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Genomic stability during cellular reprogramming: Mission impossible?

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ABSTRACT

The generation of induced pluripotent stem cells (iPSCs) from adult somatic cells is one of the most exciting discoveries in recent biomedical research. It holds tremendous potential in drug discovery and regenerative medicine. However, a series of reports highlighting genomic instability in iPSCs raises concerns about their clinical application. Although the mechanisms cause genomic instability during cellular reprogramming are largely unknown, several potential sources have been suggested. This review summarizes current knowledge on this active research field and discusses the latest efforts to alleviate the genomic insults during cellular reprogramming to generate iPSCs with enhanced quality and safety.

1. Introduction

The seminal discovery by Takahashi and Yamanaka demonstrated that a small set of transcription factors, Oct4, Sox2, Klf4 and c-Myc (dispensable for acquiring pluripotency) (OSKM), are sufficient to convert terminally differentiated cells into embryonic stem cell (ESC)-like cells called induced pluripotent stem cells (iPSCs) [1]. This revolutionary breakthrough has caused an explosion in stem cell research in the last decade. It opened up numerous possibilities for disease research and regenerative medicine. Currently, patient-derived iPSCs are used as a powerful cellular system to study many diseases, which previously were difficult to investigate [2]. Furthermore, the first clinical trial using human iPSCs started in 2014. However, along with all of this exciting progress, safety concerns have been raised. The most contentious issue is the impact of reprogramming on genomic and epigenomic stability. Although the functional consequence is debatable [3], the presence of genomic abberations in iPSCs cast a shadow over their biomedical use [4]. Genomic instability in iPSCs has been reviewed extensively elsewhere [5], therefore only a few highlights will be mentioned here. This review instead will focus on the latest efforts on understanding the source of genomic abnormalities so they might be reduced during the reprogramming process to generate iPSCs with enhanced quality and safety.

2. Genomic abnormalities in iPSCs and ESCs

Pluripotent stem cells (PSCs), including ESCs and iPSCs, have two essential properties: the capacity to self-renew and the capacity to give rise to all the different cell types within an embryo [6]. Maintaining genomic integrity in PSCs is not only crucial for faithful self-renewal and accurate embryonic development, but also vital for all of their applications, such as disease modeling, drug discovery and regenerative medicine [5].

ESCs are derived from the inner cell mass (ICMs) of a preimplantation embryo. Although the mechanism remains elusive, it is well known that ESCs accumulate genomic alterations during prolonged in vitro culturing [5,7]. These species-specific recurrent genomic abnormalities most likely impose a selective growth advantage, which suggests a suboptimal culturing system is potentially mutagenic. Thus, ESCs are susceptible to genomic instability that can reduce pluripotency.

iPSCs are generated directly from differentiated somatic cells through cellular reprogramming, a stochastic process accompanied by extensive rewiring of the epigenetic landscape and the gene expression network [8]. Cellular reprogramming is considered to be the ultimate proof of the nuclear equivalence theory [9] and the genomic and epigenomic properties of iPSCs have been under the spotlight since their initial discovery. Yet, we still do not

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know if iPScs faithfully mirror ESCs both functionally and molecularly and if they preserve the identical genome as their parental somatic cells. As elegantly enlisted in the review by De Los Angeles et al. [6], the grades of pluripotency and the quality of PSCs are assessed by multiple functional assays ranging from in vitro differentiation and teratoma formation to more stringent assays such as tetraploid complementation and single-cell chimaeras. However, the teratoma assay remains the gold standard for human iPSCs, as the most stringent in vivo methods are restricted to mouse PSCs. Lacking accurate and measurable standards impede the evaluation of human PSCs quality. Besides functional assessment, in 2011, six groups scrutinized the genome of iPSCs and revealed alarming genomic instability in these cells [10–15]. Remarkably, comparisons both to the parental somatic cells and the counterpart ESCs show that iPSCs contain a set of de novo acquired genomic abnormalities, pointing to cellular reprogramming itself as the cause of genomic instability [13].

3. Potential cause of genomic instability in iPSCs

Genomic instability in iPSCs could be generated in several steps [16]. This review will focus on instability generated during the reprogramming process. Although the molecular mechanism is unknown, a few clues have emerged from the growing understanding of cellular reprogramming. To endow changes in original cell identity, successful reprogramming requires reactivation of telomerase to adquire immortality, acquisition of the characteristic cell-cycle signature of PSCs [17], and induction of a metabolic reprogramming from an oxidative to a glycolytic state [8]. Thus, these processes could be mutagenic.

3.1. Reprogramming methods

Yamanaka's landmark paper in 2006 used retrovirus to ectopically express OSKM. There is one obvious threat to the safety of iPSCs by employing this method, as viruses damage DNA when they integrate into the genome. The integration issue was soon overcome by several non-integrative methods [18]. Indeed, the load of genomic aberrations was reduced by the use of a non-integrative system [18,19]. However, many genomic abnormalities remained irrespective of the reprogramming methods [14,18].

