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SPOTLIGHT

Programming pluripotent stem cells: Can't teach an old cell new DNA replication tricks

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Pluripotent stem cells differentiate with varying efficiencies depending on the method of reprogramming that created them. In this issue, Paniza et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.201909163) demonstrate that cells with lower differentiation potential retain some features of somatic DNA replication origin utilization and suffer more frequent DNA damage.

The ability to generate pluripotent stem cells (PSCs) from somatic cells has steadily improved in recent years. The potential for regenerative medicine using PSCs holds tremendous promise for treating degenerative disorders and age-related illness. Two reprogramming techniques generate human PSCs from differentiated somatic cells: (a) somatic cell nuclear transfer (SCNT), which transfers somatic cell nuclei into enucleated oocytes to form nuclear transfer human embryonic stem cells (NT-hESCs) that can differentiate and give rise to all cell types (1); and (b) induced PSCs (iPSCs), which introduce specific pluripotency transcription factor genes into somatic cells to generate differentiation-competent cells (2). Importantly, iPSC technologies produce patient-matched differentiated cells that are unlikely to cause immune rejection and do not require precious donor oocytes. However, key hurdles remain for the future therapeutic use of iPSCs. One challenge is that iPSCs are somewhat less capable of consistent or efficient directed differentiation than cells derived by the SCNT method. For example, iPSCs show variable differentiation to insulin-secreting β cells (3). These observations suggest that the iPSC method results in PSCs that are not as fully reprogrammed as those generated by SCNT. Thus far, however, this pluripotency difference cannot be readily explained by differences in proliferation parameters, mRNA expression, or DNA methylation profiles (Fig. 1 A; 4). Why are SCNT cells more consistently capable of directed differentiation than iPSCs? In addition to the well-known differences in cell cycle dynamics, gene expression, and chromatin composition (5), parameters related to DNA replication also differ between PSCs and differentiated cells (6). In this issue, Paniza et al. examined DNA replication as a possible contributing factor to the phenotypic differences between PSCs produced by two reprogramming methods (7).

Given their large genomes, mammalian cells must initiate DNA replication from thousands of sites known as replication origins (8). Origins are made competent for replication in G1 phase, but only a subset of origins initiate and establish bidirectional replication forks in S phase, whereas most origins remain dormant (9). Too few competent origins or too few initiation events can lead to incomplete DNA replication and genome instability (10). Replication initiation could also affect the timing of local gene expression. Both the early cell cycles of embryos and mammalian ESCs initiate many DNA replication origins, but differentiated cells initiate fewer origins per S phase (11). Paniza et al. reveal a striking difference in DNA replication origin utilization between iPSCs and NT-hESCs that correlates with their differences in differentiation potential (7).

Using both single-molecule analysis of replicated DNA at individual loci and DNA fiber spreading to examine global replication activity, the authors observed the expected higher frequency of replication initiation events in hESCs and a lower frequency in differentiated fibroblasts. Analyzing PSCs from either neonatal or adult fibroblasts that had been reprogrammed by SCNT, they detected more initiation events within some loci compared with isogenic differentiated cells. Moreover, by quantifying initiations overall, they report a replication profile closely resembling that of embryonic cells. In striking contrast, when adult fibroblasts (but not neonatal fibroblasts) were reprogrammed as iPSCs instead of using SCNT, the frequency of initiation events remained low and did not convert to the embryonic pattern (Fig. 1 B). The conclusion is that the specific combination of iPSC reprogramming from adult cells yields PSCs with incompletely reprogrammed DNA replication, particularly with respect to origin initiation.

Strikingly, the incomplete replication reprogramming of adult iPSCs also correlated with decreased genome stability. The adult-derived iPSCs displayed increased

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A

source	neonatal	neonatal	adult	adult
method	nuclear transfer	pluripotency genes (iPSC)	nuclear transfer	pluripotency genes (iPSC)
differentiation	✓	✓	✓	variable
mRNA	✓	✓	✓	✓
genome stability	✓	✓	✓	lower
cell cycle	✓	✓	✓	✓
origin firing	embryonic	embryonic	embryonic	adult

B

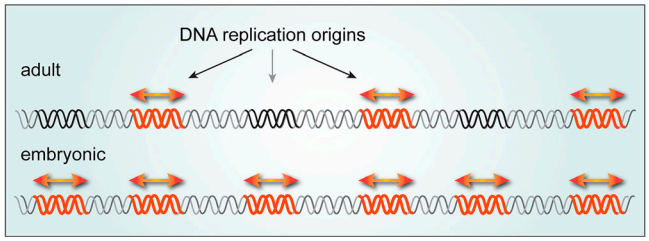

Figure 1. **Adult-derived iPSCs retain somatic cell DNA replication origin utilization. (A)** Summary of differences among pluripotent cells lines by genome source (neonatal or adult fibroblasts) and method (somatic nuclear transfer into oocytes or introduction of pluripotency transcription factor genes to generate iPSCs). The variable and lower differentiation potential of iPSCs derived by introducing the four pluripotency genes are reported in Sui et al. Differences in mRNA expression, imprinting, and mutation rates/genome stability have been reported but do not consistently correlate with differentiation potential. **(B)** Illustration of DNA replication origin utilization. Double-headed arrows indicate active origins. Embryonic cells initiate replication from more origins than adult somatic cells.

propensities for double-stranded DNA breaks, a concern for their use in therapeutic cloning. The use of reprogrammed cells prone to high levels of genomic instability could leave patients more susceptible to cancer. A potential explanation for the DNA breaks is incomplete replication resulting from fewer initiation events. Future work could determine if origin initiation frequency is the primary mechanism driving the observed genome instability or if other differences in the reprogramming methods contribute to that phenotype.

What is the nature of the molecular differences between iPSCs and NT-hESCs that drive the differences in DNA replication? As mentioned earlier, similar gene expression patterns among the different PSCs suggest that uniquely limiting replication factors in adult-derived iPSCs provide an insufficient explanation. An alternative is that adult-derived iPSCs retain adult replication checkpoint activity. Somatic cells rely on checkpoint kinase 1 (Chk1) for origin utilization control to ensure complete replication during both unperturbed replication and in response to replication inhibitors such as aphidicolin. In contrast, hESCs are impaired for Chk1 activation (12). Thus, the authors suggest that persistent lower origin initiation frequency in adult iPSCs could reflect incomplete conversion of the replication checkpoint from the adult to the embryonic state. Further work to directly compare checkpoint activity in each of the cell lines can test this model.

In addition to the reprogramming method, the source of cells used also appears to impact the efficiency of replication reprogramming; neonatal fibroblasts reprogrammed as iPSCs showed the same high initiation frequency as adult cells reprogrammed by SCNT or hESCs. What aspects of development allow the neonatal cells to convert more readily than the adult cells? From a regenerative medicine perspective, neonatal cells from a patient won't be available, but understanding why neonatal cells reprogram better may allow additional manipulations of adult cells to improve their reprogramming outcomes.

A key question raised by these findings is whether the incomplete replication reprogramming in adult-derived iPSCs is a direct cause of their low differentiation potential. Interestingly, while iPSCs display a lower differentiation potential than NT-hESCs on average, some individual iPSC cell lines differentiate nearly as efficiently as NT-hESCs (3). It would be interesting to determine if individual iPSC cell lines that differentiate comparably to NT-hESCs also have more completely reprogrammed DNA replication. If so, defining the molecular mechanisms following the work of Paniza et al. and others in the field could lead to better outcomes for regenerative medicine using the more practical, safe, and ethical iPSC technology.

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