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Gene therapy for metabolic disorders: an overview with a focus on urea cycle disorders

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Abstract Many metabolic diseases are compelling candidates for gene therapy, and are the subject of vigorous preclinical research. Successful phenotype correction in mouse models is now commonplace and research effort is increasingly being directed towards addressing the translational challenges inherent in human clinical trials. This paper places current efforts to develop gene therapy approaches to metabolic disease in historical context and describes contemporary research in the authors' laboratory on urea cycle defects, particularly ornithine transcarbamylase deficiency, in a manner that is illustrative of the general state of the field.

Overview

It is easy to forget how far our understanding of genetics in health and disease has come in less than a lifetime. Milestone events include Oswald Avery's demonstration in 1943 that genes are made of DNA, Watson and Crick's elucidation of the structure of DNA in 1953 and Nirenberg and colleagues' deciphering of the genetic code in 1961 (Judson 1996). Somewhat presciently, Nirenberg recognized the link between knowledge of the genetic code and the possibility of reprogramming cells for therapeutic purposes, but it was to be almost 30 years before other technological advances allowed clinical trials of gene therapy to be initiated. These advances included cell and tissue culture, the recombinant DNA revolution, the cloning and sequencing of human genes, and the development of relatively efficient recombinant virus-based gene delivery technology (Lyon and Gorner 1995).

Since the first authorised clinical trial in 1989 at the National Institutes of Health (NIH) in the United States (Rosenberg et al 1990), there have been in excess of 1700 trials in 30 different countries and preclinical gene therapy research has grown impressively in quality, depth and breadth (http://www.wiley.co.uk/genmed/clinical). There are now five dedicated gene therapy journals, approximately 3000 gene therapy-focused papers published per year in the international peer-reviewed literature, and promising examples of therapeutic efficacy being reported with increasing frequency. The biggest challenges remain translational.

Successful disease correction is now commonplace in murine models, including metabolic phenotypes, but there remains a paucity of large animal models and increased size places proportionally greater demands on gene transfer efficiency (Alexander et al 2008; Ginn and Alexander 2011). Indeed gene delivery has been referred to as the "the Achilles heel of gene therapy" (Verma and Somia 1997). Every gram of tissue contains up to 10^9 cells and diseases requiring highly efficient gene transfer to large numbers of post-mitotic or non-dividing cells remains at or beyond the limits of contemporary gene delivery technologies.

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Targeting replicating cell populations, such as haematopoietic stem and progenitor cells, poses additional challenges and currently necessitates the use of integrating vector systems. Integration of therapeutic sequences into host cell DNA ensures that these sequences are not lost during cell division and are faithfully copied to daughter cells. While carrying an inherent risk of insertional mutagenesis (Hacein-Bey-Abina et al 2003), this approach is exceptionally powerful as the successful modification of a small number of stem or progenitor cells has the potential to reconstitute entire cellular compartments through replicative expansion of gene-modified cells (Cavazzana-Calvo et al 2000).

The conceptual simplicity of gene therapy underpinned early expectations that success in the clinic would come easily. This view, however, was soon tempered by experience, with the first ten years (1989–1999) of clinical trial activity providing proof-of-concept for gene therapy, but little convincing evidence of therapeutic benefit. Metabolic phenotypes targeted in this early period included adenosine deaminase (ADA) deficiency (OMIM 102700), Gaucher disease (OMIM 230800), α-1-antitrypsin deficiency (OMIM 613490), mucopolysaccharidoses (MPS) types I (OMIM 607014) and II (OMIM 309900), purine nucleoside phosphorylase (PNP) deficiency (OMIM 613179), ornithine transcarbamylase (OTC) deficiency (partial) (OMIM 311250), Canavan disease (OMIM 271900) and ornithine aminotransferase (OAT) deficiency (OMIM 258870) (http://www.wiley.co.uk/genmed/clinical). Lessons learnt, highlighted in the 1995 NIH-commissioned Orkin-Motulsky report, included the need for further improvements in gene transfer technology, the need for greater research focus on the basic science of gene therapy and an improved understanding of host-vector interactions (Orkin and Motulsky 1995).

