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A fruitful liaison of ZSCAN10 and ROS on the road to rejuvenation

Clea Bárcena and Carlos López-Otín

Induced pluripotent stem cells derived from aged donors (A-iPSCs) usually show genomic instability that affects their utility and raises concerns about their safety. Now, a study highlights the importance of ZSCAN10-dependent recovery of glutathione–ROS homeostasis in counteracting the genomic defects in A-iPSCs.

The increment in lifespan expectancy achieved in the last century entails the need for a rapid development of medical strategies prepared to confront the biomedical needs of a population that is getting older. Ageing is characterized by a loss of stem cell function and a rise in degenerative diseases. Accordingly, the expansion of the knowledge of cellular and molecular pathways that intervene in the correct function of stem cells will help to fight age-related diseases[1]. The introduction of efficient cell reprogramming protocols has opened the ground-breaking field of regenerative medicine, which is allowing us to dream of an unlimited pool of histocompatible tissues and organs made *à la carte* for each patient. The generation of iPSCs from adult or aged donors (A-iPSCs) involves the rejuvenation of cells that already accumulate multiple genetic, cellular and molecular age-associated marks, such as genetic instability, cell senescence, inflammation or telomere shortening[2]. Indeed, although the reprogramming of cells from centenarian donors and progeria patients has already been accomplished[3], iPSCs derived from aged donors have not yet been proved to be completely safe[4]. In this context, different groups have observed genomic instability and frequent chromosomal alterations in human A-iPSCs[4,5]. Therefore, the identification of the molecular pathways altered in A-iPSCs would shed light on the biology of iPSCs and facilitate the design of appropriate tools for reprogramming aged cells.

Previous works have described that an excess of reactive oxygen species (ROS) causes DNA damage, but at the same time ROS are also essential to signal cellular stress and to induce the DNA damage response[6]. Therefore, a balance between ROS and antioxidants is essential to preserve genomic stability[7]. Although the importance of a correct level of ROS for restraining genomic instability in stem cells has already been assessed[8], the underlying mechanism remains to be fully elucidated. In this issue of *Nature Cell Biology*, Skamagki *et al.*[9] have explored the role of ROS and glutathione homeostasis in the generation of healthy A-iPSCs, which has led them to identify a zinc finger transcription factor, ZSCAN10, that collaborates in the preservation of this homeostasis.

Using mouse cells, Skamagki *et al.* showed that A-iPSCs accumulated genomic damage and had a defective DNA damage response when compared with iPSCs derived from young mice donors (Y-iPSCs), and with mouse embryonic stem cells (ESCs). The large number of chromosomal structural abnormalities in A-iPSCs and the resistance of these cells to different stresses suggest that these A-iPSCs were unable to activate apoptosis in response to DNA damage, due to a defective activation of the ATM pathway. Interestingly, after studying core regulators of the pluripotency regulatory network, the authors identified eight candidates that were differentially expressed in A-iPSCs compared with Y-iPSCs or ESCs. Among them, ZSCAN10 stood out for its close association with proteins involved in the DNA damage response, p53 pathway and antioxidant response[10,11]. Moreover, after overexpression of ZSCAN10 in A-iPSCs, there was a recovery in apoptosis and DNA damage response, thereby corroborating the importance of ZSCAN10 in the generation of healthy A-iPSCs (Fig. 1a). Based on this, the authors hypothesized that activation of ZSCAN10 was necessary during reprogramming to generate healthy iPSCs, and this activation was impaired in somatic cells from aged donors.

A smart bioinformatics screening of ZSCAN10 targets that could be involved in the induction of genomic instability in A-iPSCs identified glutathione synthetase (GSS) as a main contributor to this defect. Indeed, the authors found that GSS was expressed at remarkably high levels in A-iPSCs but was downregulated to levels similar to those found in Y-iPSCs and ESCs on ZSCAN10 expression. The close relationship between redox homeostasis and genomic instability[6] suggests that an alteration in glutathione homeostasis could underlie the phenotype observed in A-iPSCs. The elevation in glutathione levels is probably due to the lack of sufficient amounts of ZSCAN10 to effectively suppress GSS expression. This could eventually impair the balance between the oxidant and antioxidant responses. The consequent depletion of ROS would blunt the DNA damage response, leading to genomic instability (Fig. 1b).

Clea Bárcena and Carlos López-Otín are in the Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Instituto Universitario de Oncología (IUOPA), Universidad de Oviedo, 33006 Oviedo, Spain.
e-mail: clo@uniovi.es

