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Research review paper

Gene therapy, gene targeting and induced pluripotent stem cells: Applications in monogenic disease treatment

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ABSTRACT

Monogenic diseases are often severe, life-threatening disorders for which lifelong palliative treatment is the only option. Over the last two decades, a number of strategies have been devised with the aim to treat these diseases with a genetic approach. Gene therapy has been under development for many years, yet suffers from the lack of an effective and safe vector for the delivery of genetic material into cells. More recently, gene targeting by homologous recombination has been proposed as a safer treatment, by specifically correcting disease-causing mutations. However, low efficiency is a major drawback. The emergence of two technologies could overcome some of these obstacles. Terminally differentiated somatic cells can be reprogrammed, using defined factors, to become induced pluripotent stem cells (iPSCs), which can undergo efficient gene mutation correction with the aid of fusion proteins known as zinc finger nucleases (ZFNs). The amalgamation of these two technologies has the potential to break through the current bottleneck in gene therapy and gene targeting.

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1. Monogenic diseases

Many human diseases have now been identified to have a genetic basis, including hereditary diseases such as cystic fibrosis and thalassaemia, as well as the predisposition to certain cancers or infections (Amberger et al., 2009). While some of these may be attributed to mutations at multiple loci, a significant number of diseases are believed to be caused by mutations in single genes. These 'monogenic diseases' are also known as Mendelian disorders, due to their inheritance in accordance with Mendel's laws, and can thus be further subdivided into autosomal dominant, autosomal recessive and X-linked diseases (Table 1). According to the Online Mendelian Inheritance in Man (OMIM) database (http://www.ncbi.nlm.nih.gov/omim), as of January 2010, over 2500 disease phenotypes have been linked to specific genes, and over 1700 Mendelian phenotypes have yet to be mapped to specific loci (Amberger et al., 2009). While each of these conditions is relatively rare, the many different types of monogenic diseases together affect a substantial population (estimated to be approximately 10 in every 1000 births by the World Health Organisation). In particular, some of these diseases can be very severe and ultimately fatal, resulting in a tragically painful and shortened life.

One example of a monogenic disease is cystic fibrosis (CF) (O'Sullivan and Freedman, 2009). It is caused by recessive mutation (s) in a single gene which encodes a chloride ion channel, termed the *c*ystic *f*ibrosis *t*ransmembrane conductance *r*egulator (CFTR), commonly expressed in epithelial cells as an apical membrane protein. The mutations lead to the absence or deficiency of CFTR in the plasma membrane, which, via a number of different possible mechanisms, results in accumulation of thick mucus in the airways, as well as recurrent bacterial infections and chronic inflammation in the respiratory system. Currently, the treatment typically prescribed for

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Table 1
Examples of monogenic diseases.

Disorder (OMIM number[a])	Disorder type	Mutated gene	Function	Inheritance
Cystic fibrosis (219700)	Channelopathy	CFTR	Chloride channel	Autosomal recessive
Duchenne muscular dystrophy (310200)	Musculoskeletal	Dystrophin	Cytoskeletal component	X-linked
Fabry disease (301500)	Metabolic	α-galactosidase A	Glycosphingolipid catabolism	X-linked
Huntington's disease (143100)	Musculoskeletal	Huntingtin	Vesicular trafficking mediator?[b]	Autosomal dominant
Sickle cell anaemia (603903)	Haematological	β-globin	Oxygen carrier	Autosomal recessive
X-linked severe combined immunodeficiency (300400)	Immune dysfunction	Interleukin-2 receptor gamma subunit	Cytokine receptor	X-linked

[a] Online Mendelian Inheritance in Man numbers for disorders are included in parentheses.
[b] The function of huntingtin is still unclear, with a number of conjectures.

CF patients would include inhalation of mucolytics (e.g. dornase alfa, a recombinant human deoxyribonuclease), antibiotics (e.g. tobramycin) and/or hypertonic saline. The hypertonic saline functions as a hyperosmolar agent to draw water from the epithelial cells into the airways, thereby aiding pulmonary clearance. Lung transplantation is considered only for patients who have developed endstage lung disease, and, even where donors are available, carries considerable risk of rejection and surgery-related complications.

In cases where the monogenic disorder is caused by enzyme deficiency or dysfunction, enzyme replacement therapy is a common strategy. Caused by mutations in the gene encoding the lysosomal enzyme alpha-galactosidase A, which catalyses the catabolism of glycosphingolipids, Fabry disease is an X-linked disorder characterised by the accumulation of glycosphingolipids in the plasma and lysosomes in various tissues (Zarate and Hopkin, 2008). Such chronic accumulation eventually results in skin lesions (angiokeratomas), periodic pain crises at the extremities, and renal, cardiovascular and/or cerebrovascular complications. Childhood or adolescent onset is common, and if untreated, it can significantly shorten a patient's lifespan. Treatments, most commonly by periodic infusion of recombinant alpha-galactosidase, are costly and lifelong.

