Genome Medicine: Gene Therapy for the Millennium

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The Genome Medicine: Gene Therapy for the Millennium meeting provided a forum for discussion of scientific advances stimulated by the explosion of sequence information generated by the Human Genome Project. Genome medicine can be seen as a discipline whose focus is on genetic information that defines both the genetic basis of the disease and therapeutic modalities for optimal treatment of disease pathology. Not only does this information facilitate the functional evaluation of genes *via* biochemical analysis and through animal models, it is also the basis for development of novel therapeutic strategies.

Developments in the modification of cells by genetic intervention have led to gene therapy as a therapeutic modality. The goal of gene therapy is to correct genetic defects by gene transfer. Despite the technical difficulties associated with the transfer of therapeutic DNA, the number of gene therapy trials in humans is increasing. Most of these trials are based on viral gene transfer (e.g., adenovirus, adeno associated virus and retrovirus). The use of virus-based approaches may be limited by insertional mutagenesis and host immune responses that attenuate gene therapy efficacy [1]. While it appears that there has been some clinical success with retroviral gene therapy, other viral systems have proven more recalcitrant. Recent studies involving nonviral transfer implantation of genetically altered fibroblasts that produce Factor VIII into patients with severe haemophilia A have been encouraging [2].

The ultimate goal of gene therapy for inherited diseases is for specific and controllable studies aimed at direct rectification of mutated genes in hereditable disorders. In this respect, homologous replacement, gene repair and artificial chromosomes have particular appeal. Combined with the development of new DNA transfer vehicles and stem cell technology, there is potential for effective implementation of these therapies [3-5].

Recent advances in genome medicine

The surge of DNA sequence information from the efforts of the Human Genome Project and private industry (e.g., Celera) has led us closer to deciphering the human genetic code.Technological developments in gene isolation and DNA sequencing have been important factors contributing to the knowledge of the genes associated with numerous disease pathologies. This information has been critical for enhancing our understanding of the genetic basis of disease and the role that specific genes play in human physiology. Given this wealth of information, issues have arisen concerning how this information is disseminated and applied [6]. These issues were highlighted by R Williamson (Melbourne, Australia) in his Keynote address: 'Gene Therapy: Basic Questions - Future Implications.' To elaborate on these points, JC Kaplan (Paris, France), provided insight into the importance of acquiring functional information for specific genes as a precursor to engaging in studies aimed at genetic and phenotype correction. Using an example of neuromuscular disease, Lesch-Nyhan syndrome, T Friedmann (La Jolla, California, USA) discussed the importance of a comprehensive understanding of how the mechanisms underlying a primary defect influence and disrupt downstream functions in developing effective therapies. The genetic defect in Lesch-Nyhan syndrome results in a dysfunctional purine salvage enzyme, hypoxanthine guanine phosphoribosyltrans-ferase (HPRT) and is a clear example demonstrating the complexity of a simple genetic disorder. J Kere (Uppsala, Sweden) elegantly showed how bioinformatic analysis can be applied to identify genes giving rise to complex disorders such as asthma and psoriasis. Critical to this systems analysis are the centralization of genotype data and communication between clinicians and geneticists to ensure a standardization of data. WH Colledge (Cambridge, UK) reviewed the role animal models play in understanding the physiological consequences of disease gene mutations and in the development of therapies. He provided examples of the various classical gene targeting strategies for modification of genetic embryonic stem cells to create animal models of disease. These gene alterations ranged from ablation of expression, to introduction of missense mutations, to the development of animals with tissue-specific or temporally regulated mutant genes. This technology was initially developed in mouse, but through the advent of nuclear transfer has the potential of being applied in other species to generate large animal models of human diseases.

Ethical implications

Ethical issues regarding the generation and distribution of genetic information about individuals to private or public agencies were discussed in an open session. This discussion also involved input from family support networks like the Cystic Fibrosis Foundation, International Association of CF Adults and Spinal Muscular Atrophy Families as well as patients and parents of patients with genetic diseases. Issues regarding patient education, inclusion in clinical trials and informed consent were examined and discussed (D Wertz, University of Massachussets, USA, T Friedmann, R Williamson). The responsibility and the role of private and public institutions as well as scientists in advancing genetic information and technology into meaningful, humane and effective therapies was discussed by F Antognini (International Association of CF Adults, Switzerland), D Marchetti Jr. (Italian Federation of Spinal Muscular Atrophy Families), E Ronchi (OECD Bruxelles, Belgium), N Weisfeld (NJ Med. Society, Princeton, NJ), H Gottwers (Vienna, Austria) and R Beall (CF Foundation, Bethesda, MD, USA).

