See page 228

Small but Significant: Inter- and Intrapatient Variations in iPS Cell–based Disease Modeling

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Last year the Nobel Prize in Physiology or Medicine was awarded to John B. Gurdon and Shinya Yamanaka for their groundbreaking research on reprogramming of somatic cells.[1,2] Using a set of just four transcription factors, Yamanaka demonstrated that somatic cells could be reprogrammed into induced pluripotent stem (iPS) cells that exhibited most, if not all, of the hallmarks of bona fide pluripotent stem cells. This observation immediately raised the prospect of patient-specific pluripotent stem cells both for therapeutic applications using stem cell–based transplants and for disease modeling. However, six years after the appearance of this landmark study, the suitability of patient-specific iPS cells for disease modeling or drug screening remains challenged by the existence of clone-to-clone variability that can complicate such studies.

In this issue of *Molecular Therapy*, Thatava *et al.* describe the generation of three iPS cell lines from each of three individual patients suffering from type 1 diabetes (T1D) and the subsequent differentiation of these T1D-iPS cells into pancreatic cells.[3] Interestingly, they observed a high degree of intrapatient variability in the capacity of the T1D-iPS cells to develop into glucose-responsive insulin-producing cells. Indeed, the iPS cell lines derived from a single donor seemed to be as different from each other as individual iPS cell lines derived from unrelated donors.

These observations highlight a number of caveats to iPS cell–based disease modeling (**Figure 1**). These include sequence variations such as point mutations in single cells of the original nonclonal somatic cell population, the fidelity of nuclear reprogramming and the impact of residual epigenetic signatures derived from the original cells, potential genomic alterations during the initial expansion of iPS cell colonies into established iPS cell lines, potential subclone-related immunogenic properties, and, finally, the variability between differentiation protocols *per se* and the variability of the phenotype of the differentiated cells derived from disease-affected and control iPS cell lines.

Deep-sequencing strategies had previously demonstrated that at least half the point mutations identified in established human iPS cells were present in a very small subset of the starting cell population.[4,5] Very recently, Abyzov *et al.* described "line-manifested" copy-number variations (CNVs) among dermal fibroblasts and iPS cells derived from them, which reflected a somatic mosaicism normally present in human skin.[6] These observations underline the need for extensive genetic screening of established iPS cell lines intended for potential therapeutic applications. Moreover, such mutations might affect differentiation propensities or the cells' responses to pharmacological or genetic treatments—suggesting ramifications for disease modeling as well.

Recent data have shown that a preferential stoichiometry of reprogramming factors greatly enhances the generation of iPS cells,[7,8] and there is increasing evidence that the extent of reprogramming of the epigenetic status of the original cells toward a fully pluripotent state is also very sensitive to factor stoichiometry.[9] However, the latter can be ensured using elaborate polycistronic reprogramming constructs.[10] Bock and colleagues have developed a valuable epigenetic scorecard[11] that assesses the transcriptional and epigenetic similarity of iPS cell lines and that might prove useful in identifying individual lines with a full capacity to differentiate *in vitro*.

Notably, the degree of CNVs, as well as epigenetic and transcriptional differences, seems to be greater in early iPS-cell passages.[12,13] In these studies it was postulated that replicative stress during the initial phase of reprogramming resulted in mosaic early-passage colonies that contain cells with a high number of CNVs. Quite a few of these CNVs might give rise to a growth disadvantage that gradually removes certain clones, which could explain why later passages of iPS cell lines exhibited fewer CNVs. However, given that the number of chromosomal abnormalities increases at higher passages,[14] high-passage iPS cell lines could acquire other genetic aberrations that interfere with their capacity to differentiate or with the resulting cell type–specific phenotype.

