Abstract. Embryonic stem cells (ESCs) have unlimited expansion potential and the ability to differentiate into all somatic cell types for regenerative medicine and disease model studies. Octamer-binding transcription factor 4 (OCT4), encoded by the POU domain, class 5, transcription factor 1 gene, is a transcription factor vital for maintaining ESC pluripotency and somatic reprogramming. Many studies have established that the cell cycle of ESCs is featured with an abbreviated G1 phase and a prolonged S phase. Changes in cell cycle dynamics are intimately associated with the state of ESC pluripotency, and manipulating cell-cycle regulators could enable a controlled differentiation of ESCs. The present review focused primarily on the emerging roles of OCT4 in coordinating the cell cycle progression, the maintenance of pluripotency and the glycolytic metabolism in ESCs.

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1. Introduction

Embryonic stem cells (ESCs) are characterized by unlimited proliferation (self-renewal) and the ability to differentiate into three primary germ layers, namely the endoderm, mesoderm and ectoderm (pluripotency) (1-4). It has been established that complicated regulatory networks are present in ESCs that critically maintain the state of self-renewal and pluripotency for later development (5,6). Several transcription factors (TFs), including octamer-binding transcription factor 4 (OCT4), SRY-box 2 (SOX2) and homeobox protein NANOG (NANOG) are known to sit at the top of the regulatory hierarchy, regulating the expression of various downstream target genes (7,8). Among them, OCT4 serves an indispensable role in maintaining the pluripotency of ESCs (9,10) and in reprogramming the terminally-differentiated somatic cells back into the ESC-like cells (11-13). Furthermore, OCT4 can mediate the differentiation of murine ESCs induced by retinoic acid or Wnt/β-catenin in a manner that is independent of and distinct from other core TFs (14), indicating that OCT4 may have unique and non-substitutable roles in controlling the self-renewal, pluripotency and differentiation of ESCs.

Cell cycle progression is required for ESCs to proliferate and avoid staying in a quiescent state. Multiple studies have demonstrated that cell cycle-associated proteins can regulate various core TFs or differentiation markers (15). In a reciprocal manner, several TFs, such as NANOG and c-MYC proto-oncogene protein, can control the expression levels of multiple cell cycle-associated target genes (16,17). This review will be focused on reciprocal interplays between OCT4 and cell cycle checkpoints and their connections with the ESC pluripotency.

2. Cell cycle and pluripotency in ESCs

Cell cycle comprises four different phases; the S phase for DNA replication, the M phase for cell mitosis, and two gap phases between S phase and M phase (G1 phase for synthesis of proteins and lipids, and G2 phase for checking DNA integrity). Ample evidence has revealed that the duration of cell cycle in murine somatic cells is relatively long (>16 h), which is dominated by the G1 phase (18); in contrast, the cell
cycle of murine ESCs progresses faster (~8-10 h) (19), which is characterized by a truncated GI phase and a prolonged S phase (20). Although the duration of cell cycle in human ESCs is significantly lengthened (~32-38 h) (21), the time spent at GI phase is minimal (3 h in human ESCs vs. 10 h in human somatic cells) (15,22), indicating that the cell cycle dynamics may crucially impact on the differentiation potential of pluripotent stem cells. Indeed, ~1-5% of the total proteins differ their expression levels between ESCs and induced pluripotent (iPS) cells, and the majority of them are cell cycle proteins (23).

There is mounting evidence demonstrating that lengthening the GI phase in ESCs contributes to inducing differentiation (24-27), and distinct GI phase profiles will lead to different lineage fates. Human ESCs in early GI phase can only differentiate into endoderm, whereas in late GI phase they were limited to neuroectodermal differentiation (28). In fact, all-trans retinoic acid, a common differentiation inducer, can regulate the gene expression of Cyclin D1 (29,30) and result in GI phase accumulation (31-33). It is therefore reasonable to propose that during the GI phase, ESCs sense and integrate various extracellular and intracellular signals to make the decisions on the timing and the fate of differentiation. A shortened GI phase may minimize the exposure of ESCs to various signals, thereby preserving their pluripotency. In addition, it was demonstrated in a recent study that G2 cell cycle arrest is also required for endodermal development (34); furthermore, specific disruption of S and G2 phases will affect the pluripotent state of human ESCs in a GI phase-independent way (35-37). Gamma-ray-induced DNA damage induces G2/M blockage and the differentiation of ESCs (38,39). It is important that ESCs have a long enough G2 phase to check and restore the fidelity of the genome as a result of GI/S checkpoint deficiency.