Sickle cell anaemia (SCA) is another Mendelian disorder — it results from recessive mutation(s) in the β-globin gene, causing haemoglobin molecules to polymerise when deoxygenated (Stuart and Nagel, 2004). The polymer induces bending of the erythrocytes, giving them their signature 'sickle' shape. These red cells also become rigid and inflexible, causing occlusion in small blood vessels and poor oxygenation in tissues, which in turn result in a number of complications including pain crises, infections, acute chest syndrome and anaemia. Immunisation, antibiotics and analgesics are standard treatments for SCA patients, and hydroxycarbamide (also known as hydroxyurea), a cytotoxic drug used in cancer chemotherapy, is apparently efficacious in improving several aspects of the disease (de Montalembert, 2008; Stuart and Nagel, 2004).

Huntington's disease (HD) is a neurodegenerative disorder caused by the expansion of a trinucleotide repeat in a gene (Imarisio et al., 2008). This gene encodes a protein named huntingtin, whose cellular function has not been clearly defined. The wild-type allele of huntingtin contains an uninterrupted stretch of CAG repeats, starting from codon 18 and consisting of up to 35 CAGs, which are translated to give a polyglutamine stretch in the polypeptide. Individuals carrying more than 35 CAG repeats in one or both copies of their huntingtin gene are predisposed to HD, and because of its autosomal dominant inheritance pattern, patients have a 50% chance of passing the disease onto the next generation. Age of onset is inversely correlated to the CAG repeat length, and if the gene contains >28 CAG repeats, there is also a risk of repeat expansion on DNA replication. While the precise pathological mechanism of HD is still under investigation, it is believed that the long (>35) polyglutamine sequences are toxic to the cell. The disease is characterised by a gradual deterioration in motor coordination and mental status, and the involuntary spasmodic movements of the limbs, a condition known as chorea. Currently available treatment aims mainly to control the chorea, and a number of experimental treatments are undergoing clinical trials.

All four of these diseases share one thing in common: due to their inborn nature, lifelong treatment is necessary, often costly and not always successful, which impacts greatly upon the quality of the patients' lives. As with many other monogenic disorders, only palliative treatments are available, and an effective cure remains a distant goal.

2. Gene therapy

Over the past two decades, great effort and abundant resources have been invested into the development of effective, safe and relatively long-lasting treatments for a few monogenic diseases, but success has been limited so far. As our knowledge about recombinant DNA technology and the genetic basis of diseases accumulated, gene therapy became increasingly championed by scientists and clinicians as the ideal treatment for patients suffering from debilitating Mendelian disorders. However, despite the anticipation and hype, this technology has yet to live up to its early promise.

Gene therapy is a term used to describe the introduction of genetic material into particular cells or tissues in order to produce a therapeutic effect (O'Connor and Crystal, 2006; Somia and Verma, 2000). It encompasses a variety of strategies which nonetheless converge at the modulation of the expression of a gene intimately related to disease pathology. The most popular approach, known as gene augmentation therapy, is applicable to diseases caused by recessive mutation(s) in a single gene, including CF, Fabry disease and SCA (Table 1). It involves providing cells with an exogenous copy of the wild-type allele, expression of which will produce functional protein and should rescue the cell from the disease phenotype. Dominant genetic disorders, however, will require a different approach: HD, for example, is caused by an autosomal dominant mutation in the huntingtin gene, which cannot be complemented by an extra copy of the wild-type allele (Fig. 1). Instead, interference RNA directed against mutant huntingtin has been proposed as a means to control the disease, even though the technology of RNA delivery into the brain, where HD produces a lot of damage, remains too immature for clinical application (Harper, 2009).

In order to achieve gene therapy, a carrier, or 'vector', is required to deliver the therapeutic genetic material into the specific target cells. While there has been modest progress over the last two decades in the development of gene therapy, a safe and efficient method of gene delivery has been elusive thus far. Initially, viruses were the vectors of choice, owing to their high efficiency in gene transfer. Retroviruses and adenoviruses were commonly used, but they each posed significant health risk to the patients, even though their virulence was genetically disabled. Retroviruses (including oncoretroviruses and lentiviruses) have the ability to integrate into random sites of the host genome, which, in addition to achieving stable expression of the transgene, could also produce permanent deleterious mutations. In the case of a clinical trial for retroviral gene therapy of X-linked severe combined immunodeficiency (SCID-X1), conducted at Necker Hospital in Paris, France, 9