Protein Cell 2013, 4(2): 83–85
DOI 10.1007/s13238-012-2940-6

Protein & Cell

NEWS AND VIEWS

Reevaluation of the safety of induced pluripotent stem cells: a call from somatic mosaicism

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Recent studies have been raising doubts on the safety of induced pluripotent stem cells (iPSCs) and proposing that the process of reprogramming brought about copy number variations (CNVs) in iPSCs. However, a recent paper published in Nature provided evidence showing that most CNVs were pre-existed as somatic mosaicism but not resulted from the reprogramming. This new finding would profoundly reshape some previous thoughts and endorse the confidence of iPSCs in both research and therapy.

Induced pluripotent stem cells (iPSCs) are generated by reprogramming and exhibit essential characteristics similar to embryonic stem cells (ESCs). For this reason, iPSCs have been perceived as a promising tool for modeling disease as well as developing novel strategies of cell therapy (Hanna et al., 2010; Robinton and Daley, 2012). In recent years, several disease models and drug screening platforms have been successfully established using iPSCs (Batista et al., 2011; Brennand et al., 2011; Liu et al., 2012; Lee et al., 2012). Despite flourishing advancements, more doubts have been raised in the field regarding the safety of iPSCs, especially the possible genetic alternations introduced by the process of reprogramming (Hussein et al., 2011; Laurent et al., 2011). Recently, Hussein et al. reported that there were significantly more copy number variations (CNVs) in iPSCs comparing to ESCs or donor fibroblasts, suggesting CNVs were formed de novo during the reprogramming (Hussein et al., 2011). It had been further illustrated that human PSCs encompassed considerable number of deletions in tumor suppressor genes after reprogramming as compared to human ESCs and non-pluripotent samples (Laurent et al., 2011).

So is the reprogramming process truly responsible for sub-chromosomal differences between iPSCs and ESCs?

The answer to this question seemed to be yes, until a new paper in Nature by Abyzov et al proved that reprogramming might not generate as many CNVs in iPSCs as previously thought. Former studies had showed that some CNVs found in iPSCs could also be detected in their parental somatic cells, where other line-manifested CNVs (LM-CNVs) were undetectable. Therefore researchers deduced those LM-CNVs were generated during reprogramming. In order to figure out whether this assumption was true, Abyzov and colleagues applied a series of more comprehensive and sensitive approaches than previous studies, which are: (1) whole genome and transcriptome analysis of 20 human iPSCs lines using next-generation sequencing; (2) examination of not only iPSCs lines generated from multiple individuals but also multiple lines from the same individual; (3) a novel method based on high-sensitivity read depth analysis to predict CNVs (CNVnator) (Abyzov et al., 2011) and (4) highly sensitive PCR-based comparison of iPSCs and their parental lines on the same CNV breakpoints (Abyzov et al., 2012).

Abyzov et al. studied 20 human iPSCs lines generated from 7 individuals by whole-genome sequencing. Using CNVnator prediction, the authors firstly analyzed CNVs by comparing human iPSC samples with a reference human genome. Secondly, the group made another comparison between every human iPSC line and its respective parental fibroblasts of origin to

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detect LM-CNVs. A total of 74 LM-CMVs were identified in 20 iPSCs lines. As previously thought, these LM-CNVs might have been resulted de novo from the reprogramming, as they were not detectable by traditional methods in parental fibroblasts. However, Abyzov and his colleague further proved that at least 50% of these LM-CNVs could be traced back to the parental fibroblast population by highly sensitive PCR and digital droplet PCR amplification on CNV breakpoints. Moreover, the authors showed that the numbers of CNVs in iPSCs were not significantly increased by in vitro expansion. These results implied that most LM-CNVs might simply reflect a pre-existed somatic mosaic in the parental population, which had been masked due to low frequencies. Therefore, most LM-CNVs were not de novo consequences from reprogramming, but rather pre-existed genetic variations in rare somatic cells. Since the real LM-CNVs represented only a small part of all CNVs, the authors concluded CNVs mostly come from somatic mosaicism (Abyzov et al., 2012).