3. OCT4 and GI/S transition

The expression of Cyclin-dependent kinase 4/6 (CDK4/6) and Cyclin D is increased in early GI phase in somatic cells. Although the lack of Cyclin D expression was reported in murine ESCs (40), the mRNA levels of CDK4 and Cyclin D2 were increased in human ESCs (22,41). Further studies demonstrated that Cyclin D expression is enhanced in late GI and G1/S phases in human ESCs. Notably, knocking down Cyclin D induces endodermal differentiation, whereas its overexpression promoted neuroectodermal differentiation by inhibiting mothers against decapentaplegic (SMAD) 2/3 nuclear translocation (28). In addition, Cyclin D can also recruit transcriptional co-regulators to development-associated gene loci and modify the epigenetics of target genes (42). There is evidence demonstrating that a proper level of Cyclin D is necessary for maintaining the pluripotent state of ESCs, while overexpression of them may induce reprogramming of epidermal cells into stem-like cells with higher expression levels of OCT4 and NANOG (43). In contrast, in adult stem cells or cancer cells, OCT4 can directly bind to the promoter region of Cyclin D1, thereby regulating its transcription and controlling G1/S transition (44-46). Meanwhile, OCT4 can bind with the conserved promoter of microRNA (miR)-302 (47), increasing the level of p16(Ink4a)/p19(Ink4d) and inhibiting the interaction between CDK4/6 and Cyclin D (48). Furthermore, OCT4 can also interact with SMAD2/3 to control the pluripotent state of ESCs (49,50). Taken together, these studies suggested that OCT4 is involved in the transcriptional regulation of Cyclin D as well as other target genes (Fig. 1).

CDK2-Cyclin E is constitutively expressed and involved in the progression of GI/S transition (26). In human ESCs, inhibition of CDK2 will lead to G1 phase arrest, which is accompanied with apoptosis or differentiation. Inhibition of CDK2 can induce sustained genomic damage and elicit DNA damage response, thus contributing to apoptosis of impaired ESCs (51,52). As demonstrated in further studies, OCT4 expression can be suppressed by downregulating CDK2 (53,54), while CDK2 can enhance reprogramming efficiency by phosphorylating SOX2 at Ser-39 and Ser-253 sites (55). Although the regulation of CDK2-Cyclin A/E by OCT4 in ESCs has not been reported, OCT4 can promote tumor proliferation by activating Cyclin E (56). Thus, it remains possible that OCT4 may regulate the expression of CDK2-Cyclin A/E in ESCs.

Retinoblastoma (RB) protein is a downstream target of CDK4/6-Cyclin D, which can inhibit the transcription activity of E2F transcription factor 1 (E2F) in its hypophosphorylated state. After being hyperphosphorylated by CDK2-Cyclin E, RB can release E2F for the ultimate regulation of a number of targets involved in GI phase progression and S phase entry (Fig. 1). Therefore, it came as no surprise that the activity of RB-E2F can influence the ESC self-renewal and pluripotency (57,58). In fact, activated RB can directly bind to the promoter regions of OCT4 and SOX2, leading to their transcriptional suppression and a declined reprogramming efficiency (59); in contrast, the inactive RB allows for generation of iPSCs in the absence of exogenous SOX2 expression (60). Furthermore, RB can also regulate OCT4 level by suppressing the expression of forkhead box protein M1, which is a transcription factor promoting OCT4 expression (61,62). In addition, E2F will switch from an active state in stem cells to a suppressed state in differentiated cells through forming a complex with RB (63). Conversely, in murine ESCs, OCT4 maintains the hypophosphorylated state of RB by inhibiting the activity of protein phosphatase 1 (64), which is well-known for its role in triggering mitotic exit (65). Additionally, OCT4 can also directly bind to the promoter region of E2f3a and increase its expression level in murine ESCs, which contributes to relieving the cell growth retardation caused by OCT4 knockdown (66). As inhibition of E2F2 can impair self-renewal and cell cycle progression in human ESCs, the pluripotency is preserved in E2F2 silencing cells (67). Therefore, the effects of RB on the pluripotency of ESCs are unlikely mediated by E2F. The other roles of RB in ESCs will be discussed later.

