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NEWS AND VIEWS

Evolution of iPSC disease models

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Human induced pluripotent stem cells (iPSCs) hold unprecedented potential to model human development and genetic disorders due to their capability to self-renew and differentiate into any somatic cell type.

In the year of 2011, a number of iPSC disease models have been successfully developed for studying the mechanism of human diseases as well as establishing platforms for drug screening and testing (Batista et al., 2011; Brennand et al., 2011; Devine et al., 2011; Itzhaki et al., 2011; Koch et al., 2011; Liu et al., 2011a; Mazzulli et al., 2011; Nguyen et al., 2011; Paşca et al., 2011; Quarto et al., 2011; Tiscornia et al., 2011; Wu and Hochedlinger, 2011; Yazawa et al., 2011; Zhang et al., 2011; Zhu et al., 2011). One key hypothesis of current iPSC disease models is based on the presumption that wild type and diseased iPSCs are equal to their embryonic stem cell (ESC) counterparts. However, this hypothesis seems to be challenged by several recent findings on the striking differences between ESCs and iPSCs. At the genomic level, the reprogramming process tends to cause the accumulation of DNA mutations as well as other chromosomal abnormalities related to cancer pathways (Panopoulos et al., 2011). Whereas there is no relevant *in vivo* data indicating that these mutations are indeed linked to tumorigenesis, the genetic aberrances accumulated during reprogramming probably interfere with the cellular parameters in either iPSCs or their differentiated derivatives. If specific diseases, especially those associated with genomic instability, facilitate the accumulation of more genetic mutations during reprogramming, this could result in wrong explanation of the phenotypes of iPSC disease models. In fact, the evaluation on how aspects of specific diseases affect reprogramming-associated mutations has not been reported. Additionally, at the epigenomic level, the residual epigenetic memories of iPSCs from their original cellular environment likely represent another barrier to being a true phenocopy of their ESC counterparts. In support of this, mouse iPSCs, but not ESCs, show immune rejection upon transplantation (Zhao et al., 2011), suggesting that subtle epigenetic differences might cause substantially different cell identities. To date, it is not clear whether disease-associated epigenetic memories exist in patient-specific iPSCs. Only a few groups examined the successful resetting of abnormal epigenetic marks during reprogramming, such as histone and DNA methylation, in their iPSC disease models (Marchetto et al., 2010; Liu et al., 2011a). At the differentiation level, a number of reports have shown that iPSCs behave differently from their ESC counterparts (Buchholz et al., 2009; Bock et al., 2011; Kim et al., 2011), although researchers still don't know how this difference affects recapitulation of disease phenotypes *in vitro*. It should also be noted that certain diseased somatic cellular environments might contribute to the defective reprogramming with a higher possibility. For example, cellular defects in Fanconi anemia patient fibroblasts resulted in a complete blockage of iPSC generation (Raya et al., 2009). Hence, to avoid misinterpretation of results, it seems essential to first evaluate whether generated patient iPSCs are completely reset to a patient ESC-like status. Along this line, thorough examination of various cellular parameters in patient-specific iPSCs could be a critical step before employment of any iPSC disease model in mechanistic studies or drug testing. As complementary approaches, the relevant assays with overexpression of a mutant (e.g. for dominant mutation) or knock-down of an endogenous protein (e.g. for recessive mutation) in disease-related cell types should be included to verify the specific aspects of disease phenotypes.

Furthermore, the lack of appropriate control iPSC lines constructs another important experimental limitation for the use of patient-derived iPSCs. In fact, the "wild type" or "healthy" iPSC lines currently used as controls are derived from "phenotypically normal" populations, which nevertheless carry various genetic and epigenetic polymorphisms. The major concern with respect to these epigenetic and genetic variations is that they may cause inconsistent phenotypic