The ideal differentiation protocol should lead to an iPS cell–derived, but tissue-specific, cell that closely mimics the phenotype of its *in vivo* counterpart, with high efficiency. So far, even the most sophisticated differentiation protocols result in a quite heterogeneous cell population and lack reproducible efficiency if different pluripotent starting cells are used. With respect to endodermal and, in particular, pancreatic cell differentiation, protocols are stepwise in nature, comprising induction of definitive endoderm through a meso-endodermal progenitor cell state followed by the specification of a more tissue-specific state (pancreatic precursor cell). These precursor cells can further mature *in vivo* following transplantation and can thus give rise to results

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that are superior to those obtained when using more mature cells, which might have failed to acquire the full metabolic capabilities of the intended target cell owing to missing or inefficient cues during *in vitro* differentiation.[15] This observation casts doubt on the current concepts for *in vitro* disease modeling because an assumed maturation state of *in vitro* differentiated cells may not represent a state that provides metabolic function after engraftment *in vivo*.

Thatava and colleagues report the reproducible generation of just such a pancreatic progenitor cell from different patient-specific iPS cells. However, the further differentiation of these cells into functional (i.e., glucose-responsive) insulin-producing pancreatic β-cells was successful in only a subset of iPS cell lines.[3] This observation of high intrapatient as well as interpatient variability between functionally differentiated cell phenotypes may support a paradigm shift for differentiation strategies applied in disease modeling—rather than aim for mature cell phenotypes in a direct and rapid differentiation protocol, one might first aim to generate well-defined and expandable precursor cells as an intermediate step.[16,17] After such self-renewing progenitor cells were established, one could extensively characterize their epigenetic and transcriptional profile and identify progenitor cell lines that might most efficiently respond to subsequent differentiation cues during further *in vitro* differentiation. Then, in a second step, these cells could be differentiated toward mature cells in a more reproducible manner and presumably with greater functionality.

If the disease-related phenotype of patient-specific iPS cell derivatives was subtle compared with that of derivatives of iPS cells from healthy individuals, such intra- and intersample variation could cloud the capacity to detect disease-specific effects. Therefore, it has been postulated that syngeneic control iPS cell lines might be useful to minimize intersample differences between disease-specific and control cell lines. Such syngeneic control iPS cells could be generated by applying tailored strategies for genetic correction of disease-associated mutations, which might be achieved by homologous recombination mediated by zinc-finger nucleases or transcription activator–like effector nucleases (TALENs).[18,19] However, off-target effects such as DNA double-strand breaks could occur using both techniques,[20] which might also affect the cells' capacity to differentiate. Considering the advantages of a syngeneic source of mutated and normal stem cell derivatives, one could argue that such tailored gene-editing approaches could be utilized to introduce disease-specific mutations in well-established human embryonic stem cell lines, which would not exhibit

