Perspective

The labyrinth of nuclear reprogramming

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One of the current chief goals of regenerative medicine involves finding a way to overcome the lack of efficient treatments for thousands of diseases. Recently, increasing evidence indicates that genetic defects observed in patient somatic cells could be erased and/or alleviated by direct reprogramming toward pluripotency and recapitulated upon directed differentiation to specific cell lineages. Therefore, major efforts have been put forth to establish efficient models of disease by the use of induced pluripotent stem cells (iPSCs). In addition to disease modeling, iPSC technology also paved the way for the development of methods allowing for the generation of transplantable cellular products to reduce/halt the progression of disease symptoms or even ultimately to cure diseases. It is likely that, in the near future, these two strategies will be complementary in the discovery of new alternative treatments. However and despite enormous enthusiasm, iPSC technology still presents several pitfalls that have to be circumvented. In the present work, we will summarize the present state of iPSC technologies alongside several alternatives to the use of iPSCs that could contribute to the establishment of cell therapies.

Human embryonic stem cells (ESCs) have the capability to differentiate into all somatic cell types, a process that recapitulates the early stages of human development. However, the use of ESCs raises several controversies, particularly concerning the ethical dilemma regarding the use of human embryonic material and the need for embryo destruction. The discovery of induced pluripotent stem cell (iPSCs) technology in 2006 (Takahashi and Yamanaka, 2006) opened the door for possible application of pluripotent stem-cell-related therapies in the clinic as well as for the generation of patient-derived pluripotent stem cells suitable for disease modeling in vitro. Nuclear reprogramming is known to involve a series of steps leading to the epigenetic erasure of adult cell identity, including abnormalities responsible for disease development, by the establishment of a pluripotent network (Boyer et al., 2005; Wang et al., 2006). Although reprogramming leads to acquisition of stem-cell identity at the global transcriptome level and many similarities between ESCs and iPSCs can be observed, a number of reports have pointed out striking differences (Deng et al., 2009; Doi et al., 2009; Bock et al., 2011; Lister et al., 2011; Ohi et al., 2011). Along this line, it did not take long for the discovery of what it has become to be known as the ‘epigenetic memory’ of iPSCs. Epigenetic memory represents a state of ‘incomplete reprogramming’ in which early passage iPSCs maintain epigenetic marks characteristic of the initial cell type. Maintenance of such marks contributes to the more efficient re-differentiation of iPSCs to its lineage of origin (Kim et al., 2010; Polo et al., 2010; Bar-Nur et al., 2011; Ohi et al., 2011). To date and considering the possible medical applications, much work has been mainly focused on two different strategies to utilize iPSCs in the clinic rather than understanding the basic mechanisms of reprogramming. On the one hand, the extremely high number of publications regarding iPSCs has been mainly focused on the development of novel reprogramming strategies leading to ‘safer’ and higher numbers of iPSCs in a defined time-window. On the other hand, the development of efficient protocols for differentiation has also driven most of the iPSC research. These two main areas of investigation had two main common trigger causes. First, the standard technology applied for reprogramming per se raised serious concerns regarding the safe use of genetically modified cells bearing exogenous DNA integrated in their genome (Sun et al., 2010). Secondly, incomplete differentiation and transplantation of heterogeneous populations containing undifferentiated cells has been strongly linked to tumorigenesis in mouse models, even when iPSCs are generated in the absence of the oncogenic gene c-Myc or integrative approaches (Okita et al., 2007; Nakagawa et al., 2008; Giordetti et al., 2009; Kim et al., 2009b; Miura et al., 2009; Zhao et al., 2009). The fact is that even though a number of reports have described success in utilizing non-integrative approaches (Okita et al., 2008; Kim et al., 2009a), as well as the development of highly efficient differentiation protocols, it was not until recently and with the advent of high-resolution genomic technologies, that investigators could start to analyze the molecular consequences of the reprogramming process. By the end of 2010/beginning of 2011, a whole new wave of data and publications has once again shocked the iPSC field. First, copy number variation analysis has demonstrated the presence of a number of genomic aberrations in established iPSC lines escaping the resolution of traditional karyotyping and thus, being initially cataloged as ‘genomically correct’ (Hussein et al., 2011; Laurent et al., 2011). Furthermore, Gore et al. (2011) have
analyzed a whole set of different iPSC lines derived from different laboratories, as well as by different reprogramming approaches, including non-integrative approaches, and found a general tendency for the acquisition of mutations. Thus, the underlying mechanism of reprogramming seems to favor the accumulation of genetic aberrations in a similar fashion to cancer progression and rather independent of the integration of exogenous DNA. Along the same line, epigenetic differences between ESCs and iPSCs have also been found (Ohi et al., 2011). The presence of significant differences in global methylation patterns could partially explain the small differences observed in mRNA expression between both pluripotent cell types, however, this has not yet been conclusively demonstrated (Deng et al., 2009; Doi et al., 2009; Bock et al., 2011; Lister et al., 2011; Ohi et al., 2011).

