration of human cells, tissues, or organs to restore or establish normal function. This field can stimulate the body's own repair mechanism to heal irreparable tissues or organs. When the body cannot heal itself, it can also generate tissues and organs in vitro for transplant into the body. The main content of regenerative medicine is how stem cells develop into tissues and using stem cells for tissues replacement therapies to restore the normal structure and function of damaged tissues. By studying their growth and the differentiation and development of stem cells in molecular regulated mechanisms, we will be able to better understand the basic laws of life in organ formation and development. This can be induced in vitro to amplify and directionally differentiate, and researched the growth, migration, and differentiation of stem cells after transplanted into the body.The stem cell-derived model also can be used as an ideal research platform for drug and screening of functional genes, such as the pathogenesis of cancer, genetic diseases, neural degenerative diseases, and autoimmune diseases.

Regenerative medicine refers to a group of biomedical approaches to clinical therapies. This includes the injection of stem cells or directional differentiation of progenitor cells in the damaged tissue (cell therapy); the induction of regeneration of biologically active molecules or inhibiting factor (drug/gene therapy) and; transplantation of organs and tissues grown in vitro (tissue engineering). If a regenerated organ's cells would be derived from the patient's own tissue or cells, this would potentially solve the problem of the shortage of organs available for donation as well as the problem of organ transplant rejection.

Stem cells can be isolated from embryonic, fetal and adult tissues. Because of high self-renewal capacity and pluripotency, they are able to differentiate into all of the germ layers in vitro and in vivo. Embryonic stem cells (ES) are a candidate for regenerative medicine but are limited in clinical applications either by ethical restrictions or by the complication of using immature cells that may cause teratoma formation. By contrast, mesenchymal stem cells (MSCs) are also being used. These cells originate in the human embryo and are considered multipotent, which may have wider clinical applications. MSCs can be isolated from the bone marrow, mobilized peripheral blood, cord blood, umbilical cord (UC), placenta, adipose tissue, dental pulp, and even the fetal liver and lungs. Because of their easy isolation and low risk of neoplasia induction, they are ideal candidates for cell regeneration therapy and transplantation into the same patient from whom they have been isolated. Further clinical studies will further investigate the use of stem cells and combine genetic with pharmacological approaches to increase cell survival and recruitment of endogenous repair mechanisms.

For example, as one of the most important MSCs, UC-MSCs have many advantages. Firstly, they have faster self-renewal capacity than BM-MSCs. Secondly, it is easy to obtain a substantial

number of UC-MSCs after several passages and expansion. The largest advantage is that the collection procedure is noninvasive and ethically acceptable. UC-MSCs either do not express or have lower expression of histocompatibility tags and have low immunogenicity, leading to a lower or absent immune rejection reaction. Before use, they don't need to be strictly matched for transplant between different individuals. However, there are also disadvantages. The physicians need to confirm the baby's health as a donor because it cannot be ascertained whether the donor will grow normally without health problems in future; thus, genomic or chromosomal tests need to be performed or the baby's health monitored after birth. There is general acceptance of mesenchymal cells, such as cord blood, adipose tissues and other adult stem cell sources, but they often do not survive for more than a few weeks in patients and their effects are most likely through growth factor release, host inflammatory responses, and vascular alterations rather than replacing tissues lost in the disease. To achieve this, it will be necessary to either grow new tissues within the affected organ or transplant powerful cells that can integrate, survive, and produce new functional tissues.

Takahashi and Yamanaka showed an innovative technique in 2006 when they reported that adult mouse fibroblasts could be sent back in time to an embryonic-like state by introducing four factors, Oct3/4, Sox2, c-Myc, and Klf4, under (embryonic stem) ES cell culture conditions, a process called cellular reprogramming[13]. In 2007, they reported that iPS cells can be generated from adult human fibroblasts [14]. This is a critical consideration for choosing an appropriate cell type for reprogramming for future autologous patient-specific iPS cell production and clinical therapy. Skin fibroblasts, neural stem cells, keratinocytes, CD34+ cells from peripheral blood, melanocytes, adipose-derived stem cells, and cord blood cells were chosen with different factors (Table 1) [15].