What more can we learn from this study? Hussein et al. formerly suggested a potential relationship of CNVs in iPSCs with the somatic mosaicism, which had been found abundant in human genome (Youssoufian and Pyeritz, 2002; Hussein et al., 2011). As this relationship had not been well appreciated previously, Abyzov's study was the first report highlighting the importance. Though a more detailed investigation at higher resolution and sensitivity may still be needed to further clarify the origins of the rest manifested CNVs, this new finding made by Abyzov et al would help to ease some serious concerns related to the safety of iPSC application. Remarkably, it calls for a reevaluation of not only former studies but also the future perspectives of iPSCs in both disease modeling and regenerative medicine.

In general, iPSCs used for disease modeling are generated from multiple patients, while controls come from healthy individuals. The problem with this design is that apart from overcoming noises of genetic background from multiple individuals, even the iPSC lines generated from the same person might contain substantial variations due to the widespread somatic mosaicism. Thereby, technically it will be considerably difficult to identify the most representative iPSCs line for any given disease, or pathological mutation. Particularly, in the late age onset disorders such as Parkinson's disease, subtle phenotypical differences and increasing mosaic abnormalities are generally expected. In this case, acknowledging the existence of somatic mosaicism will likely complicate the strategical design of disease modeling studies.

In order to solve this problem, one feasible approach to avoid somatic mosaicism as well as bypass reprogramming might be generating isogenic human ESC lines with knock-in pathological mutation (Liu et al., 2011; Soldner et al., 2011; Liu et al., 2012). Recently, we and other groups have set up a targeted gene editing system to create a panel of isogenic disease-affecting ESCs lines which recapitulated Parkinson's disease (Soldner et al., 2011; Liu et al., 2012). This new approach offered an even more preferable platform for pathological disease research and therapy especially for monogenic disease. For other diseases with unknown genetic cause, novel strategy for effective modeling is still needed.

With respect to the regenerative medicine, iPSCs derived from patients hold great promise in developing autologous therapeutics as the gene corrected iPSCs would match the patient without a risk of immune rejection. Nevertheless, a recent study reported by Zhao et al. reported that undifferentiated iPSCs derived from mouse embryonic fibroblast, assuming immune-compatible in parental animals, were mostly rejected by syngeneic recipients after transplantation (Zhao et al., 2011). This claim had confounded the potential of iPSCs in therapeutic application thus was heavily debated. Interestingly, Abyzov et al's finding may offer clues for a new explanation to this question. It is possible that different gene expression profiles caused by somatic mosaicism within the parental cell population lead to the mismatching of iPSCs with recipients which evoked immune responses. Therefore, iPSCs may still be safe in delivering personalized cell therapy, upon more stringent examining and screening of iPSCs so that immune-compatible lines are acquired.

On the other hand, Abyzov et al's work also provides us an advanced platform to study somatic mosaicism in human cells with improved resolution and sensitivity. The abundant existence of somatic mosaicism has been reported in human tissues especially in the brain (Rehen et al., 2005; Baillie et al., 2011; De, 2011). Also, somatic mosaicism is evident and time-dependent in the etiology of cancer, and the frequency of mosaic abnormalities increases with aging (Jacobs et al., 2012). Benefiting from the clonal expansion technologies like iPSCs, further investigation on somatic mosaicism in human cells with the help of high-throughput approaches demonstrated by Abyzov's group may offer new insights into the understanding of neurodegenerative diseases, cancers, as well as aging.

ACKNOWLEDGEMENTS

This work is supported by the Thousand Young Talents program of China, the National Laboratory of Biomacromolecules, the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA01020312), and National Natural Science Foundation of China (Grant Nos. 81271266, 31222039 and 31201111).

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