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Human stem cell-based disease modeling: prospects and challenges

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Human stem cell-based disease models have great promise to advance our understanding of human disease. These models can be derived from patients with genetic disorders and manipulated with genome editing and myriad differentiation protocols to model pathologies *in vitro*. However, several challenges have impeded the full potential of stem cell-based *in vitro* disease modeling. Many genetically predisposed diseases take time to manifest and occur in specific tissue microenvironments, and these parameters are often not adequately modeled using conventional shorter-term monolayer cultures. These challenges must be overcome especially for cases where animal models also incompletely recapitulate the complex pathologies found in humans. As prominent ways to tackle these challenges we discuss here how advanced genome editing tools in human stem cells and human organoid cultures, specifically the example of intestinal organoids, contribute genetically defined models that recapitulate phenotypes of disease.

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The advent of stem cell-based disease modeling and current challenges

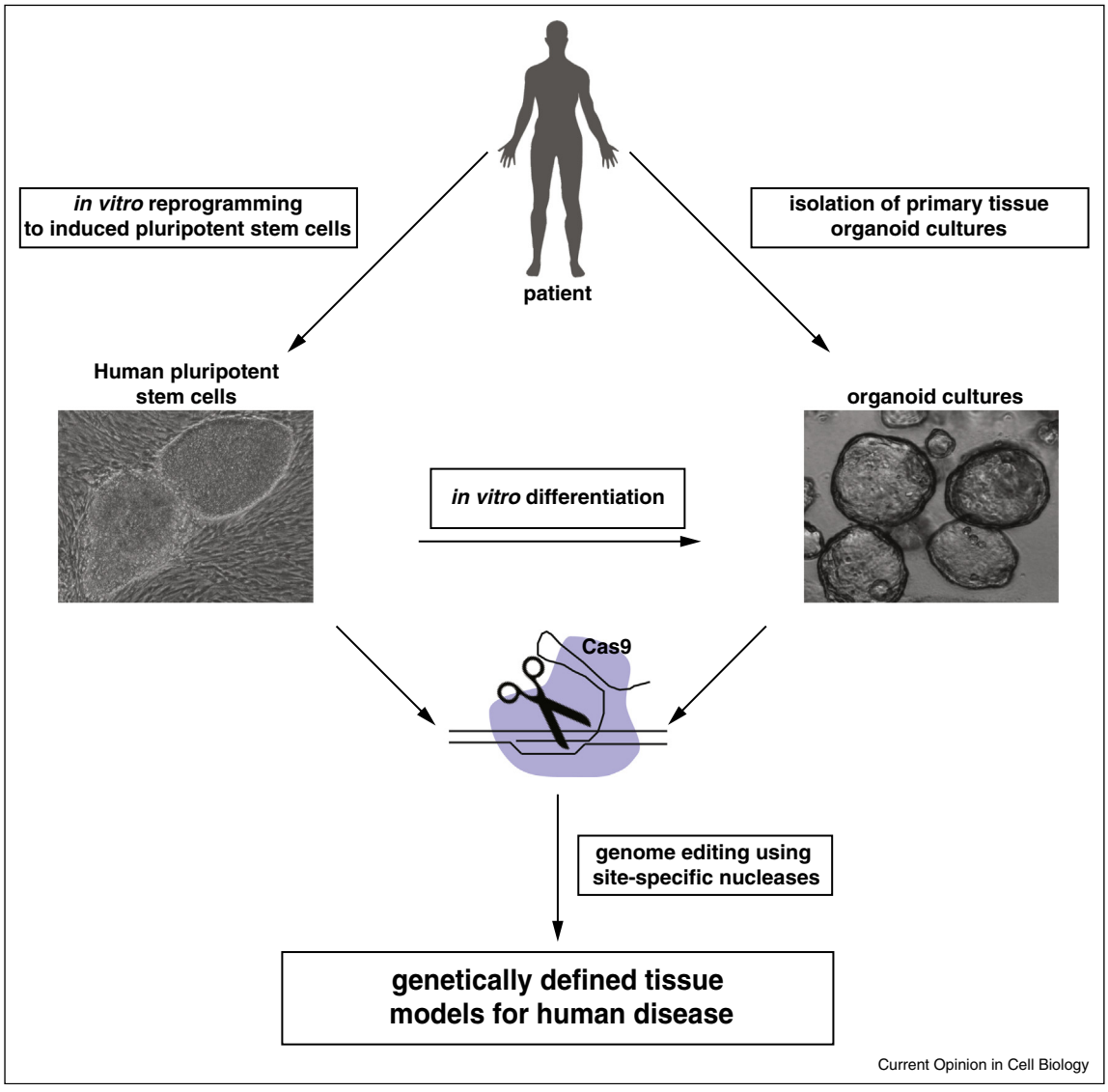
One of the most important developments in disease modeling was the generation of induced pluripotent stem cells (iPSCs) [1,2], which are functionally equivalent to embryonic stem cells (ESCs) [3–5] and are collectively referred to as pluripotent stem cells (PSCs). Cells taken from a patient with a genetic disease can be reprogrammed as human iPSCs and can subsequently be differentiated into disease-relevant cell types to uncover molecular and cellular mechanisms and to screen for drug treatment options (Figure 1).

