Cell Cycle Regulation in Pluripotent Stem Cells

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Pluripotent stem cells (PSCs) hold great promise in cell-based therapy because of their pluripotent property and the ability to proliferate indefinitely. Embryonic stem cells (ESCs) derived from inner cell mass (ICM) possess unique cell cycle control with shortened G1 phase. In addition, ESCs have high expression of homologous recombination (HR)-related proteins, which repair double-strand breaks (DSBs) through HR or the non-homologous end joining (NHEJ) pathway.

Keywords: DNA damage ; cell cycle regulation ; pluripotent stem cells (PSCs)

1. Introduction

Embryonic stem cells (ESCs) of mice [1] or humans [2] are derived from the ICM of blastocysts. They hold great promise in cell-based therapy because of their pluripotent nature and the ability to proliferate indefinitely. Since the advent of ESCs decades ago, a massive number of protocols were developed to drive them into different germ layers (endoderm, mesoderm and ectoderm) through manipulation of cell signaling pathways. For instance, we [3] and other research groups [4][5] have reported the differentiation of human ESCs (hESCs) into pancreatic lineages. Extensive work was also done to drive hESCs into trophoblast [6][7] and germ cell [8] lineages. Therefore, ESC is an excellent in-vitro model for understanding early developmental events and their interactions with external stimuli [3][9]. Notwithstanding the potential applications of ESCs in regenerative medicine, tissue rejection in recipients could be a main concern. In 2006, Yamanaka and his team reported the generation of induced pluripotent stem cells (iPSCs) from mouse embryonic and adult fibroblasts through induction of transcription factors (Oct4, Sox2, Klf4 and c-Myc) [10]. A year later, they also reported the generation of iPSCs from human fibroblasts [11]. As patient-specific iPSCs can be derived, these ground-breaking iPSCs solved the major hurdle of tissue rejection in cell-based therapy.

Understanding the biological characteristics of pluripotent stem cells (PSCs) is imperative for exploring their potential applications. In fact, both ESCs and iPSCs can proliferate rapidly. The doubling time of PSCs ranges from 8–10 h, which is much faster than that in somatic cells (around 20 h) [11][12][13]. Interestingly, the fast cell division is reminiscent of early embryonic cells, with doubling time of 5–10 h before implantation [14]. Studies suggested that the rapid proliferation of PSCs was due to their unique cell cycle regulations. For instance, PSCs have a shortened G1 phase as a result of low expression of G1 phase-specific cyclin D and cyclin-dependent kinases (CDK) 4 and 6. The interplay between cyclins, CDK and cyclin-dependent kinase inhibitors (CDKN) is important for tight regulation of cell cycle progression [15][16]. Cell cycle regulation is highly related to pluripotency in PSCs, as the master pluripotent marker Oct4 controls the progression of cell cycle [17]. In addition, the cell cycle regulators have important functions in DNA damage response (DDR) of PSCs [18]. Replication stress and DNA damage were induced by the shortened G1 phase in mouse ESCs (mESCs) [19]. More importantly, the expression levels of DNA damage marker γH2AX increased during the reprogramming process into iPSCs [20]. Thus, the DDR system in PSCs is essential for minimizing the accumulation of DNA mutations and maintaining genomic integrity in the highly proliferating cells [21].

2. Cell Cycle Regulation in Pluripotent Stem Cells (PSCs)

The mitotic cell division is the most fundamental process for all cell types. It is tightly regulated by the activation and deactivation of cyclin-dependent kinases (CDK) and the oscillatory expression of cyclins at different stages of the cell cycle. The canonical cell cycle in somatic cells consists of a DNA synthesis phase (S phase) and a cell division phase (M phase). G1 (between M and S phase) and G2 (between S and M phase) are two gap phases in between S and M phases. In each cell cycle, cyclin D and its CDK partner CDK4/6 are highly expressed in the G1 phase. Cyclin E and its partner CDK2 are predominantly active between the late G1 and S phase, while cyclin A/E with its partner CDK2 are mainly active between the S and G2 phase. Lastly, cyclin B and its partner CDK1 mainly regulate the G2 and M phase [22]. The oscillatory appearance of cyclins and CDKs are important for ensuring the correct sequences of DNA synthesis prior to

cell division, thus controlling genomic integrity. Early studies in mouse blastomeres showed that their cell cycle closely resembled that of the canonical model. Later, it was found that the mouse embryonic cells have rapid cell divisions with doubling time of 5–10 h, owing to the shortened and truncated G1 and G2 phases [23].

The fast cell cycle of mESCs is accompanied by a shortened G1 phase. Interestingly, the oscillatory expression and activities of the cyclin–CDK complexes in mESCs are quite different from that in somatic cells [24][25]. First of all, since the G1 phase is significantly shortened, the cyclin D (i.e., cyclin D1 and D3) is expressed at low levels. Throughout the cell cycle, Cdk6 becomes the predominant partner at the G1 phase with an oscillatory expression pattern. On the other hand, cyclin A/E and their partner Cdk2 are expressed at high levels in mESCs. The high expression is cell cycle independent and without oscillation. Lastly, the expression patterns of the mitotic cyclin B and its partner Cdk1 are similar to those in somatic cells; they have oscillatory expression throughout the cell cycle and are only expressed highly in the G2/M phase [24][25].