4. OCT4 and G2/M transition

In somatic cells, CDK1-Cyclin A/B is a critical cell cycle regulator that can promote G2/M transition. As has been demonstrated in multiple studies, CDK1-Cyclins serve critical roles in the self-renewal and development of ESCs. The expression level of Cyclin A, the first cloned Cyclins protein, is higher in ESCs in G2 phase than that in fibroblast cells (68), and resetting its expression level in early-passage iPSCs cells can improve the pluripotency and reduce the tumorigenicity (23). In addition, the Cyclin B1 level is also upregulated in ESCs...
in G2 phase compared with that in somatic cells. Increased expression of Cyclin B1 in G2 phase can delay the dissolution of pluripotent state in human ESCs, while knockdown of Cyclin B1 induces markedly declined expression of pluripotent markers in human ESCs (36). The same is true for CDK1. In human ESCs, down-regulating CDK1 leads to loss of pluripotency, increased differentiation markers, accumulation of double-strand breaks, as well as the inability to arrest at G2 phase and commit to apoptosis (69,70). CDK1 can enhance the binding of OCT4 to the promoter and suppress the transcription of homeobox protein CDX2, a classic differentiation marker (71). Furthermore, several markers of G2/M are expressed during the meso- and endodermal differentiation (e.g., WEE1 G2 checkpoint kinase blocks entry into mitosis by phosphorylating CDK1 at Y15), rather than the ectodermal differentiation (34). In contrast, OCT4 can inhibit the activation of CDK1 by cell division cycle 25 phosphorylation, which is independent of its transcriptional activity (Fig. 2). Thus, ESCs have to express more CDK1 to overcome the inhibitory effect of OCT4. Inhibition of CDK1 by OCT4 will lead to a prolonged duration of G2 phase, which allows for subsequent checking of genome integrity and reducing chromosomal mis-segregation (72). Indeed, inhibition of CDK1 can activate the response to DNA damage and promote nuclear translocation and activation of p53, thereby maintaining the survival of ESCs (73). The potential connection between OCT4 and Cyclin A/B has not been elucidated in any study yet, but there is evidence that SOX2, a core TF frequently associated with OCT4, can promote the expression of Cyclin A/B in cancer cells (74-76). The direct regulation of CDK1-Cyclin by OCT4 warrants further investigation.

Growth arrest and DNA-damage-inducible protein 45 (GADD45), which includes several isoforms, is crucial for protecting genome stability in G2/M transition by suppressing cell cycle and repairing DNA. GADD45ag morpholino knockdown in Xenopus can induce differentiation of neural embryonic cells by inducing various cell cycle related inhibitors, such as p53, p21 and Cyclin G1. Additionally, GADD45ag morphants exhibit increased expression of Xenopus OCT4 homologs, indicating that GADD45ag is required for early embryonic cells to exit pluripotency and enter differentiation (77). In addition, GADD45a can bind to the OCT4 promoter and promote its demethylation in Xenopus oocytes, which is accompanied with DNA repair (78,79). Furthermore, studies in human cells indicated that GADD45 G is a downstream target of OCT4, which is significantly increased in the OCT4 knockdown system (80,81).

As discussed above, RB is a tumor-suppressor gene controlling the activity of transcription factor of E2F family, which serves an indispensable role in G1/S transition. Increased activity of RB can trigger cell cycle arrest, differentiation or death of ESCs (82). However, the inactivation of RB family in ESCs can also induce G2/M arrest and cell death (57), which may be attributed to the loss of its function in maintaining the genetic stability (83-85). These findings indicated that the expression level of RB needs to be tightly controlled at a
proper level, so that the pluripotency and self-renewal of ESCs can be maintained. Furthermore, overexpression of RB in S phase can lead to G2 phase arrest (86). Additionally, RB can directly bind to cohesin and condensin II, which can regulate centromere functions and control mitosis (87-91).