A significant challenge in iPSC-based disease modeling lies in the fact that each disease-specific iPSC line is genetically distinct due to the genetic variability among patients [6]. As a consequence, phenotypes of iPSC disease models can show striking variability between individual patient-derived cell lines [7]. Moreover, variability can also be caused by the reprogramming process used to create the iPSCs [8,9]. This variability greatly challenges our ability to model disorders with mild or complex phenotypes. Recently, we and others have overcome this limitation by establishing the use of site-specific nucleases (reviewed in [10,11]) in hPSCs, allowing a level of genetic control previously limited to traditional model systems [12•,13–15]. As a result, we can now perform targeted gene knock-outs, generate tissue-specific cell lineage reporters, overexpress genes from defined loci, and introduce and repair point mutations in hPSCs. This genetic amenability of hPSCs allows researchers to generate sets of isogenic cells that differ exclusively at the site of editing. Consequently, the phenotypes identified in these cells can be attributed to the disease-relevant mutation rather than the specific genetic background of a given patient.

A proof of concept for this approach in hPSCs was the genome editing-mediated correction of disease-causing mutations in α-synuclein that cause a familial form of Parkinson's disease [16•]. Comparing isogenic cortical neurons differentiated from these iPSCs identified that α-synuclein mutations caused accumulation of nitrosative and endoplasmic reticulum stresses [17•]. Furthermore, comparing these isogenic iPSCs in a similar approach showed that α-synuclein disease-causing mutations predisposed iPSC-derived dopaminergic neurons to mitochondrial stresses from environmental toxins known to be associated with Parkinson's disease [18].

An elegant approach to increasing the efficiency of gene repair of disease alleles *in vitro* combines genome editing with the use of *piggyBac* transposase to correct patient-derived iPSCs for a point mutation in the α(1)-antitrypsin gene, which causes α(1)-antitrypsin deficiency [19]. This gene repair approach utilizes antibiotic selection of a zinc finger nuclease (ZFN)-mediated correction of the disease-causing allele using a selection cassette. Overexpression of *piggyBac* transposase can later be used to 'scarlessly excise' the selection cassette once a corrected hPSC clone is isolated and genotyped. This strategy yields efficient bi-allelic changes in patient-derived iPSCs, and restores enzymatic function of iPSC-derived and transplanted hepatocytes.

Fig. 1



Model systems to elucidate the mechanism of disease. Somatic cells derived from a patient afflicted with a genetically predisposed disease can be reprogrammed into induced pluripotent stem cells (iPSCs). These iPSCs can be differentiated into tissue-specific organoid cultures, which can also be derived from tissue samples of the patient. Modern genome editing technologies can be used in iPSCs and organoids to establish genetically defined models for disease. These model systems can be used to understand the influx of information from GWAS and then derive understanding of the epistatic relationship of genetic variants on pathology. Further work has allowed these model systems to employ the complexity of the organism in xenografts, which will facilitate the understanding of complex disease and will be important for the screening of clinically relevant drugs.

A more general translational application of genome editing that increased the versatility of iPSC-based disease modeling has been demonstrated for trisomy 21. Jiang *et al.* [20] showed that Down syndrome patient-derived iPSCs could be engineered to insert an inducible gene for the Xist lncRNA into chromosome 21. Induction of Xist in the edited iPSCs transcriptionally represses the third copy of chromosome 21 and thereby reverses cellular disease phenotypes *in vitro*.

Since these initial studies utilizing ZFNs [12•,21••] and transcription activator-like effector nucleases (TALENs) [13,22–24], the advent of the 'Cas9 revolution' — the establishment of site specific nucleases based on the bacterial adaptive defense system CRISPR (Clustered Regularly Interspersed Short Palindromic Repeates)/Cas9 (Cas9) — has made genetic engineering of stem cells a widely available and standard tool in human disease modeling. Since the founding work by Jinek *et al.* [25•], Cas9 has