Induced pluripotent stem cells and senescence: learning the biology to improve the technology

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The discovery that adult somatic cells can be reprogrammed into pluripotent cells by expressing a combination of factors associated with pluripotency holds immense promise for a wide range of biotechnological and therapeutic applications. However, some hurdles—such as improving the low reprogramming efficiencies and ensuring the pluripotent potential, genomic integrity and safety of the resulting cells—must be overcome before induced pluripotent stem cells (iPSCs) can be used for clinical purposes. Several groups have recently shown that key tumour suppressors—such as members of the p53 and p16^{INK4a}/retinoblastoma networks—control the efficiency of iPSC generation by activating cell-intrinsic programmes such as senescence. Here, we discuss the implications of these discoveries for improving the safety and efficiency of iPSC generation, and for increasing our understanding of different aspects of basic biology—such as the control of pluripotency or the mechanisms involved in the generation of cancer stem cells.

Keywords: iPSC; senescence; reprogramming; tumour suppressors; cancer stem cell

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See Glossary for abbreviations used in this article.

iPSCs: promises and challenges

Somatic cells—unlike their cancer derivatives—cannot divide indefinitely. This limited proliferative potential is critical for maintaining the integrity of their progeny. Conversely, embryonic stem cells (ESCs) have the ability to self-renew and can undergo numerous cell divisions, giving rise to identical undifferentiated daughter cells. ESCs are also pluripotent, as they can differentiate into all the cell types of the embryo. The idea of deriving pluripotent cells from somatic cells by somehow reversing the natural differentiation process that occurs during development has been long explored. Although the transfer of nuclei from somatic cells—first in frogs and later in mammals (Gurdon, 1962; Hochedlinger & Jaenisch, 2002; Wilmut *et al*, 1997)—and cell fusion studies (Weimann *et al*, 2003; Ying *et al*, 2002) have informed us about the biology of these processes, the real quantum leap in this field was the generation of induced pluripotent stem cells (iPSCs). Four years ago, Takahashi

and Yamanaka reported that somatic cells could be reprogrammed by the expression of four factors associated with pluripotency, the so-called ‘Yamanaka factors’: OCT4, SOX2, KLF4 and c-MYC (Takahashi & Yamanaka, 2006). This finding implies that one would be able to generate pluripotent cells starting from any cell of choice, including patient cells, with all the implications that this has for regenerative medicine, disease modelling and drug screening in relevant cellular systems. However, the reprogramming to iPSCs still suffers from several hurdles that have to be overcome for their practical application. Among them, the efficiency of reprogramming needs to be improved and iPSCs need to be generated that are safe and ideally devoid of any exogenous sequences. These processes are inherently linked—for example, the inadequate silencing of some genes can be sufficient to impair the pluripotent potential of the putative reprogrammed cells. Similarly, exogenous sequences inserted in the iPSC genome can be responsible for increased tumorigenicity owing to insertional mutagenesis or incomplete transgene silencing. In addition, the quality of the iPSCs—that is, their ability to contribute to germ-line transmission or differentiate into different lineages—must be controlled, as qualitative differences seem to exist between iPSCs obtained using different methods (Han *et al*, 2010). A lot of effort has been put into improving and understanding the reprogramming process, a topic that has been reviewed excellently elsewhere (Feng *et al*, 2009). Here, we focus on the fact that tumour-suppressive mechanisms are activated during the reprogramming process, indicating that senescence is a barrier to iPSC generation.

Fine-tuning reprogramming

Significant improvements have been made to increase the efficiency and safety of iPSCs since their discovery. For example, alternative approaches to the original retroviral vectors have been explored to deliver the reprogramming factors. These include methods that minimize or eliminate the permanent modification of the genome, such as polycistronic vectors (Kaji *et al*, 2009; Woltjen *et al*, 2009), transient transfection (Okita *et al*, 2008), transposon vectors (Kaji *et al*, 2009), adenoviral vectors (Stadtfield *et al*, 2008), episomal vectors (Yu *et al*, 2009), ‘minicircle’ vectors free of bacterial DNA elements (Jia *et al*, 2010) or recombinant proteins (Zhou *et al*, 2009). By avoiding permanent alterations in the genome, the risk of transformation or incomplete pluripotency due to insufficient transgene silencing is reduced.