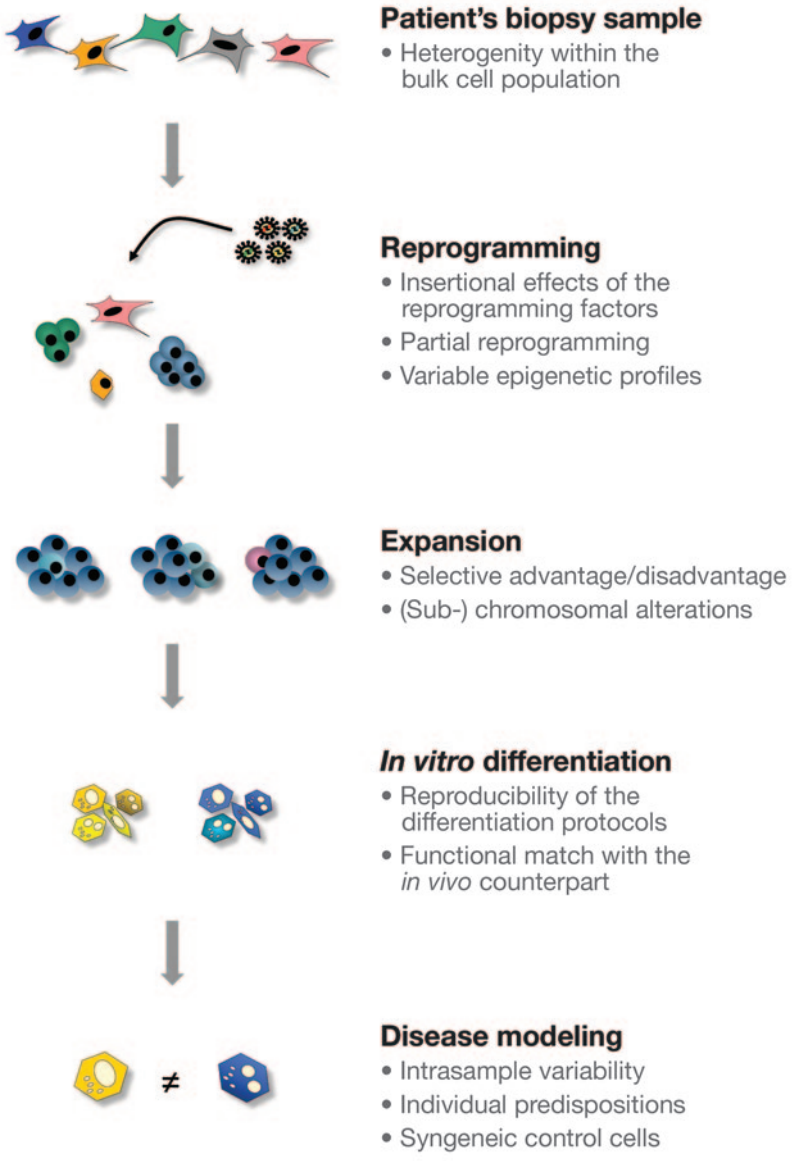

Figure 1 Disease modeling of patient-specific iPS cells. Within the heterogeneic bulk cell population of a patient's biopsy sample, cells with point mutations or copy-number variations providing a selective advantage may preexist and account for a significant number of intrasample variations of induced pluripotent stem (iPS) cells derived from the same donor. During reprogramming, insertional effects and off-target effects of the reprogramming factors can affect the pluripotency-associated transcriptome or epigenome. During expansion and establishing of late-passage iPS cells, disadvantageous mutations could be counterselected, but cell cycle–accelerating or other chromosomal alterations could also emerge. Upon differentiation of these iPS cell lines into the functional cells of interest, genetic alterations or misregulated epigenetic modifications may result in heterogeneous populations of differentiated cells. If these cells were applied for disease modeling, the intrasample variability and other individual predispositions by themselves could cause significant phenotype differences and thus could challenge the fidelity of the specific disease phenotype-related assay. Hence, syngeneic control cells generated with tailored genetic engineering tools should be considered for translational research studies.

epigenetic or transcriptional aberrations related to iPS cell generation *per se*. But this approach fails to take into account that a particular genetic mutation would not necessarily cause the same severe phenotype in individual patients with different genotypic backgrounds and that the onset of the particular disorder could further depend on other individual factors.

In conclusion, Thatava *et al.* provide good evidence that human disease–specific iPS cell lines exhibit considerable intra- and intersample variability that must be addressed if tissue-specific functional iPS cell derivatives are to be used for basic or translational research on T1D. Future research may identify additional factors that could provide improved tools that generate iPS cells with less intra- and intersample variation. Advanced profiling of the iPS cells' transcriptome and epigenome may not only assay the establishment of fully reprogrammed pluripotent stem cells but also more adequately predict the differentiation ability of a given iPS cell line such that cell lines with aberrant differentiation potential could be excluded. If more subtle disease-related phenotypes are investigated, syngeneic cell sources for the disease-specific and the control cells may provide an advantageous system, provided that relevant intersample genetic alterations could be excluded by applying array–comparative genomic hybridization or deep-sequencing techniques.

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