Benefiting from these lessons, and other disease-specific insights, the first unequivocal success for gene therapy in the treatment of a genetic disease was reported in 2000 (Cavazzana-Calvo et al 2000). This particular trial, addressing the X-linked form of severe combined immunodeficiency (SCID-X1) (OMIM 300400) exploited integrating gamma-retroviral vector technology to target haematopoietic stem and progenitor cells, and took advantage of *in vivo* selection of gene-corrected cells imposed by the inherent pathophysiology of SCID-X1. Successful treatment of a second genetic disease, ADA deficiency, was reported soon after using a very similar approach (Aiuti et al 2002). Collectively these successes illustrate the importance of carefully matching the specific challenges posed by the target disease phenotype with the capabilities of the gene transfer technology used. Building on this progress, therapeutic benefit has now also been reported for six other genetic diseases including a form of blindness caused by retinal pigment epithelial-specific 65 kDa protein (RPE65) deficiency (Bainbridge et al 2008; Cideciyan et al 2009; Simonelli et al 2010) (OMIM 204100), X-linked adrenoleukodystrophy (ALD) (Cartier et al 2009) (OMIM 300100), lipoprotein lipase deficiency (Mingozzi et al 2009) (OMIM 238600), Wiskott-Aldrich syndrome (Boztug et al 2010) (OMIM 301000), chronic granulomatous disease (Santilli et al 2011) (OMIM 306400) and β-thalassaemia (Cavazzana-Calvo et al 2010) (OMIM 141900). Trials for metabolic phenotypes including Pompe disease (OMIM 232300), α-1-antitrypsin deficiency, late infantile neuronal ceroid lipofuscinosis (NCL) (OMIM 204500), metachromatic leukodystrophy (MLD) (OMIM 250100), galactosialidosis (OMIM 256540), MPS I and GM_2 gangliosidosis (OMIM 272750) are approved or ongoing, with further incremental progress expected (http://www.wiley.co.uk/genmed/clinical).

Gene therapy for urea cycle defects (UCDs): from mouse to man

With the exception of N-acetylglutamate synthetase (NAGS) deficiency (EC 2.3.1.35), mouse models exist for each of the six urea cycle enzymes (Deignan et al 2008). These include the OTC (EC 2.1.3.3) deficient *spf* and *spf*^{ash} mouse models which have residual enzyme activity (Doolittle et al 1974; Qureshi et al 1979), and knock-out models for carbamyl phosphate synthetase (CPS 1) (EC 6.3.5.5), argininosuccinate synthetase (ASS) (EC 6.3.4.5), argininosuccinate lyase (ASL) (EC 4.3.2.1) and arginase (EC 3.5.3.1). For ASS deficiency, a naturally occurring bovine model has also been described (Dennis et al 1989).

Reflecting disease prevalence and severity in humans, the majority of pre-clinical studies have focused on OTC deficiency. The earliest of these exploited adenovirus-mediated gene transfer in OTC deficient *spf* and *spf*^{ash} mice, with phenotype correction first reported in 1996 (Ye et al 1996). In conjunction with additional gene transfer studies focused on vector refinements in mice and primates (Raper et al 1998), this early pre-clinical success soon led to an ill-fated phase 1 clinical trial for partial OTC deficiency in 1999 (Raper et al 2003). Whilst the first 17 patients recruited to this vector dose escalation safety study were treated uneventfully, the 18th, Jesse Gelsinger, developed a fatal systemic inflammatory response with disseminated intravascular coagulation and multi-organ failure. This tragic event was subsequently linked to the immuno-biology of adenoviral vectors and their capacity to elicit powerful innate immune responses. The immediate effects on the developing gene therapy field were salutary and led to a greater focus on the pre-clinical evaluation of vector safety and renewed emphasis on the need to balance the relative risks and potential benefits to trial participants in the development of early phase gene therapy trials (Wilson 2009).

As is the case more generally for the entire gene therapy field, ongoing progress towards gene therapy for urea cycle defects has been underpinned by developments in gene

transfer technology. Most notably, vectors based on the dependent parvovirus, adeno-associated virus (AAV) show particular promise for liver targeted gene transfer (Alexander et al 2008). Not only are AAV vectors proving efficacious in the correction of mouse liver phenotypes including OTC (Moscioni et al 2006), but importantly have already reached human clinical trials, and to date have been free of serious adverse events (Manno et al 2006; Mueller and Flotte 2008; Nathwani et al 2011).

Work in our laboratory, focusing on the development of gene therapy for urea cycle defects, particularly OTC deficiency, is illustrative of the general state of the field and the translational challenges being confronted in moving successful correction of mouse phenotypes through to clinical trials.

Consistent with the promise of AAV-based vectors outlined above, we have based our research efforts around this vector system. As a first step we explored gene delivery to the adult mouse liver using vectors with an AAV2/8 configuration. This nomenclature indicates that the vector consists of a recombinant genome derived from AAV2 and a capsid from AAV8. The type 8 capsid is particularly liver-tropic in the mouse, such that modest vector doses delivered by simple intra-peritoneal (i.p.) injection are capable of transducing essentially every hepatocyte in the adult mouse liver with life-long persistence in the majority of cells. Similar efficiencies can be achieved in newborn mice, but unintegrated (episomal) vector genomes are rapidly lost as a consequence of hepatocellular proliferation occurring in concert with liver growth. As a result, only the small subset of vector genomes that undergo genomic integration persist through to adulthood (Cunningham et al 2008; Dane et al 2009).