In addition to the timing of expression and silencing of the reprogramming factors, the efficiency of vector transduction and factor

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Fig 1 | Generation of secondary iPSCs. Reprogramming factors are expressed from doxycycline-inducible vectors in primary somatic cells. Induction of reprogramming into primary iPSCs is achieved by treatment with doxycycline, colony selection and treatment withdrawal. The resulting iPSCs are then differentiated *in vitro* to somatic cells that carry the DOX-inducible factors; alternatively, chimeric animals can be produced from which to obtain somatic cells—such as B cells or fibroblasts. Secondary somatic cells—which all contain integrated reprogramming factors—can undergo reprogramming by DOX induction to produce secondary iPSCs with greater efficiency. DOX, doxycycline; iPSCs, induced pluripotent stem cells; KLF4, Kruppel-like factor 4; OCT4, octamer 4; SOX2, SRY-box 2.

stoichiometry affect the efficiency of reprogramming (Hanna *et al*, 2009; Yamanaka, 2009). This issue has been addressed by the use of polycistronic vectors that express the four factors (Kaji *et al*, 2009), or systems in which ‘secondary iPSCs’ are generated from iPSC-derived differentiated cells (Fig 1). In the latter strategy, fibroblast-like cells (or other somatic cell types) are obtained after *in vitro* differentiation of iPSCs that harbour doxycycline-inducible Yamanaka factors or, in the case of mouse cells, by the generation of chimeric mice (Maherali *et al*, 2008; Wernig *et al*, 2008). The differentiated cells are then treated with doxycycline to induce their reprogramming into secondary iPSCs. However, although the viral transgenes are reactivated in most of the cells and there is a 50–100-fold increase in the reprogramming efficiency, the overall frequency of reprogramming remains low at 1–3% (Maherali *et al*, 2008). This is probably due to the existence of additional barriers that limit the reprogramming efficiency.

Understanding reprogramming

To make reprogramming a more efficient process, we need to understand the mechanism by which it is enabled and relate it to our

knowledge of stem-cell biology and pluripotency. The best example of that strategy is the original work from Takahashi and Yamanaka, in which they chose 24 factors relevant for the maintenance of stem-cell identity as a starting point for their reprogramming experiments (Takahashi & Yamanaka, 2006). The Thomson group devised a similar strategy, using 15 factors expressed in ESCs and relevant for pluripotency as a starting point to define that the combination of OCT4, SOX2, LIN28 and NANOG reprogrammes human somatic cells (Yu *et al*, 2007). The importance of studying pluripotency to understand reprogramming is exemplified by the recent finding that AID-dependent DNA demethylation is crucial for the epigenetic reprogramming of primordial germ cells (PGCs; Bhutani *et al*, 2010). Not surprisingly, AID-dependent DNA demethylation is also required for the reprogramming of single heterokaryons towards pluripotency (Popp *et al*, 2010).

