Opinion

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# Concealing cellular defects in pluripotent stem cells

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**Inherent and acquired defects in gene expression, protein homeostasis, metabolic pathways, and organelle function are linked to aging and a wide range of human diseases. Although concealed or dormant in the embryonic stage, they often manifest later in life. We review and discuss recent observations on how somatic cells bearing specific phenotypic defects can be reprogrammed into a pluripotent state where most phenotypic abnormalities can be reset or tolerated. Gaining insights into the tolerance of cellular defects in pluripotent stem cells will facilitate our understanding of the properties of reprogrammed cells and may provide theoretical guidance for induced pluripotent stem cell based disease modeling and clinical therapies.**

## The ability of pluripotent stem cells (PSCs) in concealing cellular defects

PSCs, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), hold far-reaching potential in disease modeling and regenerative medicine. These cells can be propagated *in vitro* while retaining their differentiation potential. So far, iPSCs have been generated from various human and murine somatic cells by ectopic overexpression of different combinations of Oct4, Sox2, Klf4, and c-Myc, or other transcription factors [1]. Interestingly, recent studies on reprogramming aged or diseased somatic cells suggested an erasure, dormancy, or tolerance of somatic cell associated cellular phenotypic defects in PSCs reprogrammed from phenotypically aberrant somatic cells. Here we summarize recent evidence supporting the ability of PSCs to (i) reconfigure aberrations during the reprogramming process, and (ii) tolerate or desensitize cellular defects inherited from original somatic cells. The notion that somatic cell associated defects consisting of various structural and functional changes in cellular components could become dormant in the pluripotent state may help to establish new methodological bases for using iPSCs to model human diseases and develop therapies. Here, we focus on the recently published 'genetic', 'epigenetic', 'energy', and 'structural' parameters in wild type and diseased PSCs, and discuss the potential biological relevance of the 'concealing' capabilities of PSCs (Table 1).

## Concealing genetic aberrations in PSCs

Genomic integrity is a fundamental property of cells. In somatic cells, genomic instability is correlated with tumorigenesis, cellular aging, and various other phenotypic aberrations. PSCs appear to have a strong ability to conceal genetic aberrations. Fragile X syndrome (FXS), one of the most common inherited intellectual retardation disorders, and which results from expansion of CGG trinucleotide repeats in the *FMR1* gene, would be an example. Such pathological expansion appears more significant in somatic cells because, in human ESCs isolated from preimplantation FXS-affected embryos, the *FMR1* gene is transcriptionally activated with no CGG repeat expansion [2]. Recently, FXS-specific iPSCs have been generated in four independent laboratories [2–5]. Three groups demonstrated that the *FMR1* gene still contained long repeats and remained silenced after reprogramming. The length of CGG-repeat remained unchanged even after further differentiation of FXS-iPSCs into neurons [2,3,5]. By contrast, another study reported that the lengths of CGG trinucleotide repeats in the *FMR1* gene were shorter in patient iPSCs than in their parental fibroblasts [4]. In fact, homogeneous FXS fibroblasts give rise to heterogeneous iPSC lines with different lengths of repeats [4]. Therefore, it appears that iPSCs have a unique cellular status allowing differential *FMR1* expression levels associated with differential expansion of CGG repeats. Importantly, all of these FXS-iPSC lines generated from different laboratories demonstrate common ESC-like characteristics with great potential for differentiation. The ability of iPSCs either to tolerate (maintain original CGG expansion) or reset/erase (decrease CGG expansion) genetic defects supports the notion that somatic aberrations could become dormant at the PSC stage. In the context of modeling

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**Table 1. Representative somatic cell defects that are reset or tolerated in PSCs**

