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Review

Nuclear Reprogramming by Defined Factors: Quantity Versus Quality

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The generation of induced pluripotent stem cells (iPSCs) and directly converted cells holds great promise in regenerative medicine. However, after in-depth studies of the murine system, we know that the current methodologies to produce these cells are not ideal and mostly yield cells of poor quality that might hold a risk in therapeutic applications. In this review we address the duality found in the literature regarding the use of 'quality' as a criterion for the clinic. We discuss the elements that influence reprogramming quality, and provide evidence that safety and functionality are directly linked to cell quality. Finally, because most of the available data come from murine systems, we speculate about what aspects can be applied to human cells.

iPSCs and Directly Converted Cells in Regenerative Medicine

Cell and organ transplantation is the conventional medical treatment for lost/damaged cells or tissues and for end-stage organ failure. However, the field of regenerative medicine is redefining how transplantation occurs, by growing cells, tissues, and organs in the laboratory and implanting them into patients [1]. One of the most attractive cell types for regenerative medicine is embryonic stem cells (ESCs) because they are capable of long-term growth, self-renewal, and can give rise to every cell type [2]. However, two major bottlenecks to realizing such potential are allogenic immune rejection of ESC-derived cells by recipients and ethical issues involving the destruction of a 'live' embryo.

The discovery that murine and human fibroblasts can be converted into stable and fully functional embryonic stem-like cells, termed **induced pluripotent stem cells** (iPSCs, see Glossary), by the ectopic expression of **key master regulators** Oct4, Sox2, Klf4, and Myc (OSKM, also known as Yamanaka factors) [3,4] has encouraged scientists to look beyond ESCs for regenerative medicine, as well as to re-evaluate the terminology 'terminally differentiated state' and the notion of cellular plasticity [5]. Since their discovery, researchers have attempted to directly convert various adult cells to different cell types, by avoiding the pluripotent state, using a unique combination of cell type-specific key master regulators [6–8]. Several medically-relevant cell types have been generated, including hematopoietic cells [9,10], different neuronal cells [11–13], cardiomyocytes [14], hepatocytes [15,16], embryonic Sertoli cells [17], endothelial cells [18], neural crest cells [19], and pancreatic β cells [20]. Furthermore, the first clinical trials using iPSC technology have been launched [21,22]. However, despite remarkable progress in characterizing the reprogramming process and the resulting iPSCs and directly converted cells [23–27], it remains to be seen if these converted cells are safe and of sufficiently high quality to warrant their immediate use in the clinic.

Trends

Despite the great promise that iPSCs and directly converted cells hold for regenerative medicine, concerns regarding the safety and functionality of these cells currently hold back their use in the clinic.

Many criteria affect the quality of the converted cells, such as genome integrity, complete somatic epigenetic erasure, histone deposition, and expression of long terminal repeats of endogenous retroviruses.

While the quality of murine iPSCs can best be assessed by their ability to form 'all iPSC' mice, this assay is not practicable for human iPSCs, thus highlighting the need for other quality control measures.

The choice of reprogramming factors and their stoichiometry, the use of non-integrating agents, and specific culture conditions provide routes by which iPSC quality may be improved.

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Theoretically, iPSCs and **directly converted** cells are ideal for regenerative medicine and for disease modeling [28–31]. In contrast to ESCs, their use does not involve ethical issues and, because they can be derived from patients, they should not be rejected by the host [32]. However, rigorous functional assays in the mouse system show that, unlike ESCs – which are relatively uniform in their differentiation capacity – the quality of iPSCs varies widely between different colonies [33]. Some colonies can contribute to chimeras but are unable to generate a healthy 'all-iPSC' mouse using the stringent pluripotency assay, **tetraploid complementation** (4N), while others may differentiate to the three germ layers *in vitro* but do not contribute to the embryo *in vivo* [34–37]. These experimental differences clearly demonstrate a significant gap in the quality of the various iPSC colonies in mice. Furthermore, many mouse assays such as the 4N test and **chimera contribution** are not applicable with human iPSCs (hiPSCs), highlighting the need for alternative and reliable quality measures for testing hiPSCs.

