

The silver lining of induced pluripotent stem cell variation

Valentina Fossati, Tanya Jain, Ana Sevilla

The New York Stem Cell Foundation Research Institute, New York, NY 10023, USA

Correspondence to: Valentina Fossati. The New York Stem Cell Foundation Research Institute, New York, NY 10023, USA. Email: vfossati@nyscf.org.

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Abstract: Induced pluripotent stem cells (iPSCs) are being generated using various reprogramming methods and from different cell sources. Hence, a lot of effort has been devoted to evaluating the differences among iPSC lines, in particular with respect to their differentiation capacity. While line-to-line variability should mainly reflect the genetic diversity within the human population, here we review some studies that have brought attention to additional variation caused by genomic and epigenomic alterations. We discuss strategies to evaluate aberrant changes and to minimize technical and culture-induced noise, in order to generate safe cells for clinical applications. We focus on the findings from a recent study, which compared the differentiation capacity of several iPSC lines committed to the hematopoietic lineage and correlated the differential maturation capacity with aberrant DNA methylations. Although iPSC variation represents a challenge for the field, we embrace the authors' perspective that iPSC variations should be used to our advantage for predicting and selecting the best performing iPSC lines, depending on the desired application.

Keywords: Induced pluripotent stem cells (iPSCs); epigenetic variation; aberrant methylation; differentiation capacity

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As induced pluripotent stem cell (iPSC) research is entering its second decade, the field is deeply marked by the realization of pioneering clinical trials using iPSC-derived cells for various disorders (1). Therefore, understanding the variability between different iPSC lines is no longer a basic biological question but rather an important need towards safety and efficacy of clinical applications. Several studies in the past have begun to address this question by evaluating whether iPSCs, which are obtained through *in vitro* reprogramming, are truly equivalent to embryonic stem cells (ESCs) or represent a distinct subpopulation of pluripotent stem cells. Contradictory articles have been fueling a longstanding debate that is not completely solved yet, mainly because of the limited number of cell lines analyzed in each independent study.

The current predominant interpretation is that iPSC lines cannot be clearly distinguished from ESCs,

nonetheless they show a greater inter-line heterogeneity in their genetic and epigenetic profiles (2-5). Thus, the focus has mostly shifted towards understanding the molecular basis of the differences among iPSC lines and the implications for their differentiation potential. The ability to discriminate confounding technical/methodological variations from biological variations is certainly a priority and makes it problematic to compare iPSCs generated from independent laboratories that have adopted different reprogramming technologies (e.g., retrovirus, Sendai virus, mRNAs, episomal vectors), using different protocols. Several efforts have been made to address this issue. The Progenitor Cell Biology Consortium, for example, performed an extensive analysis of 58 iPSC lines from 10 laboratories that followed rigorously standardized procedures to minimize variations (6). Additionally, at the New York Stem Cell Foundation

Research Institute, we established a unique, fully automated platform to support large scale reprogramming of patient samples into iPSCs. We have shown that this system generates high quality, stable iPSC lines and can reduce the line-to-line variation by 30% when compared to manually derived iPSC lines (7).

The recent paper from the Yoshida laboratory “Epigenetic variation between human pluripotent stem cell lines is an indicator of differentiation capacity” explored the main factors involved in line-to-line variations with a compelling, novel focus toward the differentiation potential. Three factors are recognized as major causes of iPSC variability. One is the DNA methylation signature from the original somatic cell that remains after reprogramming, which is referred to as “Somatic Memory”. The second are aberrations introduced by the reprogramming process and tissue culture techniques, and the third factor is the genetic inter-individual diversity. The existence of a somatic memory that would point to great variability depending on the cell source has been somehow controversial. Many studies have provided evidence that epigenetic memory affects the differentiation capacity (8-10), however these studies recognize the fact that the results may have been influenced by cell passage number, transfection techniques, and variability due to manual handling procedures across different labs. A recent large study of 200 iPSC lines suggests that, independently of the cell source, iPSCs exhibit altered epigenetic patterns caused by early aberrant hypermethylation, that decrease over time in culture (11). In line with these findings, the Yoshida group found that the origin of the cells to be reprogrammed and the reprogramming method were not key factors in further differentiation efficiency of those lines. Instead, they showed that high expression of certain genes like insulin-like growth factor 2 (IGF2) was a good indicator of iPSC cells starting their conversion into hematopoietic cells. This correlation was surprising because IGF2 itself is not a gene directly related to the hematopoietic lineage, but its expression turns on signaling-dependent chromatin accessibility at those genes that are. The study compared 35 iPSC lines made using human dermal fibroblasts, blood cells from cord blood and peripheral blood, dental pulp cells and keratinocytes, using retrovirus, Sendai virus and episomal vectors as reprogramming methods. The conclusion arising from their analyses is that acquisition of aberrant hypermethylation in some differentially methylated regions (DMRs) is more crucial of a factor in differentiation capacity, than the cell type origin. This also explains why