The mechanisms involved in reprogramming somatic cells to a pluripotent-like state remain largely unknown. One of the approaches to understanding how reprogramming operates has been to produce genome-wide profiles of gene expression and chromatin status in iPSCs and compare them with those of somatic cells, ESCs and partially reprogrammed iPSCs (pre-iPSCs; Mikkelsen *et al*, 2008; Sridharan *et al*, 2009). These studies have shown that iPSCs of different origins, derived independently or through different protocols are markedly similar at the molecular level. Importantly, iPSCs and ESCs are similar in their gene expression profile, epigenetic status (Mikkelsen *et al*, 2008; Sridharan *et al*, 2009) and even in the status of tissue-specific enhancers (Xu *et al*, 2009), although subtle differences exist (Chin *et al*, 2009). **Perhaps more surprising** is the high similarity observed between different pre-iPSCs, suggesting they have a common intermediate origin. One of the differences between pre-iPSCs and iPSCs is their degree of chromatin remodelling; for example, the number of genes with bivalent chromatin domains (that is, those containing both activating and repressive epigenetic modifications) is lower in pre-iPSCs than in iPSCs or ESCs, which correlates with a larger number of hypermethylated promoters. Among the promoters showing an aberrant methylation pattern in pre-iPSCs are those of the pluripotency-associated factors OCT4, NANOG and REX1 (Mikkelsen *et al*, 2008). These studies are starting to illuminate the reprogramming process and can be used to rationally improve the technology. **Indeed, treatment with an inhibitor of DNA methylation**, such as AZA, not only increases the efficiency of reprogramming to iPSCs but also converts a percentage of pre-iPSCs into iPSCs (Mikkelsen *et al*, 2008). Similar results were obtained after suppressing the expression of the methyltransferase DNMT1 with short hairpin RNAs (Mikkelsen *et al*, 2008). In addition, the use of other drugs that affect the general state of chromatin, such as several HDAC inhibitors—for example, VPA, TSA or SAHA—or BIX-01294, an inhibitor of the G9a histone methyltransferase, also enhances the efficiency of reprogramming (reviewed in Feng *et al*, 2009). Conversely, some pre-iPSCs fail to repress lineage-specific transcription factors and, therefore, the combined silencing of several of those factors—such as GATA6, PAX7, PAX3 and SOX9—can increase reprogramming (Mikkelsen *et al*, 2008).

Transcriptional profiling also revealed the upregulation of genes involved in DNA replication (*POL1*, *RCF4* and *MCM5*) and cell cycle progression (*CCND1* and *CCND2*; Mikkelsen *et al*, 2008)—in addition to the upregulation of several antiproliferative genes, such as the CDK inhibitors p21^{Cip1} and p16^{Ink4a}—in pre-iPSCs or as an immediate response to the expression of the reprogramming factors

Glossary

AID	activation-induced cytidine deaminase
AZA	5-azacytidine
DNMT1	DNA methyltransferase 1
γ H2AX	phosphorylated histone H2AX
HDAC	histone deacetylase
hTERT	human telomerase reverse transcriptase
KLF4	Kruppel-like factor 4
MEF	mouse embryonic fibroblast
MDM2	mouse double minute 2
OCT4	octamer 4 (also termed POU class 5 homeobox 1)
RB	retinoblastoma
SA- β -Gal	senescence-associated β -galactosidase staining
SAHA	suberoylanilide hydroxamic acid
SAHFs	senescence-associated heterochromatin foci
SOX2	SRY-box 2
TSA	trichostatin A
Terc	telomerase RNA component
SV40	simian vacuolating virus 40
VPA	valproic acid

(Mikkelsen *et al*, 2008; Banito *et al*, 2009; Sridharan *et al*, 2009). These results show an unexpected activation of the antiproliferative response during reprogramming (Fig 2; Banito *et al*, 2009). Several groups analysed whether the expression of the reprogramming factors is sufficient to trigger directly an antiproliferative response and showed that the expression of the four Yamanaka factors, or combinations of only OCT4, SOX2 and KLF4, in human or mouse fibroblasts is enough to induce p53 and p21^{Cip1} (Hong *et al*, 2009; Banito *et al*, 2009; Kawamura *et al*, 2009). The expression of the individual factors or a combination of OCT4 and SOX2 seems also sufficient to trigger the activation of p53 and/or p21^{Cip1} (Banito *et al*, 2009; Kawamura *et al*, 2009).