5. OCT4 and p53-p21 checkpoints

The p53-p21 signaling pathway is a major checkpoint in cell cycle of G1/S and G2/M transition. The expression level of p53 is kept low in ESCs, which is predominantly present in the cytoplasm. The extremely low level of p53 in the nucleus is also inactivated. p53 will translocate to cell nucleus and initiate the transcription of its target genes in the event of DNA damage (92). In addition, p53 can promote the translocation of active Bcl-2-associated X protein from the Golgi to mitochondria to initiate apoptosis under DNA damage stresses (93). It is demonstrated that p53 deficiency will lead to genomic instability in ESCs (94). In contrast, the activated p53 in ESCs will result in differentiation (31,95,96) or apoptosis (73,97). However, it has also been demonstrated in other studies that p53 has anti-differentiation effects in ESCs (98), indicating that p53 exerts its functions in a context-dependent manner, and that proper intracellular levels and subcellular localization of p53 are critical for its roles in maintaining the pluripotent state in ESCs.

In addition, p53 can regulate the expression of various key TFs in ESCs. For example, knockdown of p53 can lead to downregulated NANOG expression (99). As a common differentiation inducer of ESCs, p53 expression is activated after exposure to retinoic acid, which drives the expression of miR-34a and miR-145 and reduces the OCT4 expres-

sion (31). In addition, the differentiation-activated p53 can recruit UTX and lysine-specific demethylase 6B (JMJD3), the H3K27me3-specific demethylases, bind to the promoter regions of developmental transcription factors that are repressed by OCT4, and increase the expression of various differentiation genes (100). p53 is also the downstream target of OCT4 (Fig. 2). Studies have revealed that silencing OCT4 will lead to p53 activation and induce differentiation (101-103). For instance, silencing OCT4 significantly reduces the expression of SIRT1, a deacetylase known to inhibit p53 activity and the differentiation of ESCs, leading to increased acetylation of p53 at lysine 120 and 164 that is required for its stabilization and functionality (104). In addition, OCT4 can bind to the promoter region of CD49f (integrin subunit α6), which can also decrease the level of p53 (105).

p21, a downstream target of p53, can inhibit the activation of CDKs and result in cell cycle arrest (Fig. 2); in addition, it can also be regulated in a p53-independent way. It has been revealed in studies that p21 is involved in DNA repair, transcriptional regulation, differentiation and apoptosis. In ESCs, the expression level of p21 is compromised due to epigenetic modification (106), and the lack of p21 function is required for maintaining the pluripotent state (107). Ionizing radiation-induced DNA damage can lead to elevated p21 mRNA level and cell cycle arrest at G2 phase (108). Upregulation of p21 in human ESCs will induce G1 phase arrest and
subsequent differentiation into multiple lineages (109). This result is consistent with the finding that p21 has multiple fuctions in both G1/S and G2/M checkpoints (110,111). p21 can also mediate apoptosis in murine ESCs that are exposed to dihydroliopoic acid (112). In addition, increased p21 expression leads to decreased reprogramming efficiency in somatic cells (113). Conversely, OCT4 can inhibit the activity of p21 by directly binding to its promoter region or by indirectly up-regulating DNA (cytosine-5)-methyltransferase 1, a DNA methyltransferase, which can inhibit lineage differentiation (114-116).

6. OCT4 and ESC metabolism

A large amount of energy is generated in ESCs to meet the requirements for biosynthesis and cell cycle progression. The energy metabolism mode of primed ESCs is similar to that of other adult stem cells or cancer cells with a high glycolytic flux rather than oxidative phosphorylation (OXPHOS), which is known as the ‘Warburg effect’ (117-121). This phenomenon can be partly attributed to the immature structure and function of mitochondria and a hypoxic niche (5% of physiological level) (122,123). Though glycolysis produces less ATPs than OXPHOS, it has faster rate of ATP generation, which makes it competent to support active cell proliferation. Additionally, pyruvate, the product of glycolysis, together with other intermediate products of tricarboxylic acid (TCA) cycle, can be used for biosynthesis (such as DNA, protein and lipid) in ESCs as well as in cancer cells for shortening the G1 phase (123-125).