Technologies for gene replacement and gene targeting

The limited success of the first-generation, cDNA-based gene therapy strategies has influenced development of genetic therapies for treatment of genetic disease. There has been significant progress in the last few years in the development of alternative gene therapy approaches for treatment of inherited disease. Advances in development of gene targeting strategies using DNA fragments [4,5] as well as those employing chimeric DNA/RNA [7,8] or triplex forming oligonucleotides [9] have shown substantial potential as genetic therapies. Studies by PH Thorpe (Edinburgh, UK) indicated a relatively low efficacy of gene repair (10^{-4}) with RNA/DNA oligonucleotide (RDO) correction of a nonsense mutation (W399X) in the GFP reporter gene. Comparison between the RDOs and small fragment homologous replacement (SFHR) suggested a greater cell type range of site-specific modification with SFHR than with the RDOs. The RDO strategy was further elaborated upon by B Kren (Minneapolis, MN, USA), who showed effective long-term modification of the Factor IX gene in rat heptozytes *in vivo*. However, when compared to single-stranded DNA (ssDNA) oligonucleotides, as is also the case with SFHR, RDOs showed no real advantage. Studies by M Rice (Newark, DE, USA) and O Igoucheva (Philadelphia, PA, USA) also compared RDOs to ssDNA oligonucleotides showing similar effects. A Colosimo (Chieti, Italy) demonstrated that SFHR could effectively correct ~ 4% of an antibiotic resistance gene (Zeocin) in a transiently transfected plasmid [10]. SFHR was also used by R Kapsa (Melbourne, Australia), to repair a nonsense mutation in the dystrophin locus of the *mdx* mouse [11]. The high level of correction obtained *in vitro* (15–20% of *mdx* loci) has yet to be obtained *in vivo* by direct gene injection in muscles. In *ex vivo* transfection studies targeting the human β-globin locus KK Goncz (Burlington, VT, USA) was able to reproducibly demonstrate SFHR by introducing 'targeting' fragments into the nucleus of hematopoietic cells by microinjection and electroporation. This study demonstrates the potential of using SFHR in *ex vivo* gene therapy (e.g., autologous transplantation of SFHR-modified stem cells). In another application of SFHR, E Bruscia (Rome, Italy and Burlington, VT, USA) generated isogenic cell lines that have different SFHR-generated CFTR alleles. The parental cells are homologous for the ΔF508 mutation, the SFHR daughter cells are heterozygous with one wild type allele and one ΔF508 allele. These 'isogenic' cell lines also express a correct wild type mRNA. Another gene targeting approach, guided homologous recombination (GOREC) was illustrated by JS Sun (Paris, France) and is based on triple helix forming oligonucleotide-directed site-directed mutagenesis.

Chromosome engineering

Another genome-based gene therapy involves the development of artificial chromosomes. As with the gene targeting approaches, artificial chromosomes provide another means to maintain gene integrity after correction of the mutation associated with the disease causing phenotypic dysfunction. P Warburton (New York, NY, USA) gave a comprehensive overview of artificial chromosome construction and the role that centromeric regions play in these constructs. B Grimes (Cleveland, OH, USA), Z Larin (Oxford, UK) and H Matsumoto (Nagoya, Japan) discussed the specifics of human artificial chromosome construction in the context of α-satellite DNA. D Schindelhauer (Munich, Germany) presented data on improvements in the generation of human artificial chromosomes carrying genomic CFTR DNA. Studies presented by C Farr (Cambridge, UK) demonstrated how mini-chromosomes can be used to decipher X-chromosome structure. MH Sheu (Edinburgh, UK) showed that these mini chromosome constructs are stable during mitotic and meiotic segregation by demonstrating their transmission through the mouse gene line. It is clear that, while artificial chromosomes are still under development, significant progress has been made in the translation of the this technology to treatment of genetic disorders.