The main determinants of p53 activation during reprogramming have not been clearly established. Differences in how p53 is activated might exist between cells of human and mouse origin, as occurs during oncogene-induced senescence (OIS). For example, a role for p19^{Arf} in the activation of p53 during reprogramming in MEFs can be inferred from recent results (Kawamura *et al*, 2009), but whether p14^{ARF} has a similar role in activating p53 during the reprogramming of human cells remains to be determined. Nevertheless, several groups have observed that a DNA damage response (DDR) is mounted coinciding with the expression of the reprogramming factors (Banito *et al*, 2009; Kawamura *et al*, 2009; Marion *et al*, 2009a). The triggering factor for this DDR is still unclear, although it has been suggested that the observed pan-nuclear pattern of γ H2AX staining is compatible with DNA replication-induced DNA damage (Marion *et al*, 2009a). This would be similar to what seemingly occurs during OIS, when aberrant DNA replication accounts for the activation of the DDR. However, the expression of the four Yamanaka factors in human fibroblasts results in the accumulation of 8-oxoguanine adducts—which are commonly the result of oxidative stress—and c-MYC induces DNA damage in a mainly ROS-dependent (Vafa *et al*, 2002) rather than DNA replication-dependent manner (Egler *et al*, 2005). Therefore, the DNA damage that occurs on reprogramming could be caused not only by aberrant DNA replication but also through the generation of ROS, which would explain why reprogramming is more efficient under low oxygen conditions (Utikal *et al*, 2009; Yoshida *et al*, 2009).

Fig 2 | Alternative cell fates limit reprogramming efficiency. During successful reprogramming, the expression of reprogramming factors in somatic cells results in the generation of iPSCs. In some cases, the reprogramming process is not complete and partially reprogrammed iPSCs that have undergone incomplete chromatin remodelling are obtained. Alternatively, the expression of the reprogramming factors can cause senescence (RIS), apoptosis, or contribute to the oncogenic transformation of the resulting cells. ESC, embryonic stem cell; iPSCs, induced pluripotent stem cells; KLF4, Kruppel-like factor 4; OCT4, octamer 4; RIS, reprogramming-induced senescence; SOX2, SRY-box 2.

In addition to the activation of p53, our group has also observed that p16^{INK4a} is induced early on expression of the four Yamanaka factors in human fibroblasts (Banito *et al*, 2009). Although the mechanism behind this upregulation of p16^{INK4a} needs to be explored further, it involves chromatin remodelling, including the loss of H3K27me3 marks around the *INK4b/ARF/INK4a* locus. The upregulation of the histone demethylase JMJD3 observed on reprogramming could be partly responsible for this phenotype (Banito *et al*, 2009), which markedly resembles the one observed during RAS-induced senescence (Agger *et al*, 2009; Barradas *et al*, 2009). The *INK4b/ARF/INK4a* locus is repressed epigenetically by Polycomb group proteins in both iPSCs and ESCs (Banito *et al*, 2009; Li *et al*, 2009), raising the question of whether the *INK4b/ARF/INK4a* locus is reprogrammed properly in iPSCs or if there is pressure to accumulate mutations or silence it through aberrant DNA methylation. In this regard, work from the Serrano group indicates that it undergoes proper reprogramming; iPSCs reset the epigenetic status of

the *INK4b/ARF/INK4a* locus, acquiring similar epigenetic marks to those observed in ESCs (Li *et al*, 2009). This situation resembles the resetting of telomeres during reprogramming; the telomeres of iPSCs are elongated when compared with those of the somatic cells from which they are derived and also undergo a remodelling of their epigenetic marks to resemble those of ESC telomeres (Marion *et al*, 2009b). Although these observations indicate that reprogramming is possible without insurmountable pressure to accumulate genetic alterations in loci that are critical for cell proliferation, it will be necessary to determine whether mutations occur in any of the crucial antiproliferative tumour suppressor genes that are activated during reprogramming by analysing their epigenetic status or sequencing them in larger samples of reprogrammed iPSCs.