Table 1: Different cell origins used for reprogramming with different factors.

Cell source	Derivation	Reprogramming factors
Skin fibroblasts	Skin Biopsy	OSKM, OSK, OSNL
Fetal Neural Stem Cells	Cadavers at 13 weeks of gestation	OK, O
Keratinocytes	Skin Biopsy	OSKM, OSK
CD34 Blood Cells	Peripheral blood undergo G-CSF stimulation	OSKM
Adipose Stem Cells	Lipoaspiration	OSKM
Melanocytes	Skin Biopsy	OSKM, OKM
Cord Blood Cells	Collected at birth from cord blood	OSKM, OSNL, OSK, OS
O:Oct4;S:Sox2;K:Klf4;M:c-MYC;N:Nanog;L:Lin-28.		

To generate iPS cells, most of the current studies have used lentiviruses or retroviruses containing reprogramming factors. Other methods include cre-recombinase excisable lentiviruses, epstein-Barr nuclear antigen-1 based episomal vectors, piggyBac transposon expression vectors, plasmid based techniques, and minicircle vectors. To generate safer iPS cells, adding small

molecules, small interfering RNAs (siRNAs), or micro RNAs (miRNAs) to the reprogramming cocktails represents an important alternative future direction [15]. In general, with the rapid progress and improvement of the induced pluripotent cells derivation method, the production of safe, non-virus, high efficiency iPSCs is feasible.

The Potential Cellular Therapies with MSC and iPS

MSCs can renew and turn over the mesenchymal tissue including cartilage, bone, fat, and muscle when tissue injury occurs. They can provide an essential endogenous therapeutic tool. Cell engraftment is a one of the best treatments for orthopedic disorders in which physiological endogenous tissue repair is impaired. For example, in osteogenesisimperfecta (OI) [16], hypophosphatasia[17], osteonecrosis[18], nonunion, delayed union, and mal-union of bones[19], and osteoarthritis (OA)[20], transplant MSCs can migrate and differentiate, contributing to improved bone structure.

MSCsin the treatment of diseases of the nervous system have focused on human multiple sclerosis (MS)[21], Alzheimer's and Parkinson's diseases[22], and brain and spinal cord injuries resulting from stroke [23], Amyotrophic lateral sclerosis (ALS) [24], trauma, and spinal cord injury (SCI) [25]. There are two basic strategies for stem cellular therapy of neurodegenerative diseases. One is to culture the stem cells and induce the desired differentiated neuronal cell type before implantation. The second is to implant the stem cells directly. Although there are a large number of reports regarding stem cell transplantation in treatment of nerve injury disease, it is still in its infancy stage and requires further in-depth clinical research to find the signals between injury and gene regulation in nerve cells. Nerve regeneration studies with positive effects have been conducted in rodents such as rats, but need to be further tested and verified in more advanced animals such as dogs, pigs, sheep, and other large animals.

In the developed and developing world, heart ischemic diseases are the leading cause of mortality. Their pathological processes are due to obstructive lesions that prevent blood flow into the heart muscle, causing cardiomyocyte death. Both cultured and freshly isolated MSCs have provided some evidence for attenuation of cardiac dysfunction in animals with myocardial injury [26]. Moreover, there is report in the clinical trials that stem cell therapy can improve recovery from myocardial ischemia [27]. A series of in vivo and in vitro differentiation strategies have given support for MSCs ability to differentiate into cardiac cells and possibly promote regeneration of the heart [28], however, this is still hotly contested in the field [29,30].