Gene transfer technologies

Advances in the development of gene transfer vehicles have facilitated cell and organelle-specific targeting as well as the delivery of oligonucleotides and artificial chromosomes. The modification of these artificial vehicles with cell-type specific ligands have been critical for transferring DNA into specific cells, facilitating its escape from endosomes and/or entry into the nucleus. A Ziady (Cleveland, OH, USA) showed that DNA-poly-L-lysine complexes modified with peptide ligands C105Y and C1315 change the binding capacity of the therapeutic complex to cellular targets in CF airway epithelia. Similarly, JS

Remy (Strasbourg, France) presented his studies using polyethylenimine (PEI) in targeting the lung *in vivo* and in the role that purification of PEI/DNA complexes play in enhancing transfection *in vivo*. Another group (I Fajac, Paris, France) reported results on the effect of glycosylating polylysine complexes. SL Hart (London, UK) presented data from studies utilizing peptides, isolated from phage display libraries, to facilitate uptake of DNA lipid complexes. M Magnani (Urbino, Italy) presented data on the potential of using erythrocytes as a delivery system for drugs, peptides and modified oligonucleotides [12]. Data presented on a Phase I/II study suggested that this may be an effective approach for delivering dexamethasone to cystic fibrosis patients. E Wagner (Munich, Germany) gave an overview of DNA therapeutics using synthetic delivery systems. He stressed that progress in DNA therapeutics is based on several factors that include:

- the clinical target
- the gene
- the technology dictated by the therapeutic target
- the optimization of delivery and expression

Non-embryonic stem cells

Recent studies on non-embryonic stem cells have demonstrated that pluripotent stem cells can not only be isolated from bone marrow but from other tissue as well. These stem cells have been shown to have the capacity to repopulate multiple organ systems and display differentiated features characteristic of the organ they repopulate [13]. This has significant implications for the development of *ex vivo* gene therapy strategies for numerous inherited diseases and for autologous transplantation of modified cells. A special Satellite Meeting addressed issues surrounding the use of non-embryonic stem cells in gene therapy. Strategies directed at using genetically modified autologous stem cells for sickle cell anemia were presented by P Malik (Los Angeles, CA, USA). Genetic manipulation of hematopoietic stem cell growth (RK Humprhies, Vancouver, BC, Canada) and elucidation of the cellular basis of regeneration of skeletal muscle were covered by T Partridge, (London, UK) respectively. M Heddrick (Los Angeles, CA, USA) presented data on the isolation of pluripotent stem cells from adipose tissue, G Cossu (Milan and Rome, Italy) demonstrated that vessel-associated stem cells (meso-angioblasts) were also multipotent and self renewing. Of particular significance were the studies by D Krause (New Haven CT, USA) who showed that stromal stem cells could repopulate multiple organ systems including brain, liver and lung epithelium and muscle.

Expert opinion and conclusions

Technological advances are progressing towards the day that the genomic basis of inherited diseases can be corrected [14]. The individual genome-based gene therapy strategies (i.e., sequence-specific gene modification and artificial chromosomes) have the potential of providing comprehensive genetic therapies that are efficacious and maintain genomic integrity. The actual limitations of gene therapy that have been encountered can be overcome by development of an integrated approach that addresses gene structure, phenotypic variation and therapeutic DNA delivery. It will be particularly important to evaluate the enzymatic mechanisms that underlie homologous replacement and sequencing to optimize these gene targeting strategies rectifying the pathologies associate with genetic lesions. We hope that readers will share our excitement and optimism which epitomize how diverse and dynamic gene therapy research is at the start of the 21st century.

In the context of these advances in genome-based therapies and their potential for clinical application, as a component of genome medicine, it will be equally important to define the ethical and social parameters with which genetic information is disseminated and applied. Given this framework and the candor with which the technological and social issues were discussed, we are very optimistic and enthusiastic about the future of gene therapy and the role that it can play in medicine. We are clearly at the beginning of a new epoch in medicine.

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14. SUMMARY TABLE: Human gene therapy protocols, last updated: 8/10/01. *Hum Gene Ther.* 12, 2019-2020 (2001).

•• **The current status of human gene therapy: trials and protocols.**