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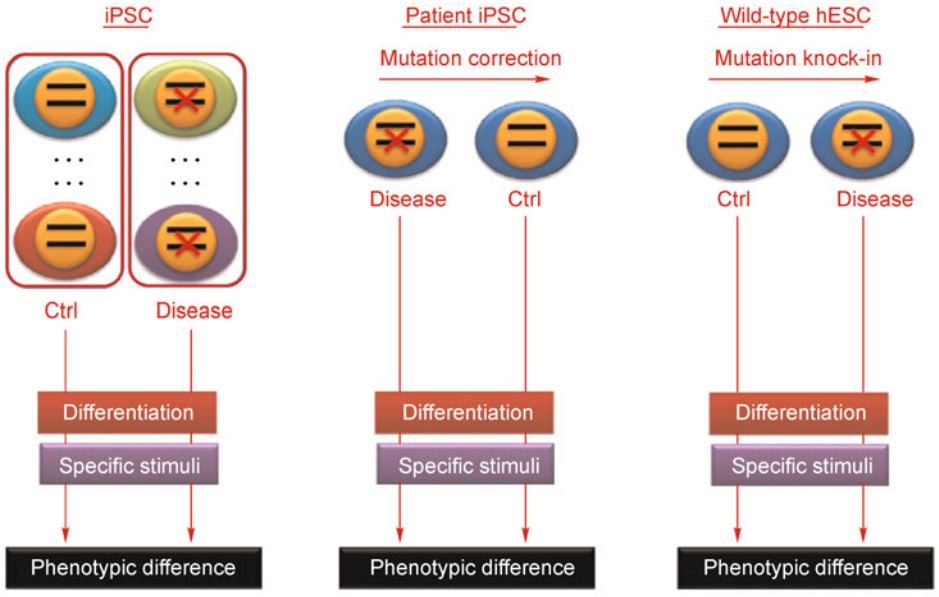

Figure 1. Studying human diseases with pluripotent stem cell (PSC) models. Classic iPSC disease models utilize multiple control and diseased iPSC lines to recapitulate disease phenotypes (left). Correction of disease mutation in patient iPSCs (middle) or knock-in of disease mutation in hESCs (right) is able to generate isogenic PSC lines for disease modeling.

outputs. Although it could be helpful to use many iPSC lines from different individuals to overcome some variations (Fig. 1), high genetic background noise derived from multiple individuals would mask the subtle phenotypic differences in iPSC disease models, especially for diseases with low penetrance or diseases related to aging. Correction of known disease-specific mutations in patient iPSCs, which is now becoming feasible, probably represents a superior approach to generate isogenic iPSC controls for disease modeling (Fig. 1) (Deyle et al., 2011; Hockemeyer et al., 2011; Howden et al., 2011a, 2011b; Li et al., 2011; Liu et al., 2011b, 2011c; Pan et al., 2011; Sebastiano et al., 2011; Soldner et al., 2011; Yusa et al., 2011; Zou et al., 2011). Nevertheless, these studies are still based on a presumption that patient iPSCs can faithfully recapitulate disease phenotypes upon differentiation like their ESCs counterparts.

A more unbiased way to model diseases would be to genetically manipulate ESCs and introduce disease-specific mutations (Fig. 1). Recently, Jaenisch group have successfully generated Parkinson disease-specific ESCs by introducing related genetic mutations with Zinc Finger Nuclease (ZFN) technology (Soldner et al., 2011), opening an avenue to model diseases with isogenic mutation-bearing ESCs. The engineered human ESCs with disease-associated mutations may represent the most reliable model for disease study. Direct knock-in of disease-specific mutations in ESCs would bypass the reprogramming steps as well as reprogramming-associated side-effects. This would not only short-cut the necessity for strict validation of patient-derived iPSCs but also avoid the potential misconclusions that could arise from the use of a defective iPSC disease model. Indeed, differential pathological characteristics between patient specific iPSCs and ESCs have been recently observed in specific disease contexts (Urbach et al., 2010). For instance, human ESCs and iPSCs behave differently in modeling fragile X syndrome triggered by extended copies of CGG trinucleotide repeat in chromosome X-encoded *FMR1* gene. In this case, *FMR1* is transcriptionally activated in patient ESCs, but epigenetically silenced in diseased iPSCs (Urbach et al., 2010), raising a concern on whether iPSC disease models could faithfully reflect the process of diseases. In contrast, the disease phenotypes of Marfan syndrome were recently successfully recapitulated by using both patient iPSCs and ESCs (Quarto et al., 2011), arguing that iPSC models could act faithfully at least in certain disease contexts. Therefore, isogenic mutant ESCs generated by reverse genetic manipulation indeed provide superior tools for modeling single-gene genetic disorders, although iPSC-based models are irreplaceable for studying specific human diseases especially those with unknown biological clues, such as schizophrenia (Brennand et al., 2011).

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