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Developing precision medicine using scarless genome editing of human pluripotent stem cells

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Many avenues exist for human pluripotent stem cells (hPSCs) to impact medical care, but they may have their greatest impact on the development of precision medicine. Recent advances in genome editing and stem cell technology have enabled construction of clinically-relevant, genotype-specific ``disease-in-a-dish'' models. In this review, we outline the use of genome-edited hPSCs in precision disease modeling and drug screening as well as describe methodological advances in scarless genome editing. Scarless genome-editing approaches are attractive for genotype-specific disease modeling as only the intended DNA base-pair edits are incorporated without additional genomic modification. Emerging evidentiary standards for development and approval of precision therapies are likely to increase application of disease models derived from genome-edited hPSCs.

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Introduction

Improving pre-clinical disease models to more faithfully predict clinical effectiveness and identify toxicity is anticipated to lower the current 90% clinical trial failure rate [1]. This is especially important in the context of **precision medicine**, because disease models need to be tailored to specific **biomarkers** or genetic variations (bolded terms in Box 1). Human **pluripotent** stem cell (hPSC) based disease models are good candidates to meet this challenge, since they can be tailored through genome editing for the rigorous evaluation of genotype-to-disease phenotype relationships in biologically relevant human cells.

Human induced pluripotent stem cells (hiPSCs) are **reprogrammed** from routine clinical samples (e.g., blood draws, skin biopsies) and can be reliably expanded in culture [2,3]. Importantly, hiPSCs retain their patient specific genotype throughout the reprogramming process. This feature enables hiPSCs to be differentiated towards disease affected cell types in order to recapitulate a patient's disease phenotype [4] and to evaluate patient-specific therapeutic response in a controlled cell culture environment [5] (Fig. 1A). Stem cell-derived disease models are most applicable for modeling diseases with quantifiable cell autonomous disease phenotypes observed in well-defined cells. With continued advancement of cell differentiation protocols, the ''disease-in-a-dish'' paradigm has been applied to an array of neurodegenerative, cardiovascular, and other diseases [6].

As the process of reprogramming cells has become more efficient, bio-banks of hiPSC cohorts that are disease specific or representative of the general population have been created

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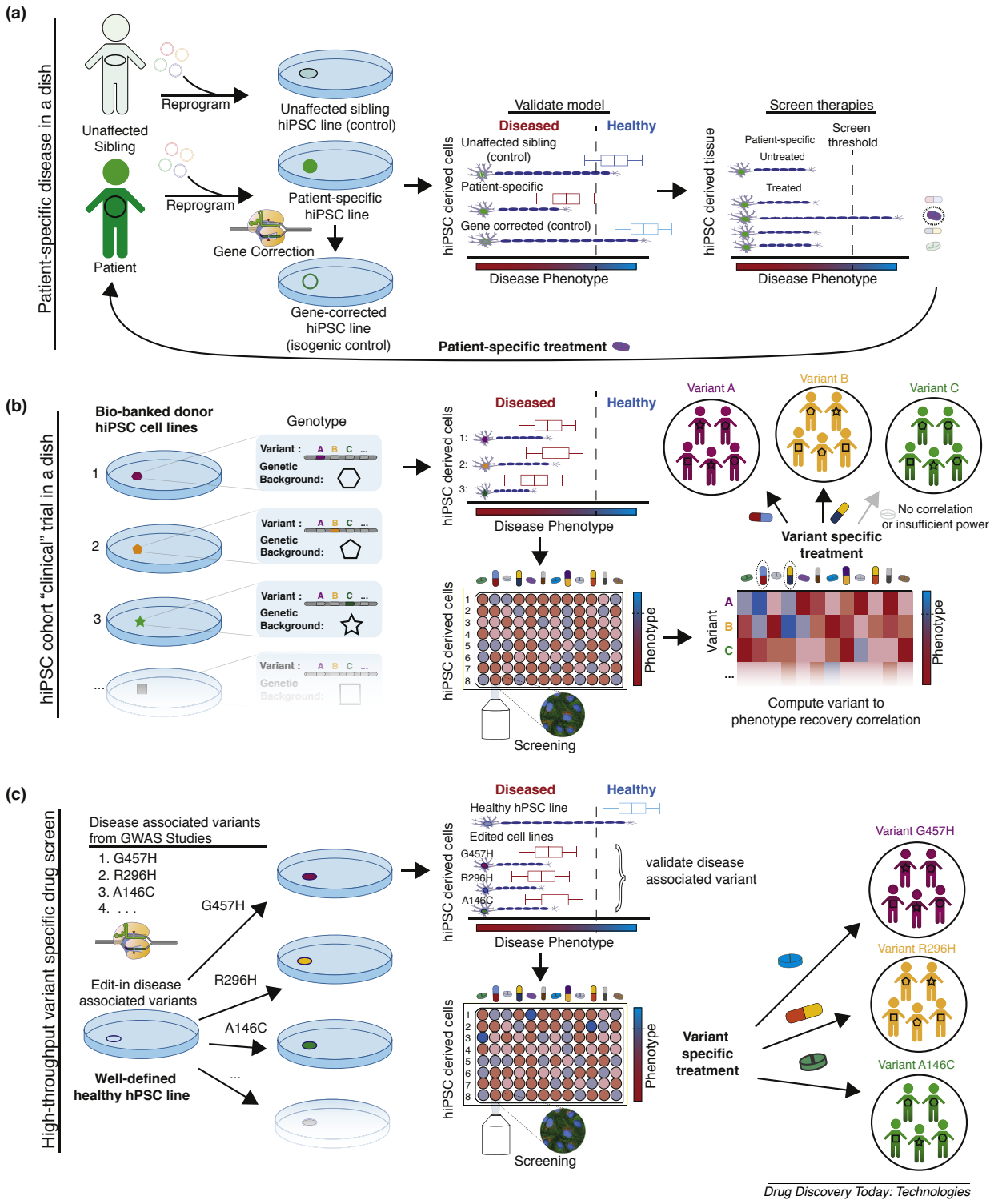

Fig. 1. Paradigms of stem cell disease modeling for therapeutic screening. (A) Patient-specific disease in a dish models. Unaffected siblings share only ~50% genetic inheritance with affected patients and the field has largely moved towards isogenic controls to validate phenotypic outputs (diseased vs. healthy). These screens are not generalizable (i.e., can only inform treatment for a specific patient) in isolation due to potentially confounding effects of background gene modifiers. (B) hiPSC cohort clinical trial in a dish . Cohorts of bio-banked iPSCs, with various disease associated variants/biomarkers and various genetic backgrounds, are differentiated and cell line to phenotypic recovery outcomes are identified for candidate drugs. Computational analysis is performed to identify externally valid (i.e. results can be used to inform treatment for patients not in cohort) drug specific variant/biomarker to phenotypic recovery correlations. Cohort approaches are ideal for diseases with complex inheritance and access to patient hiPSCs. (C) High-throughput, variant-specific therapeutic screening. Disease associated variants can be introduced into a well-defined hPSC line with scarless genome editing to create an array of isogenic cell lines. Direct assessment of variant/biomarker specific therapeutic response relationships can be observed with high-content screening (or other high-throughput) assays. This approach is ideal for rare monogenic diseases where access to patient-specific hiPSCs is limited.

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