Phenotype correction studies in urea cycle deficient mice directly reflect these findings. The *spf*^{ash} mouse model of OTC deficiency has 5-7% residual enzymatic activity. While not hyperammonaemic, these mice have persistent orotic aciduria which provides an excellent biomarker for phenotype correction. Using an AAV2/8 vector encoding a liver-specific enhancer/promoter and the murine OTC cDNA, we found that a *single* i.p. injection of 5×10^{10} vector genomes (vg) into young adult male *spf*^{ash} mice restored liver-wide OTC activity to 60% of physiological values and normalised urinary orotic acid levels for life. At higher doses up to 1.5×10^{12} vg per mouse, OTC activities up to 17-fold above physiological levels could be readily achieved (Cunningham et al 2009), a result that bodes well for human translation. A limitation of the *spf*^{ash} mouse model, however, is the absence of hyperammonaemia, control of which is the clinically relevant therapeutic end-point. To address this limitation we developed an shRNA-based strategy to convert the mild *spf*^{ash} phenotype into a severe hyperammonaemic phenotype, and were able to show that vector doses 5-fold lower than those required to control orotic aciduria were sufficient to control hyperammonaemia (Cunningham et al 2011). Again this result bodes well for human clinical translation.

While these results in adult mice are encouraging, success in the context of severe neonatal disease is considerably more difficult. We therefore next set out to correct a severe neonatal phenotype and exploited the knock-out mouse model of citrullinaemia (ASS deficiency) in the absence of an available OTC knock-out model (Patejunas et al 1994). Three major challenges were encountered. Firstly, rapid onset of hyperammonaemia and death within 24 hours of birth rendered it impossible to achieve adequate levels of ASS expression with sufficient rapidity, despite vector delivery soon after birth. This challenge was overcome by vector delivery in utero in late gestation. In humans this would be unnecessary, as veno-venous haemofiltration is effective for the management of acute hyperammonaemia in infants during the first days of life, and buys time to institute alternate therapies, whether conventional (Lanpher et al 1993) or experimental. Secondly, loss of vector genomes in concert with liver growth necessitated vector re-delivery and thirdly, recurrent exposure to vector led to induction of anti-capsid antibodies which reduced the efficacy of vector re-delivery. This in turn was overcome by changing the vector capsid and other measures (manuscript in preparation). Collectively these interventions allowed successful rescue of the severe neonatal phenotype and survival to adulthood with normal or near normal plasma ammonia levels. The challenge now is to move successful treatment of urea cycle defects in mice through to human therapy. Many of the key issues to be addressed have been clearly elucidated in the literature and in the mouse studies described above. These include the loss of episomal vector genomes as a consequence of liver growth and the resultant need to undertake vector re-delivery, which in turn introduces the limitations imposed by induction of immune responses against the gene delivery vector. Other translational challenges include the increased size of the human liver, unpredictable differences in host-vector biology across species, such as capsid tropism for the liver, and the need to rigorously evaluate vector safety.

We are currently taking advantage of technology that allows primary human hepatocytes, including OTC deficient hepatocytes obtained from explanted livers, to be expanded in the mouse liver (Azuma et al 2007) to identify optimal AAV capsid types for human liver therapy, to confirm phenotype correction with vectors configured for human use and to evaluate aspects of vector-related safety in the context of human cells. Already this “humanized” mouse approach is delivering unexpected insights that are complementing studies being undertaken by others in non-human primates in advance of human trials for urea cycle defects. Data from human trials for haemophilia B using AAV vectors, which are already well advanced, are also proving invaluable (Nathwani et al 2011).

Conclusions

Based on progress to date it can be predicted that, over the coming decade, there will be a steady increase in human gene therapy trials showing therapeutic benefit, including trials for metabolic disease phenotypes. History reminds us, however, that progress will be hard-won and not without unforeseen difficulties. Certainly the field is at the point where correction of mouse phenotypes is increasingly straightforward and there is now a pressing need to increase research focus on the many translational challenges involved in human therapy. Paramount among these is the safety of trial participants. Progress should be cautious with due consideration given to the potential risks and benefits of therapy (Deakin et al 2010). Close working interaction between physicians and scientists, expert in metabolic disease and the science of gene therapy, respectively, is the way forward.

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