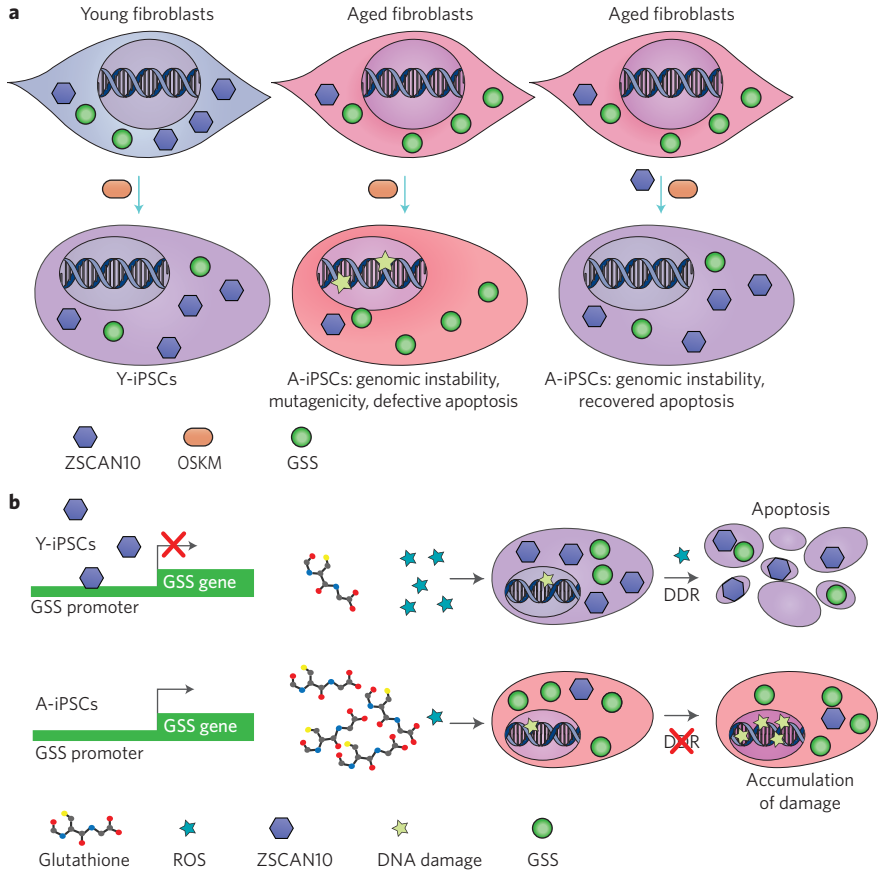

Figure 1 ZSCAN10 preserves genomic stability in iPSCs through the maintenance of ROS levels. (**a**) Reprogrammed cells from aged donors show increased levels of genetic damage than those from young donors. Genomic stability and apoptotic response are recovered after induction of ZSCAN10 during reprogramming. OSKM: OCT4, SOX2, KLF4 and MYC. (**b**) ZSCAN10 binds the promoter region of GSS, inhibiting its transcription. In the absence of sufficient levels of ZSCAN10, an excess amount of glutathione induces scavenging of ROS, which are otherwise necessary for the activation of the DNA damage response (DDR).

Consistent with this, the authors found that knockdown of GSS in A-iPSCs was sufficient to reduce glutathione levels, increase ROS and recover the DNA damage response. Similarly, after ZSCAN10 expression, glutathione and ROS levels were normalized to those observed in Y-iPSCs and ESCs.

To test if the relationship between glutathione–ROS homeostasis and DNA damage response during reprogramming is conserved in humans, Skamagki *et al.* analysed ZSCAN10 levels in different human iPSC clones from aged donors (A-hiPSCs), and found low levels of ZSCAN10 and high levels of GSS with a concomitant loss in the DNA damage response in multiple but not all clones. Interestingly, chromatin-immunoprecipitation quantitative PCR (ChIP–Q-PCR) confirmed that ZSCAN10 binds to the human GSS promoter in A-hiPSCs, suppressing its transcription. Finally, the authors proved that overexpression of ZSCAN10 during reprogramming normalized the glutathione–ROS balance, restored the DNA damage response and increased genomic stability in both mouse and human A-iPSCs.

It has been recently described that clonal variation among hiPSCs is due to genetic variability between individuals[12], and mutations present in iPSCs derived from aged donors disturb the archetype behaviour of reprogrammed cells, reducing their pluripotency or even contributing to the tumorigenic potential of the differentiated cells derived from them[5]. For this reason, identifying the genetic drivers of ageing that may hamper the correct rejuvenation of aged cells is imperative for the development of efficient reprogramming strategies. With this study, Skamagki *et al.* discovered a previously unrecognized molecular driver of ageing, the downregulation of ZSCAN10, whose expression regulates the glutathione–ROS balance, helping to preserve the DNA damage response and, therefore, genomic stability. These findings are reminiscent of what was observed in cancers where increased levels of glutathione in cancer-associated fibroblasts from the tumour microenvironment induced scavenging of ROS and mediated resistance to chemotherapy by avoiding the DNA damage response and apoptosis of tumour cells[13]. With the current trend promoting the wide use of antioxidants in nutritional supplements, body lotions and rejuvenating drinks, it is imperative to keep in mind that ROS also perform important biological functions that cannot be dismissed.

The findings reported by Skamagki *et al.* further support previous observations indicating that the use of antioxidants suppresses somatic reprogramming of fibroblasts[14], and even suggest the putative use of oxidizers — such as hydrogen peroxide — in stem cell research. Moreover, after intense years of research trying to find effective ways to boost glutathione levels, this study also indirectly opens the path to the design of GSS inhibitors. Still, the importance of keeping limited levels of ROS for the maintenance of different types of stem cells, such as haematopoietic stem cells, indicates the need to carefully study the role of ROS in iPSC technology and stem cell biology in different tissues and cell types[15], as the observations described herein might not be extrapolated to all conditions. Despite these caveats, the interesting findings reported by Skamagki *et al.* will help us to expand the knowledge of the role of ROS homeostasis in stem cell biology, contributing to the development of improved and personalized stem cell therapies.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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