3.2. Replication stress (RS)

Cellular reprogramming is a rare, multi-step process, which shares many biological and molecular pathways with tumorigenesis [20]. Firstly, important tumor suppressors, p53 and Ink4a/Arf, serve as a major barrier for cellular reprogramming, most likely through regulation of proliferation, apoptosis and senescence [21,22]. Secondly, each of the four classical factors has been shown to be oncogenic in mice. c-Myc and Klf4 have well established roles in tumorigenesis, and Oct4 is an important initiator for germ cell tumors [20]. Recently Sox2 was identified as an amplified oncogene in human squamous cell carcinomas of the lung and esophagus and small-cell-lung carcinoma [23]. As oncogene activation is a major driver of genomic instability, Pasi et al. questioned the genomic status of iPSCs generated by overexpression OSKM, particularly by c-Myc. By analyzing copy number variations (CNV) in iPSCs generated with either three factors (OSK) or four factors (OSKM), Pasi et al. detected the presence of genomic abnormalities, such as deletions and amplification [10], which were much more prominent when c-Myc is included. In cancer biology, it is speculated that the cascade of oncogene-induced genomic instability is initiated by hyper-replication, which provokes the generation of replication stress (RS) [24]. RS is a type of damage defined by stalled or collapsed replication forks, which usually results in persistent formation of single-stranded DNA (ssDNA). The pan-nuclear phosphorylation pattern of histone H2AX, reminiscent of RS [25], was observed in reprogrammed cells [26]. Noteworthy, the genomic structural variations detected in iPSCs were highly enriched at the fragile sites, a hallmark of RS [10,13,15]. In addition, acquiring iPSCs' unique cell cycle structure during the reprogramming process required increased proliferation [17], which would also generate abundant RS. Recently, Ruiz et al. further observed increased RS levels after OSK induction, by measuring γH2AX expression (indirect marker of RS) and replication fork speed (direct maker of RS) [27], and the RS level was further induced with c-Myc [28]. Taken together, these studies collectively demonstrated that reprogramming factors induce RS, which contributed significantly to the de novo generation of genomic instability in the iPSCs. Moreover, they also highlighted the role of c-Myc in inducing RS and genomic abnormalities. Although c-Myc is a universal amplifier of transcriptional signals and an enhancer of cellular reprogramming processes, it is dispensable for iPSCs generation. Due to its significant impact on genomic stability of iPSCs, omitting c-Myc should become a requirement for generating hiPSCs for clinical applications.

3.3. Reactive oxygen species (ROS) and oxidative stress

ROS are the natural by-products of the mitochondrial respiratory chain, which increase dramatically upon environmental stress. If they cannot be removed efficiently by the radical-scavenging system, excess ROS will cause oxidative stress and damage macromolecules like DNA and protein [29]. It is well known that ESCs have less and also immature mitochondria compared to differentiated cells [30], due to the hypoxic environment in the ICM, which corresponds to their distinct metabolic requirement [31]. Upon cellular reprogramming, cells undergo a metabolic shift from an oxidative to a glycolytic state as iPSCs' mitochondria reset back to an ESCs stage [8,31,32]. However, during the cellular reprogramming process, progressively reduced mitochondria activity cannot cope with the increased energy demand imposed by accelerated proliferation, which increases ROS production. Indeed, multiple studies detected elevated levels of oxidative stress and DNA damage highlighting the metabolic imbalance during reprogramming [33,34]. Noteworthy, hypoxic culture conditions (3–5% O_2) are known to reduce oxidative stress, restrain the accumulation of DNA mutations, prevent differentiation and promote survival of multiple cell types, including PSCs [35,36]. Interestingly, hypoxia was shown to enhance the generation of iPSCs, most likely by accelerating the metabolic switch required for acquisition of pluripotency [37,38]. However, it is unknown whether hypoxia could enhance the quality of iPSCs by protecting cells from oxidative stress and DNA damage during reprogramming.

3.4. Telomere maintenance

The telomere is a distinct structure consisting of repetitive DNA sequences found at the end of every chromosome. It protects chromosome ends from degradation and fusion. Due to the "end replication problem", telomere would shorten with every cell division. Telomerase is the enzyme responsible for telomere elongation, which is exclusively expressed in stem cells (including PSCs and adult stem cells) and reactivated in cancer cells. Telomere maintenance is not only important for genomic stability but also critical for cancer and ageing [39]. There are two differences between PSCs and differentiated cells in regard to telomere biology: telomere length and telomerase activity. It has been shown that telomerase is reactivated during reprogramming and both the length and epigenetic status of the telomere is rejuvenated in iPSCs similar to those found in ESCs [40]. Importantly, short telomeres