Some of the signature genes activated during reprogramming are common to different antiproliferative responses—such as apoptosis, senescence or other forms of cell-cycle arrest—raising the question of which antiproliferative response(s) is triggered on reprogramming. The answer is probably complex. Mouse and human fibroblasts expressing reprogramming factors suffer a cell-cycle arrest that presents multiple characteristics of senescence, such as a high percentage of cells that have SA- β -Gal activity or SAHFs. In addition, the upregulation of p16^{INK4a} further indicates that the arrest resembles senescence, as p16^{INK4a} is upregulated specifically during senescence but not in other types of cell-cycle arrest. Thus, we argue that it makes sense to talk of reprogramming-induced senescence (RIS; Banito *et al*, 2009). In addition to senescence, the reprogramming factors can also trigger apoptosis (Fig 2); for example, BAX is upregulated in response to the expression of OCT4, SOX2 and KLF4, and expression of its antagonist molecule BCL2 results in enhanced reprogramming efficiency (Kawamura *et al*, 2009). Other reports suggest that the expression of the reprogramming factors synergizes with induction of DNA damage to trigger apoptosis, especially in cells that have critically short telomeres or are sensitized by exposure to exogenous DNA-damaging agents—such as ultraviolet or ionizing radiation. In such a scenario, the expression of BCL2 restores the ability of these cells to be reprogrammed to levels similar to control cells (Marion *et al*, 2009a). Therefore reprogramming is limited by both antiproliferative responses—as happens during tumour suppression, in which both senescence and apoptosis are implicated.

Modulating reprogramming by controlling senescence

A corollary to the fact that senescence and apoptosis are triggered during reprogramming is that the inhibition of those responses could increase the efficiency of reprogramming. Indeed, Park and colleagues were among the first to describe the reprogramming of human adult somatic cells—such as fibroblasts—to ESC-like pluripotent cells by using the four Yamanaka factors in combination with the SV40 large T antigen (SV40 LT) and/or hTERT (Park *et al*, 2008); both additional factors are involved in senescence control. Several groups have shown subsequently that knocking down p53 in human or mouse cells can significantly increase the efficiency of reprogramming (Banito *et al*, 2009; Hong *et al*, 2009; Kawamura *et al*, 2009; Marion *et al*, 2009a; Utikal *et al*, 2009; Zhao *et al*, 2008). The expression of MDM2 or a dominant-negative mutant of p53 also results in enhanced reprogramming, whereas the activation of p53 through different strategies reduced the reprogramming efficiency (Kawamura *et al*, 2009; Marion *et al*, 2009a), emphasizing the importance of controlling p53 activity to modulate reprogramming. Similarly, low levels—or absence—of p16^{INK4a} or p21^{CIP1} expression

leads to more efficient and faster reprogramming in mouse and human cells (Banito *et al*, 2009; Li *et al*, 2009; Utikal *et al*, 2009).

The Jaenisch group has shown recently that reprogramming is a stochastic process amenable to acceleration, by using a secondary iPSC system (Fig 1) and clonal expansion of B cells (Hanna *et al*, 2009). They concluded that all cells have the potential to be reprogrammed to pluripotency. Interestingly, the authors suggest that the acceleration of reprogramming by inhibition of the p53/p21 axis is strictly dependent on their ability to regulate cell-cycle progression, which could be the case in the secondary iPSC reprogramming system (Hanna *et al*, 2009). However, other experiments suggest that the long-term proliferative potential of these cells—and perhaps additional factors—also contributes to this effect, as MEFs devoid of p53 and grown in the presence of 0.5% serum give rise to more iPSC colonies than wild-type MEFs grown on 15% serum, despite the more vigorous growth of the latter (Utikal *et al*, 2009).