In addition to the tight regulations of the expression of the cyclin–CDK complexes, another key cell cycle regulator is the retinoblastoma protein (RB). In somatic cells, RB is unphosphorylated and active in the G1 phase. The active RB couples with E2F and binds to the promoters of target genes expressed in the G1/S phase, such as components of the cyclin A/E-Cdk2 complex. The binding of E2F at the promoters leads to histone deacetylation and repression of gene expression. When the cells are ready to enter the S phase, the RB is phosphorylated and does not repress the cyclin A/E–Cdk2 complex when entering the S phase [26]. The hypo-phosphorylation of RB at the G1 phase and the hyper-phosphorylation of RB at the S phase add another level of cell cycle checkpoint to ensure proper cell cycle progression. Interestingly, the inactive hyper-phosphorylated RB is found in mESCs throughout different phases of the cell cycle. The reason is due to the high expression and activities of the cyclin A/E–Cdk2 complex, which lead to phosphorylation of RB [27].

Human ESCs, though derived from the ICM of human blastocysts, are considered as at a primed state similar to the mouse epiblast stem cells (mEpiSCs). On the other hand, mESCs are generally defined as at a naïve state [28]. It is therefore reasonable that there are major differences in cell cycle regulation between mESCs and hESCs. Indeed, hESCs have longer and functional G1 phase compared to mESCs. Therefore, the Cyclin Ds (i.e. Cyclin D1, D2 and D3) have intermediate expression in the G1 phase in hESCs, though their expressions are still lower than that in somatic cells. Unlike the mESCs, the expression of Cyclin D partner CDK4 is higher than CDK6 in hESCs [13, 15]. Another major difference between mESCs and hESCs is that the expression pattern of Cyclin A/E-CDK2 complex is cell-cycle dependent in hESCs but it is constant in mESCs. Moreover, the functional RB checkpoint at G1 phase guarding the correct entry into S phase occurs in hESCs but not in mESCs [15]. On the other hand, there is similarity between mESCs and hESCs; the oscillatory patterns with high expressions of the mitotic related Cyclin B and its partner CDK1 are observed at the G2/M phase.

3. DNA Damage Response and Cell Cycle Regulation

Apart from the cyclin–CDK complexes and the RB protein, the cyclin-dependent kinase inhibitors (CDKN) are also important for cell cycle progression. Generally, there are two major groups of CDKNs, including the CDK interacting protein/kinase inhibitory protein (CIP/KIP) and the inhibitors of the CDK4/alternate reading frame (INK4/ARF) family. The CIP/KIP family consists of subunits *P21*(*CIP1*), *P27*(*KIP1*) and *P57*(*KIP2*), while the INK4/ARF family consists of *P16*(*INK4A*), *P15*(*INK4B*), *P18*(*INK4C*) and *P19*(*INK4D/ARF*) [25]. CDKNs inhibit the activities of cyclins and CDKs. As the levels of CDKNs in PSCs are low [27], the expression of cyclin–CDK complexes can be maintained at high levels for fast cell cycle progression. A study even suggested that some CDKNs might not be functional in mESCs, as the cyclin D3-Cdk6 expression was not affected by overexpression of its upstream CDKN regulator *p16* [28].

The CDKNs not only act as cell cycle checkpoints but also are heavily linked to DNA damage response. For instance, *P53* is a tumor suppressor gene and responsible for regulating genome stability. In response to DNA double-strand breaks (DSB), *P53* can directly activate the ataxia-telangiectasia mutated (ATM) kinase through phosphorylation. The ATM kinase is then recruited to the site of DNA damage for repairing, either through homologous recombination (HR) or non-homologous end joining (NHEJ) [29]. More importantly, *P53* also activates *P21* , which in turn inhibits cyclin A/E–CDK2 activity, leading to blockage of G1/S phase entry [30]. In PSCs, *P53* is activated during DNA damage. The induction of P53 in turn suppresses pluripotent marker (*OCT4* and *NANOG*) expression in mESCs and hESCs, leading to their differentiation [31].

A precise DNA repair system is imperative in PSCs. The system allows the cells to cope with DNA lesions and maintain their genomic integrity during rapid cell cycle progression. In PSCs, the system is tightly controlled by DNA damage response (DDR) signaling. Generally, PSCs respond to DNA damage or lesions through DDR, leading to cell cycle arrest

and increased expression of DNA repair genes [32]. Cell cycle arrest at either the G1/S or the G2/M-phase allows incorporation of different DNA repair mechanisms, including mismatch repair (MMR), base excision repair (BER), nucleotide excision repair (NER), HR and NHEJ.

The PSCs have a short G1 phase that can help to minimize the induction of differentiation-related signaling. Therefore, some of the DNA repair mechanisms occurring at the G1 phase checkpoint in somatic cells are bypassed in PSCs. The prolonged S phase makes the PSCs utilize HR preferentially over other DNA repair pathways during DDR [33]. The genes involved in HR, including *RAD51* and *RAD52* , are highly expressed in the S phase of DNA repair. In addition to these genes, the MRE11- RAD50-NBS1 (MRN) complex is also involved in DNA repair through HR and NHEJ. They serve as a DNA damage sensor and generate single stranded DNA regions that activate the checkpoint responses. Following the activation, the checkpoint transmits and amplifies the signal to downstream targets such as the cyclin–CDK complex and other DNA repair-related genes [34][35].

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