| Cell type | Cause(s) of somatic cell defects | Pluripotency[a] | Defective phenotype(s) concealed in PSCs | Refs |
|---|---|---|---|---|
| **Genome** | | | | |
| Human iPSCs | Expanded CGG-repeats in 5′-UTR[b] of the *FMR1* (fragile X syndrome) | ✓ | Tolerance to long CGG-repeats and *FMR1* silencing | [2,3,5] |
| | Oncogenic mutations | ✓ | Tolerance to carcinogenic mutations | [8–10] |
| Mouse iPSCs | Oncogenic mutations | ✓ | Tolerance to carcinogenic mutations | [6,7] |
| | BubR1 insufficiency | ✓ | Tolerance to whole-chromosome instability | [11] |
| | RanBP2 insufficiency | ✓ | Restoration to normal chromosome segregation | [11] |
| Human ESCs | Triple genome | ✓ | Avoid lethality | [16] |
| Mouse ESCs | Haploid genome | ✓ | Avoid lethality | [12–14] |
| **Epigenetics** | | | | |
| Human iPSCs | Abnormal methylation of the $p16^{INK4A}$ promoter | ✓ | Epigenetic reactivation of $p16^{INK4A}$ | [17] |
| | Mutant *MECP2* (Rett syndrome) | ✓ | Reactivation of X-chromosome and *MECP2* expression | [20,24] |
| | | ✓ | Tolerance to silenced *MECP2* | [19,21,22] |
| Mouse ESCs | Loss of Eed | ✓ | Avoid lethality | [50] |
| **Telomere** | | | | |
| Human iPSCs | Inactivation of the dyskerin gene (DC[b]) | ✓ | Telomere elongation | [38] |
| | | ✓ | Insensitivity to telomere shortening | [37] |
| **Nuclear architecture** | | | | |
| Mouse ESCs | $Lmnb1^{-/-}Lmnb2^{+/+}$ $Lmnb1^{+/+}Lmnb2^{-/-}$ $Lmnb1^{-/-}Lmnb2^{-/-}$ | ✓ | Retain normal nuclear architecture and ESC identity | [31] |
| **Aging-related** | | | | |
| Human iPSCs | *LMNA* mutation (HGPS[b]) | ✓ | Removal of progerin, resetting nuclear defects | [27–30] |
| | Physiological aging-associated cellular changes in centenarians | ✓ | Erasing age-related cellular physiology (telomere, gene expression profiles, and mitochondrial metabolism) | [34] |

[a]Pluripotency is designated by ✓.

[b]Abbreviations: DC, Dyskeratosis congenita; HGPS, Hutchinson–Gilford progeria syndrome; 5′-UTR, 5′-untranslated region.

FXS pathology, FXS-specific ESCs represent an invaluable reference system to recapitulate the progressive appearance of CGG trinucleotide repeats as development proceeds. Although the failure to reactivate the mutant *FMR1* gene in diseased iPSCs is inconsistent with the early developmental characteristic of FXS, the abnormal CGG expansion present in FXS-iPSC-derived neurons may still be suitable for studying FXS-associated neurological pathology [2].

Another important observation to discuss is the fact that iPSCs can overlook carcinogenic mutations. Transformed or cancer cells, including Epstein–Barr virus (EBV)-immortalized B lymphocytes and those from melanoma and gastrointestinal cancers, have recently been reprogrammed into iPSCs or converted to ESCs by nuclear transfer [6–10]. In contrast to their parental cells of tumor origin, the generated iPSCs acquired ESC-like characteristics and were free of phenotypic defects [6–8]. These results collectively support the notion that pluripotent cells are able to conceal carcinogenetic genetic changes, and the reprogrammed cancer cell derived iPSCs appear to be more similar to iPSCs derived from wild type cells than their transformed counterparts [6,8].

In addition to concealing subtle genomic aberrances that are undetectable via karyotyping, PSCs can tolerate severe chromatin abnormalities. It has recently been reported that reprogramming to pluripotency can tolerate whole chromosome instability in somatic cells caused by specific genetic defects, and aneuploid cells can be selected for or against by the reprogramming process [11]. More strikingly, haploid mouse ESCs have been successfully derived from parthenogenetic and androgenetic embryos respectively [12–15]. These haploid ESCs are pluripotent like their diploid counterparts, and can be stably maintained *in vitro* [12–15]. In addition, oocytes have been used to reprogram human somatic cells, resulting in the generation of pluripotent cells with triple genomes [16]. The existence of haploid and triploid PSCs, and the phenotypic resemblance between haploid and diploid mouse ESCs, provide strong evidence for the ability of pluripotent cells to carry genetic abnormalities. Stemming from this knowledge, it will be interesting to investigate how PSCs can globally tolerate increased or decreased loading of gene expression products.

## Resetting epigenetic aberrances in PSCs

Cell transformation and senescence are usually associated with epigenetic alterations at specific genomic loci. Aberrant epigenetic silencing of tumor-suppressor genes, such as $p16^{INK4A}$ and p53, and activation of tumor-promoting genes, such as Ras, could promote tumor initiation and progression. Somatic reprogramming has been shown to effectively reset disease- and tumor-associated aberrant epigenomes [17]. Although $p16^{INK4A}$ was epigenetically silenced in immortalized human embryonic lung fibroblasts, its expression and promoter-associated DNA methylation were completely restored through somatic reprogramming and subsequent redifferentiation [17]. Furthermore, analysis of global DNA promoter methylation in human sarcoma cells and their reprogrammed counterparts showed that epigenetic abnormalities associated with oncogenes and tumor suppressors in the original tumor cells