Assessing quality in direct conversion models is more problematic because many of them do not reach a stable and complete reprogramming state [8,38,39]. While iPSCs can grow independently of exogenous factors and are almost indistinguishable in their epigenome and transcriptome to their ESC counterparts, in the vast majority of direct conversion models the converted cells express only a fraction of the relevant markers and are dependent either fully or partially on their transgenes (Table 1) [8,38,39]. This observation raised the possibility that a high nuclear resetting state can be achieved only in stem cell populations. However, an incomplete reprogramming process was also noted in the generation of neuronal stem-like cells [40,41] and hepatic stem-like cells [42]. Taken together, these data suggest that the currently prevailing technology to reset the somatic nucleus by a defined number of factors is not ideal and mostly yields cells of poor quality. This is not surprising given that a normal reprogramming process (i.e., the reprogramming of a sperm nucleus by an egg) involves a large number of proteins that are present within the cytoplasm of a fertilized oocyte [43] as well as a unique nuclear chromatin condensation and epigenetic state of the sperm that make it adequate for early embryonic development [44]. Nevertheless, does cell quality really matter? After all, we are not trying to clone a human being, but instead to generate safe and functional cells for therapeutic applications.

In this review we focus on the current ability to analyze and compare the quality of murine iPSCs and directly converted cells, their validity, and the elements that affect the reprogramming quality. We address the duality found in the literature regarding the use of 'quality' as a parameter for the clinic and provide evidence that quality is directly linked to safety, stability, and functionality of the cells. Finally, we touch upon the limitations in assessing the quality of human cells and possible solutions.

Table 1. Properties of High-Quality iPSCs and Directly Converted Cell Types

Criterion	iPSCs (Related to High-Quality Cells)	Directly Converted Cells (Related to Studies Described)
Transgene dependency	No	Yes (either fully or partially)
Activation of the entire endogenous circuitry	Yes	No or only partially
Functionality	Fully equivalent to ESCs	Partial
Show full rescue in mouse model	Yes	Partial
De novo mutations/copy-number variation	Yes, but still debatable	N/D[a]
'Epigenetic memory'	Yes, very few loci	Yes, many loci
Transcriptome	Highly similar	Partially similar
Superenhancer activation	Yes	N/D

[a]N/D, not determined.

Glossary

Chimera contribution: an assay for pluripotency evaluation. ESCs/iPSCs are injected into blastocysts that are then transferred into pseudopregnant females. Developing embryos, pups, and mature mice are analyzed for the contribution of the cells. High-grade chimeras represent high-quality ESCs/iPSCs.

Directly converted cell: adult cell that uses a unique combination of cell type-specific key master regulators to undergo a specific nuclear reprogramming process to acquire the identity of a different cell type. The process avoids the pluripotent state.

Epigenomic assembly: a process that includes complete erasure of the epigenomic landscape of the donor cell followed by the acquisition of a new epigenome, including histone acetylation, methylation, and chromatin organization, that is similar to the targeted cell.

Ground state: the basal proliferative state of ESCs, which is free of epigenetic restriction and has minimal requirements for extrinsic stimuli. Cells in ground state are fully pluripotent and can generate an entire embryo.

Hyperdynamic chromatin state: a state of dynamic chromatin characterized by hypermobility of chromatin-associated proteins in pluripotent cells.

Induced pluripotent stem cells (iPSCs): somatic cells that underwent a nuclear reprogramming process to resemble ESCs, by introduction of a defined transcription factor combination such as Oct4, Sox2, Klf4, and Myc (OSKM); or Sall4, Nanog, Esrrb, and Lin28 (SNEL).

Key master regulators: potent cell type-specific transcription regulators that, when highly expressed in a parallel cell, can initiate a cellular program that alters cell fate.

Somatic cell conversion models: this term relates to all conversion experiments including directly converted cells and iPSCs.

Somatic memory: remnants of epigenome and transcriptome marks of the donor cells.

Teratoma assay: a teratoma is a nonmalignant tumor comprised of cells from all three embryonic germ-layers. In the teratoma assay, ESCs/iPSCs are implanted under the skin