Senescence and reprogramming are deeply intertwined processes: a direct comparison of the ability of young and old cells to be reprogrammed shows that the closer cells are to the onset of senescence—and therefore the higher the levels of p16^{INK4a} and p21^{CIP1} they express—the more difficult it is to reprogramme them. Fibroblasts from older mice, which express high levels of the *Ink4b/Arf/INK4a* locus products, are less efficient in generating iPSCs (Li *et al*, 2009), further linking ageing with decreased reprogramming efficiency. Similarly, experiments performed in late generation *Terc*^{−/−} MEFs, which have critically short telomeres, emphasize the difficulty of reprogramming cells that are already aged or stressed (Marion *et al*, 2009b). These results have implications for the derivation of iPSCs for therapeutic purposes from older individuals. The reprogramming of cells from patients with defects in telomere biogenesis, such as those with dyskeratosis congenita (DC) is also inefficient (Agarwal *et al*, 2010). DC is caused by a mutation in the dyskerin gene, which is necessary for stabilizing TERC levels and maintaining telomere homeostasis. Surprisingly, iPSCs successfully derived from DC cells can still upregulate the expression of TERC, restoring telomere elongation (Agarwal *et al*, 2010).

The relationship between reprogramming and tumour suppressors is interesting at different levels and highlights how stressful the process of reprogramming must be for the cell. Although the efficiency of reprogramming can be improved markedly by interfering with crucial antiproliferative genes, the consequence of dismantling cell-intrinsic tumour suppressive mechanisms is too detrimental to consider, as it would affect the safety of the resulting iPSCs. Indeed, iPSCs derived from *p53*^{−/−} MEFs have increased chromosomal instability (Marion *et al*, 2009a). Complex mouse models have suggested that we could theoretically exploit the positive effects of senescence in tumour suppression while avoiding unwanted side effects such as premature ageing (Serrano & Blasco, 2007). Thus, a sensible alternative could also exist to benefit from disabling senescence to enhance reprogramming efficiency without compromising the integrity and safety of the resulting iPSCs. One possibility could be to transiently inhibit senescence, by using either small interfering RNAs or chemical compounds. Another possibility is to affect specific pathways involved in triggering senescence during reprogramming. In this regard, reprogramming cells in low oxygen conditions (Utikal *et al*, 2009; Yoshida *et al*, 2009) or in the presence of antioxidants—such as vitamin C (Esteban *et al*, 2010)—has been shown to enhance the generation of iPSCs. Vitamin C alleviates RIS, suggesting that antioxidants or other compounds that transiently inhibit senescence, without permanently

disabling tumour suppression, could be used to safely improve reprogramming. We anticipate that the pursuit to avoid the anti-proliferative barriers present during reprogramming will be the focus of intense research in the field, in parallel with the development of complementary strategies aimed at enhancing reprogramming.

Pluripotency, reprogramming and transformation

Beyond the technological implications, the aforementioned results have unveiled the relationship between reprogramming and tumorigenesis, illuminating interesting biological processes. Most tumours have defects in the p53 and p16^{INK4a}/RB pathways, which can enhance and accelerate reprogramming. It is thus tempting to speculate that this ability is necessary for tumour initiation or maintenance. The belief is that in many cancer types, tumour growth and propagation is sustained by the so-called cancer stem cells (CSCs). CSCs were first identified in leukaemias (Lapidot *et al*, 1994) and later shown to be crucial for certain solid tumours (Gupta *et al*, 2009), although their relevance for other tumours—such as melanoma—remains unclear (Quintana *et al*, 2008). CSCs are a subpopulation of tumour cells with the ability to self-renew and maintain tumour growth (Clarke *et al*, 2006). It is therefore plausible that alterations in the p53 or the p16^{INK4a}/RB pathways have an impact on tumorigenesis, not only by affecting proliferation, but also by contributing to reprogramming somatic cells to a more dedifferentiated state or increasing the pool of CSCs. Indeed, the loss of p53 has been linked with the acquisition of some CSC properties, such as regulating the polarity of self-renewing divisions (Cicalese *et al*, 2009). Interestingly, deletion of the three RB family proteins triggers the reprogramming of MEFs to generate CSC-like cells, and cells that resemble CSCs can even be generated from MEFs that only lack RB if they are forced to grow beyond contact inhibition (Liu *et al*, 2009).