blood-derived iPSCs have a lesser risk of gaining aberrantly methylated sites, since blood cells would require fewer changes in methylation to successfully reprogram.

Another recent study (12) used blood- and fibroblast-derived iPSCs, reprogrammed using the Sendai virus system, with samples from only female donors to reduce gender-related variability, analyzed at late passages. They concluded that the majority of different transcriptional, epigenetic, and differentiation propensities are donor dependent, and not tissue-source dependent. Though this study confirms similar claims by the Yoshida group, it does bring up a rather important point missing in the Yoshida study, which is the sex factor. During cell reprogramming, female-derived somatic cells must overcome an additional barrier compared to male-derived cells, which is the reactivation of the inactive X chromosome. Moreover, it is known that there are more X chromosome-localized DMRs in the female iPSCs than in males (13) and indeed sex is a major contributing factor to explain differential methylation patterns among iPSC lines (6). It is surprising that this point was not addressed by Yoshida and colleagues while describing the cell lines used and the potential causes for variation between them. Another factor related to aberrant DMRs is the age of the donor, and it would be interesting to stratify the different iPSC lines by age, as other studies have done (14).

When focusing on the reprogramming techniques, the Yoshida study found that retroviral transfection of the reprogramming factors leads to the highest degree of aberrant methylation and lowest maturation capacity compared to Sendai-virus and episomal strategy. Considering that the retroviral system has been largely replaced by non-integrating, zero fingerprints methods, the inclusion of iPSC lines made with mRNAs technology is desirable for the future. We assume that the mRNA method was excluded in the present study because blood is one of the somatic tissue sources that to date has not been reprogrammed by mRNA lipofection.

While our understanding of the molecular basis of iPSC diversity is still incomplete and will require additional large-scale studies, the importance of this work resides on practical applications. Indeed, the authors suggest that the analysis of the iPSC's epigenetic signature, including aberrant DMRs, could become the new paradigm to predict the differentiation potential and select the most suitable lines for various applications. Blood cells have been recently emerging as the preferred source for reprogramming over skin biopsies, because collection of blood is by some

means easier and many cryopreserved samples from previous studies already exist (the downside of blood cells as a reprogramming source is the potential for unwanted T-cell receptor gene rearrangement, although this can be tested for). The work from the Yoshida group reinforces this direction by showing that iPSCs from blood have less propensity to acquire aberrant DMRs compared to the other sources tested. A second practical consideration involves the use of teratoma formation to evaluate the quality of iPSC lines. The validity of this assay has been largely questioned and alternative tests that do not require sacrifice of animals have been proposed (3,6). It is likely that moving forward, the teratoma formation assay will be replaced by *in vitro* differentiation, analysis of karyotype, genetic and epigenetic profiles to evaluate stability and quality of iPSC lines. Due to the considerable costs of these tests, the stem cells community should agree on the minimal standard requirements for legitimate research purposes. However, we strongly advocate for a rigorous and more in-depth analysis of iPSC lines for clinical applications to identify genetic and epigenetic alterations, including mitochondrial DNA mutations (15) copy number variations (CNVs) (16) and histone tail modifications (17), at least until we achieve a clear understanding of the safety profile. Of note, the first clinical trial using iPSC-derived cells was put on hold because the genetic analysis revealed presence of new CNVs in the cells differentiated for transplantation.

In summary, we have accumulated a large body of evidence for genomic and epigenomic alterations that frequently appear during reprogramming or expansion of iPSCs. This should not discourage the stem cell community from using the iPSC technology for clinical applications, but rather promote further in-depth studies, like the Yoshida group has performed, that capitalize on the iPSC heterogeneity for enhancing the differentiation potential while generating safe cells for therapeutic purposes. The key to success will be to minimize technical variations by adopting rigorous standard protocols in large-scale experiments.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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