stem cell research&therapy

COMMENTARY

Stem cell-based therapy for α_1-antitrypsin deficiency

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Abstract

Human induced pluripotent stem cells offer the possibility of generating unlimited quantities of cells for autologous transplantation. By correcting the genetic defect underlying Z-allele α_1-antitrypsin deficiency, we recently provided the first proof of principle for application of human induced pluripotent stem cells in the treatment of inherited genetic disorders. Several important safety concerns will need to be addressed before this can be translated into clinical practice.

α_1-Antitrypsin is synthesised in the liver and released into the circulation, where it is the most abundant circulating protease inhibitor. Most individuals carry two copies of the normal M allele. One in 25 Caucasians of North European descent, however, carry the Z allele that results from the substitution of a lysine for glutamic acid at position 342 in the polypeptide chain. Over the past 20 years we have used a range of techniques to show that the Z allele causes α_1-antitrypsin to misfold and form ordered polymers that are retained within the endoplasmic reticulum of hepatocytes [1-4]. Individuals who are homozygous for the Z allele develop cirrhosis as a consequence of α_1-antitrypsin entrapment-induced hepatocyte death. The lack of circulating α_1-antitrypsin predisposes to early-onset emphysema.

We have focused our efforts on a cure for α_1-antitypsin deficiency by designing peptides and small molecules that can block polymerisation *in vitro* [5]. An alternative approach to this and other related inherited metabolic disorders of the liver is to use whole cells for therapy. Animal models have demonstrated that wild-type hepatocytes have a selective advantage when transplanted into the liver of a mouse with a genetic abnormality [6]. However, the translation of such studies into the human context has been disappointing [7]. This disappointment is due to the scarce availability of large numbers of high-quality hepatocytes. An additional problem, common to whole-organ transplantation, is the continued requirement for lifelong harmful immunosuppression. Seminal work by Takahashi and Yamanaka raised the exciting possibility that induced pluripotent stem cells (iPSCs) could be used to generate large quantities of high-quality cells for autologous transplantation [8].

We explored the prospect of iPSC-based cell therapy by deriving fibroblasts from skin biopsies of individuals who are homozygous for Z-allele α_1-antitrypsin deficiency (PiZ). These cells were reprogrammed with retroviral constructs that overexpressed key pluripotency-associated transcription factors to produce patient-specific iPSCs. The stem cells were then differentiated using a novel in-house protocol to produce hepatocytes that recapitulated many of the features of the clinical phenotype [9]. Specifically the hepatocyte-like cells from PiZ homozygotes formed polymers that were retained within the endoplasmic reticulum [9]. This potentially limitless supply of cells provided us with a useful new model since, unlike previous cell models, the mutant α_1-antitrypsin is under the control of the endogenous promoter.

The use of iPSCs within the context of treating individuals with PiZ would require correction of the underlying genetic abnormality in a manner fully compatible with clinical applications. The genetic defect responsible for PiZ (Glu342Lys) was therefore targeted using a combination of engineered zinc finger nucleases and a piggyBac donor vector in patient-specific human iPSCs [10]. Through this approach we successfully demonstrated for the first time an efficient gene-editing technique capable of restoring normal structure, function and secretion of α_1-antitrypsin in subsequently derived liver cells. This genetic correction did not leave residual exogenous sequences in the targeted iPSC genome. The drawback of this approach is that retroviral reprogramming vectors remain within the genome. We therefore derived human iPSC lines using a nonintegrating RNA (Sendai) virus and produced corrected hepatocytes with stable karyotypes in almost completely chemically defined culture conditions. Given the concern surrounding the safety of stem cell products, however, more effort was required to understand the genetic stability of human iPSC lines. Lines were characterised not only at the

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chromosome number level (by G-banding), but also for copy number variation (using array-based comparative genomic hybridisation) and single base pair resolution (using whole exome sequencing). A large proportion of Sendai virus-reprogrammed human iPSCs carried the correct numbers of chromosomes but had significant copy number variation detected by array-based comparative genomic hybridisation. Lines genetically stable by array-based comparative genomic hybridisation could be identified and these were subjected to whole exome sequencing. This analysis revealed that whilst the gene correction technique did not perturb the genome, the initial derivation of nonretroviral human iPSC lines induced 29 exomic point mutations.

The biological relevance of the point mutations in iPSC-derived hepatocytes is unclear as none of the new mutations occurred in genes known to predispose to cancer. Moreover, to the best of our knowledge such an in-depth analysis of human cell lines has not previously been performed. We therefore investigated the behaviour of the cells *in vivo* by injecting genetically corrected iPSC-derived liver cells into a mouse model of liver injury. This assay confirmed the functional capacity of our cells and importantly demonstrated that the point mutations did not cause catastrophic carcinogenic sequelae since none of the mice developed tumours. In total, our results provided proof of principle for the potential of combining human iPSCs with gene therapy techniques to generate cells for autologous cell-based treatment of individuals with α_1-antitrypsin deficiency.

Several challenges remain before this technology can be applied within the context of a clinical trial. First, the cell type produced *in vitro* remains of a foetal nature in terms of its functionality, and great efforts must now be made to improve protocols of differentiation to realise the final step of maturation in order to achieve truly adult-like cells. The final step of maturation, however, may perhaps only be achieved within an extracellular niche of the human liver. If this was to be the case then, as long as the iPSC-derived hepatocytes can be shown to be safe, a move towards the clinic seems rational. Defining safety will require considerable discussion amongst scientists and clinicians alike and will need more comprehensive animal data than we had the time to perform in our study. In the interim, our work opens the possibility that, with the correct level of screening, iPSC-derived products may soon realise their much anticipated use for the treatment of human disorders.

Abbreviations

iPSC, induced pluripotent stem cell; PiZ, Z-allele α_1-antitrypsin deficiency.

Competing interests

The authors declare that they have no competing interests.

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