Reprogramming of Pluripotency-Specific microRNA Signatures Is Not Essential to Generate Inducible Pluripotent Stem Cells

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To the Editor:

A recent clinical trial reported that autologous transplantation of retinal pigment epithelium differentiated from patients' own induced pluripotent stem cells (iPSCs) halted the progression of age-related macular degeneration (Mandai et al., 2017). However, the same study also reported genomic aberration of iPSCs derived from some aged donors (A-iPSCs), which deters the use of A-iPSCs of such patients (Mandai et al., 2017). Because aged donors are most likely beneficial from cell replacement therapy, genomic instability associated with A-iPSCs poses a significant risk in clinical settings.

Using mouse embryonic stem cells (ESCs, the gold standard for pluripotency) and A-iPSCs (derived from skin fibroblasts from 1.5-year-old adults) in a genetically controlled mouse model, we discovered that excessive glutathione production in A-iPSCs contributes to genomic instability of A-iPSCs. We also examined differences in other pluripotency-specific signatures in ESCs and A-iPSCs, including microRNA expression patterns (Fig. 1A, B).

We found that, despite expressing pluripotency markers and passing multiple pluripotency tests such as teratoma formation assays and chimera analysis (Skamagki et al., 2017), A-iPSCs exhibited an aberrant microRNA expression signature compared with ESCs (Fig. 1A, B). Our data demonstrated that A-iPSCs expressed lower levels of pluripotency-promoting microRNAs such as members of the miR-302, -106, -363, and -290/294, clusters (Fig. 1A, B).

MicroRNAs are small noncoding RNAs that play essential roles in early embryo development and tissue differentiation (Greve et al., 2013). Members of several microRNA families or clusters, such as miR-302, -106a, -363, or -290/294, have been shown to enhance iPSC reprogramming efficiency. A previous study also demonstrated that expression of the miR-302, -200c, -302, and -369, without the Yamanaka factors, is sufficient to achieve iPSC reprogramming (Greve et al., 2013).

However, the role of microRNAs as a whole during iPSC reprogramming has not been clearly demonstrated, with in-

dividual families or clusters acting as enhancers, sufficient sole drivers, or inhibitors. In addition, whether microRNAs are necessary for iPSC reprogramming was unclear. In our previous study, we demonstrated that somatic cells lacking *Dgcr8*, which encodes a factor required for the biogenesis of miRNAs, can be reprogrammed into iPSCs at reduced efficiencies by the Yamanaka factors (OCT4, SOX2, KLF4, and MYC) (Liu et al., 2015). These data, therefore, suggest that, although miRNAs facilitate reprogramming, microRNAs may be dispensable in converting somatic cells into iPSCs and the maintenance of iPSCs (Liu et al., 2015).

Our study unexpectedly discovered that A-iPSCs that pass standard pluripotency tests (Skamagki et al., 2017) could be obtained in the absence of the complete pluripotent stem cell-specific microRNA expression signatures present in ESCs (Fig. 1A, B). This microRNA signature was confirmed again using an RNA sequencing-based microRNA profiling method in addition to A-iPSC clones (Fig. 1C). These data support our previous discovery that microRNAs are not essential for somatic cell reprogramming (Liu et al., 2015).

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Supplementary Material

Supplementary Data

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FIG. 1. MicroRNA profiling analysis of SCs, A-iPSCs, and ESCs. (Refer to the Supplementary Data.) **(A)** Clustering analysis of the microarray-based microRNA profiling data from two independent clones (average value) of SCs (skin fibroblast), ESCs, and A-iPSCs. Pluripotent stem cell-specific microRNAs were identified as those differentially expressed between ESCs and SCs. Heat maps include all microRNAs in ESCs expressed at higher levels than in SCs, and the top 100 and top 20 highly expressed microRNAs in ESCs compared with SCs. The majority of pluripotent stem cell-specific microRNAs show poor expression in A-iPSCs, which clustered with SCs. Heat maps were generated using the Broad Institute-morpheus tool. Hierarchical clustering of the cell types was generated using the Euclidean distance metric. Clustering analysis of top 100 microRNAs was generated using the Heatmapper tool. **(B)** Pluripotent stem cell-specific microRNA expression is aberrant in A-iPSCs compared with ESCs. Previously reported pluripotent stem cell-specific microRNAs are marked with red letters in top 20 highly expressed microRNAs in ESCs compared with SCs (Greve et al., 2013). Expression levels of the top 20 microRNAs and fold differences in expression levels between ESCs and A-iPSCs/SCs are shown. **(C)** Confirmation of the aberrant microRNA signatures in A-iPSCs using RNA sequencing-based microRNA profiling analysis with additional A-iPSC clones. Two independent representative clones were used. A-iPSC, aberration of induced pluripotent stem cell derived from some aged donors; ESCs, embryonic stem cells; SCs, somatic cells.

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