Importantly, no functional validation or analysis of the potential tumorigenic effect of these aberrations has been precisely studied (Panopoulos et al., 2011). Just to make things a bit more exciting, iPSC reprogramming by miRNA expression has come into play (Anokye-Danso et al., 2011; Liao et al., 2011; Miyoshi et al., 2011; Subramanyam et al., 2011). Indeed, the finding that mere expression of several miRNAs could mediate and/or facilitate reprogramming by themselves has once again raised enormous expectations and hopes. The fact that miRNAs can lead to much higher reprogramming efficiencies, alongside the shorter time required for iPSC generation, might actually contribute toward the generation of safer iPSCs (Anokye-Danso et al., 2011). However, a closer look and careful interpretation has to be undertaken, moreover considering the lack of high-resolution epigenetic analysis of the generated lines to date. First, up to 10 times higher efficiencies achieved were due to continuous expression of mir302/367 clusters upon lentiviral infection and thus, the presence of exogenous DNA still remains. Secondly, a more recent report has demonstrated the ability to reprogram cells by transient transfection of mature miRNAs, yet all the above-mentioned advantages including higher efficiencies and shorter timing were lost (Miyoshi et al., 2011). It is tempting to speculate that acquisition of genomic mutations has two major contributors, on one side, the presence of exogenous and randomly integrated DNA and, on the other, a positive selection of randomly acquired mutations during reprogramming and culture. Accordingly, miRNA reprogramming could actually lead to safer iPSCs if the ideal situation combining non-integrative approaches and fast, high-efficient reprogramming can ever be achieved. Thus, a number of questions regarding the potential safety of these cells still remain. Interestingly, and perhaps somehow inspired from such studies, alternatives bypassing the pluripotent state have received increased attention and served for the re-discovery of an approach first described by Davis et al. (1987), the direct conversion of one cell type into another or transdifferentiation. Direct lineage conversion is based on the use of specific cocktails of transcription factors (TFs) defining the identity of the target cell lineage. Along this line, a number of other studies have demonstrated, and continue to outline, the possibility to exchange cell identity, by either introduction of a single (Kulesza et al., 1995; Laiosa et al., 2006) or a combination of TF(s) (Ieda et al., 2010; Vierbuchen et al., 2010; Caiazzo et al., 2011; Sekiya and Suzuki, 2011), as well as by in vivo ablation of TF(s) (Nutt et al., 1999). More recently, a similar conceptual approach has been described in murine cells. It is generally believed that acquisition of the endogenous regulatory loop between pluripotency-related TFs is achieved in the first week of reprogramming (Brambrink et al., 2008; Stadtfeld et al., 2008). In such a scenario, cells lacking the traditional hallmarks of pluripotency, and accordingly classified as non-iPSCs might yet bear certain epigenomic plasticity allowing for their direct differentiation by specific media conditions and lack of identity-specific TFs. Indeed, exposing cells for a short time period to the conventional cocktail of pluripotency inducers before directed differentiation has proven successful in the generation of mouse cardiomyocytes and neuronal cells (Efe et al., 2010; Kim et al., 2011). While the safety of this ‘shortcut-approach’ has not been reported yet, such studies demonstrate the plasticity of the cells and their potential to be driven to specific lineages in the absence of ES-like colony formation as well as full manifestation of other hallmarks of pluripotency. It is still unknown whether the cells induced by such approaches bear pluripotency properties or rather a more limited multilineage potential. Interestingly, the first demonstration that miRNAs can also be used for direct lineage conversion came out only few months after the first report describing full reprogramming to pluripotency (Yoo et al., 2011), thus demonstrating the versatility of miRNAs for epigenetic reprogramming and bringing one more tool to the researcher’s toolbox in the search for therapeutically relevant cell products. In summary, the stem cell and regenerative medicine fields seem to dance an unpredictably syncopated groove in which every new discovery leading to the general enthusiasm of the community is followed by another one alerting us to the potential pitfalls that early clinical translation might face. As an example, autologous transplantation of mouse-derived iPSCs has been demonstrated to provoke immune rejection due to aberrant expression of fetal genes recognized as exogenous products (Zhao et al., 2011). Although it is probable that transplantation of iPSCs will generate teratomas, teratocarcinomas, as well as immune rejection by infiltrating T-cells, the question remains on whether efficient protocols of differentiation coupled to further transplantation of highly pure differentiated populations will present tumorigenic and/or immunogenic activities in vivo. Furthermore, the discovery of alternative approaches to what, considering the pace of this field, we can already call ‘traditional’ iPSC technology might represent additional means for cell therapy-based clinical applications. Altogether, it is still to be seen how many doors will be opened and how many others will be closed in the labyrinth linking nuclear reprogramming to the clinic or even if the maze will ever be solved.

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