The relationship between pluripotency and tumorigenesis is intricate and delicate. ESCs are derived from the cells of the inner cell mass of the embryo, which are forced to grow in culture. Thus, they could represent a cell type that does not exist in nature; indeed, they share characteristics with tumour cells. ESCs—the tumorigenic counterparts of which are embryonic carcinoma cells—cause teratomas when injected into nude mice, a test that is used as the ultimate proof of pluripotency. The fact that c-MYC and KLF4 are well-known oncogenes linked to the generation of iPSCs and to stem-cell self-renewal emphasizes further the parallels between pluripotency and tumorigenicity. In addition, other reprogramming factors, such as SOX2 and LIN28, have been shown recently to be oncogenes in lung and oesophageal squamous carcinomas and germ-cell tumours, respectively (Bass *et al*, 2009; West *et al*, 2009). Aggressive, poorly differentiated tumours also express high levels of ESC-associated factors (Ben-Porath *et al*, 2008). The expression of some oncogenes—such as c-MYC—in epithelial tumours is sufficient to reactivate an ESC-like transcriptional signature. Together, these observations suggest that reprogramming to a more dedifferentiated state occurs during tumour progression (Wong *et al*, 2008) and might be favoured by alterations in crucial tumour suppressors. If the acquisition of stem-cell-like properties goes hand-in-hand with tumorigenesis, it is reasonable to think that the mechanism triggered on expression of the reprogramming factors—which ultimately leads to reprogramming—elicits the tumour suppressor pathways that protect cells against uncontrolled growth. In fact, the cellular response to the expression of the reprogramming factors

Fig 3 | Similarities between reprogramming to pluripotency and oncogenic transformation. (A) Aberrant oncogene expression triggers senescence (OIS) in primary cells, which limits oncogenic transformation. The expression of the reprogramming factors also triggers senescence (RIS), limiting the efficiency of reprogramming. (B) As a consequence, when senescence is disabled, cells are more susceptible to either oncogenic transformation or reprogramming. OIS, oncogene-induced senescence; RIS, reprogramming-induced senescence.

or stem-cell-specific genes mimics the senescence response observed during OIS, emphasizing the parallels between RIS and OIS (Fig 3). It would be clearly unsafe to use iPSCs therapeutically until we fully understand this deleterious connection between pluripotency and oncogenic transformation.

Conclusions

The initial analyses of direct cellular reprogramming to pluripotency have yielded some mechanistic clues about how the process works, which we are starting to use to improve the technology. Coincidentally, the study of the reprogramming process is unexpectedly revealing new information on, for example, the links between pluripotency and transformation (Sidebar A). Perhaps the study of

Sidebar A | In need of answers

- (i) Do cells destined to become reprogrammed not activate senescence? Or are they blocked at senescence and able to eventually bypass it?
- (ii) Do the pathways that lead to pluripotency overlap with those leading to cancer?
- (iii) Can we modulate the senescence pathways specifically to safely improve reprogramming efficiency?
- (iv) Which are the dynamics of induction and repression of the *INK4b/ARF/INK4a* locus during reprogramming? What are the mechanisms responsible for these dynamics?
- (v) Does senescence limit physiological and pathological processes—such as the reprogramming that occurs in the fertilized egg and primordial germ cells, or the acquisition of stem-cell-like properties by somatic cells during tumorigenesis?

reprogramming—which has been seen mostly as a markedly artificial technology with promising applications—will also increase our understanding of crucial aspects of basic biology (Ramalho-Santos, 2009). The fact that the same alterations that drive oncogenic progression can influence the reprogramming of somatic cells to CSCs or iPSCs suggests that the derivation of safe iPSCs could present enormous challenges.

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