Commentary

Stem Cell INVESTIGATION

Aging *vs*. rejuvenation: reprogramming to iPSCs does not turn back the clock for somatic mitochondrial DNA mutations

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The process of cellular reprogramming is believed to be able to "turn back the developmental clock" by allowing somatic cells to acquire a state that is normally associated only with embryonic stem cells (ESCs) (1). Indeed, human induced pluripotent stem cells (iPSCs) can be obtained from aged individuals and still show the key properties of ESCs, including self-renewal, elongated telomeres, and round-shaped mitochondria with underdeveloped cristae (2). However, it remained to be determined whether reprogramming to pluripotency could actually erase aging-associated signatures and thus represent a rejuvenation route. A new paper by Mitalipov and colleagues (3) now clearly demonstrates that iPSCs not only do not erase the signs of aging but, due to their clonal origin, may even reveal aging-related defects in the mitochondrial DNA (mtDNA) that were not detectable in the whole parental tissues.

Using iPSCs derived from both skin fibroblasts and peripheral blood mononuclear cells (PBMCs), Kang *et al*. show that all iPSCs exhibited mtDNA mutations that could not be observed in the whole-tissue DNA extracts of the parental cells (3). This is in agreement with previous data demonstrating the occurrence of novel mtDNA mutations in iPSCs in comparison to their parental foreskin fibroblasts (4). These mutations were originally considered as negative by-products of reprogramming as a consequence of oxidative stress-mediated genomic damage (4). However, Kang *et al*. demonstrate that also skin fibroblasts grown as individual clones exhibit mtDNA mutations that are not seen in the pooled fibroblast population (3). Hence, individual cloned fibroblasts and iPSCs may both represent the progeny of a single parental fibroblast cell, thereby enabling the detection of mtDNA mutations that were already present in the original fibroblast population but remained undetectable due to their relatively low presence.

Several studies indicate that mtDNA mutations, including large-scale deletions, increase with aging (5). In accordance, Kang and coauthors detected increased presence of mtDNA mutations in fibroblasts and iPSCs derived from aged individuals compared to young individuals. Moreover, the identified mutations in somatic cells and derived iPSCs were mostly located in coding genes, while ESCs displayed mtDNA variants primarily within the non-coding D-loop (3). This gives further support to the notions that the majority of mtDNA alterations seen in adults is of somatic rather than embryonic origin (6).

An important point to be addressed was the functional consequence of the detected mtDNA mutations. The presence of mtDNA alterations that were not seen in the pooled parental fibroblasts were previously found to not cause major bioenergetic defects, as all generated iPSCs could efficiently undergo the extensive metabolic shift that is associated with cellular reprogramming (4). However, the detailed analyses carried out by Kang *et al*. unveil diminished metabolic function in iPSCs carrying high heteroplasmic mtDNA mutations (3). Hence, in order to correctly employ patient-derived iPSCs for disease modeling and therapeutic studies, it will be imperative to include the detection of mtDNA integrity as part of the basic characterization toolkit. This will be especially relevant when dealing with patients of advanced age who may harbor increased amount of mtDNA mutations.

It is interesting to mention that the findings may have special significance for iPSC-based modeling of inherited mtDNA disorders. In fact, several previous works detected differences in the level of heteroplasmy in various iPSC lines with respect to the original parental fibroblasts derived from patients affected by mtDNA diseases (7-9). The work of Mitalipov and colleagues suggests that the

changes observed in iPSCs may be caused by the clonal expansion that is inherent in iPSC derivation. Patient iPSCs may thus still represent a faithful model system of mtDNA disease, at the condition that all the different clones generated undergo a detailed mitochondrial genome assessment. Reassuringly, the present and current works similarly demonstrate that the mtDNA mutation level remain relatively constant during extensive culture and upon differentiation into different lineages. Therefore, once different iPSC lines carrying a defined mtDNA mutation level are obtained, it will be possible to investigate the functional effects of this mutation in various differentiated cell types and potentially determine a cell type-specific genotype-phenotype correlation.

Overall, the manuscript by Kang *et al*. (3) strongly confirms that, in addition to nuclear genome integrity (10), mitochondrial genome integrity will become a key parameter to investigate for all medical applications of iPSCs. Furthermore, it highlights the strength of single-cell studies, which may reveal the real biological variability that pooled population studies have so far prevented to be identified. In conclusion, in order to allow faithful and meaningful discoveries, future analysis of iPSCs and their derivatives should not shy away from mitochondrial genome monitoring and single-cell technology.

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Footnote

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