Other clinical applications include differentiation into islet cells for diabetes [31], liver cells for treat liver disease [32], endothelial cells for angiogenesis and endothelial tissue engineering transplants [33], and steroid cells to treat partial androgen deficiency in middle-aged and elderly men [34].

iPS cells will provide an autologous source of cells for a multitude of regenerative medicine therapies in future clinical trials. iPSCs can make many types of neural cells. iPSCs could be

directed to large quantities of immature astrocytes for the study of brain development and function, understanding the roles of astrocytes in disease processes, and developing novel treatments for neurological disorders [35]. Human iPS-derived oligodendrocyte progenitor cells could differentiate in vitro and in vivo into myelinogenicoligodendrocytes and astrocytes to treat disorders of myelin loss [36]. Furthermore, there is a very simple way to produce a readily expandable neural stem cell that grows as a spherical suspension culture that we termed EZ spheres due to their ease of growth [37].However, there are still many obstacles when iPSCs are applied in clinical treatment. First of all, because the brain is an immune isolated organ, T cells can't arrive and play an anti-tumor role. The iPSCs have potency and the tumor rate could be greatly increased. Secondly, the iPSCs would be generated from the patient's own cells, which is consistent with the same genotypes when differentiated into nerve cells. Due to its genetic susceptibility of the nerve cells in the treatment of genetically related diseases of the nervous system, there is a risk of recurrence.

The most significant recent advances have been made in the eye. Because of the deficits in the retinal pigmented epithelium (RPE), diseases such as macular degeneration and retinitis pigmentosa can be treated. RPE cells can be easily derived from human pluripotent cells and survive transplantation [38]. Through simulate eye cup development in the embryoidbodies, it forms the structure of retinal with pigment epithelium and neural retina.

hES or hiPS can differentiate into islet beta cells in vitro or in vivo. This can make patients with pancreas returned to normal function as a source of tissue for treating Type I diabetes. Type I diabetes is an autoimmune disease and iPSCs from somatic cells in patients for induced islet cells have the same genetic material. So the induced islet cells also have the possibility of its own immune attack. The iPS used in the field of diabetes is mainly in the pathogenesis of diabetes and the development of new drugs [39].

Human iPSC-derived cardiomyocytes have been shown to beat in a culture dish and show some important markers but do not display all of the expected phenotypes of mature cells. Nor do they electrically couple, which can cause arrythymias, they survive very poorly following transplantation [40].

Some studies provide evidence that the generation of patient customized cells is feasible in vivo, providing materials that could be useful for transplantation, human antibody generation, and drug screening applications [41].

In summary, iPSCs promote the development of the new research field and represents a revolution for medicine. However, the reprogramming process still very inefficient and the reprogramming mechanism is still not very clear. There must be further study of the basic molecular mechanisms that will lead to more effective methods of in vitro cell reprogramming, induced differentiation of iPSCs in vitro, and diseased tissue repair and regeneration in order to move forward with treatment.

CONCLUSION

GWAS can detect the SNP locus compared to control groups and discern the mutant allele frequency in the entire genome. These advantages enable GWAS to avoid the limitations of candidate gene strategies that rely on the presupposition of disease-causing genes and further open the door to study complex diseases. However, due to linkage disequilibrium phenomenon, obtaining a large sample of genetic data is very difficult in GWAS studies even if the cost of sequencing is very low.

Cas9, as the core of gene editing technology, is still quite novel. Cas9-mediated treatment interventions are likely to travel two routes. The first is by using genome editing to correct genetic disorders and destroy the invading virus genome. The second is by using small molecule drugs to target and regulate the genome. However, in addition to the main problems of the "miss", there are many technical difficulties have not yet been addressed, particularly the safety and efficacy of clinical applications.

Approaches in regenerative medicine such as current trials of stem cell therapy with mesenchymal and induced pluripotent stem cells will help realize the dream of repairing trauma and curing pathological tissue as well as end-stage disease for humans. However, the platforms involved in the study of this technology have not yet been fully established and there are ethical concerns that must still be addressed.

In summary, although the rapid development of technology still presents difficulties, we expect that modern medical biological technology will improve much like the industrial revolution to result in fundamental changes to human life.

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