A high glycolytic flux metabolism in hypoxia may reduce the damages to DNA caused by reactive oxide species (ROS), which may impair the pluripotency ESCs and induce their differentiation (126,127).

Initial evidence indicated OCT4 may be involved in regulating metabolism as its knockdown resulted in increases in TCA cycle activity and decreases in glycolytic flux (117). Further studies demonstrated that OCT4 can directly regulate the transcription of hexokinase 2 (HK2) and pyruvate kinase (PK) M2, the two key glycolytic enzymes that determine the rate of glycolysis. Overexpression of HK2 and PKM2 contributes to sustaining the high glycolysis level and preserving the pluripotency of ESCs (128). Notably, PKM2 can directly bind to OCT4 and enhance OCT4-mediated transcription (129,130).

7. Conclusion

It has been known for a while that ESCs are characterized by an abbreviated G1 phase and a prolonged S phase. However, the underlying mechanisms remain largely elusive. Emerging evidence has implicated a direct role of the master pluripotency factor OCT4 in controlling the transcription of several key cell cycle regulators. In general, OCT4 appears to directly or indirectly activate the transcription of cell cycle machineries that promote G1/S transition and avoid differentiation (Fig. 1). Meanwhile, by suppressing multiple cell cycle genes, OCT4 controls proper duration of G2 phase to ensure the genomic integrity via both the transcription-dependent and -independent mechanisms (Fig. 2). Reciprocally, the cell cycle regulators especially CDK1 can directly interact with OCT4 and promote its suppressive binding to the differentiation genes and thereby maintaining the ESC pluripotency.

Another important feature of ESCs is their high glycolytic metabolism under hypoxic conditions that may minimize the oxidative damage of ROS to genetic material. Recent studies revealed that OCT4 can promote glycolysis by transcriptionally upregulating the expression of several key glycolytic enzymes, directly linking ESC metabolism to their self-renewal and pluripotency. Given the convergence of ESC pluripotency and cell cycle control on OCT4, it would be of interest to investigate in future studies how OCT4 and other master pluripotency factors coordinate ESC metabolism with their cell cycle progression.

The rapid cell cycle progression of ESCs requires high-fidelity DNA replication and repair mechanisms. The investigation into the potential connection between ESC cell cycle control and DNA replication/repair is just at its infancy, and it remains to be seen if the master pluripotency factors such as OCT4 may also serve a role in these events.

Acknowledgements

The present review was supported by the National Key Research and Development Program of China (grant no. 2016YFA0100303) and the National Natural Science Foundation of China (grant no. 31601103).

References

1. Wu J and Izpisua Belmonte JC: Dynamic pluripotent stem cell states and their applications. Cell Stem Cell 17: 509-525, 2015.
2. Smith AG: Embryo-derived stem cells: Of mice and men. Annu Rev Cell Dev Biol 17: 435-462, 2001.
3. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS and Jones JM: Embryonic stem cell lines derived from human blastocysts. Science 282: 1145-1147, 1998.
4. Martin GR: Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci USA 78: 7634-7638, 1981.
5. Yousfi M, Hajhoseini V, Jung W, Hosseinpour B, Rassoulli H, Lee B, Baharvand H, Lee K and Salekdeh GH: Embryonic stem cell interactivations: The beginning of a long road to biological function. Stem Cell Rev 8: 1138-1154, 2012.
6. Wang L and Chen YQ: Signaling control of differentiation of embryonic stem cells toward mesendoderm. J Mol Biol 428: 1409-1422, 2016.
7. Martello G and Smith A: The nature of embryonic stem cells. Annu Rev Cell Dev Biol 30: 647-675, 2014.
8. Das S and Levasseur D: Transcriptional regulatory mechanisms that govern embryonic stem cell fate. Methods Mol Biol 1029: 191-203, 2013.
9. Nichols J, Zevnik B, Anastassiadi K, Niwa H, Klewe-Nebelius D, Chambers I, Schöler H and Smith A: Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell 95: 379-391, 1998.
10. Niwa H, Miyazaki J and Smith AG: Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat Genet 24: 372-376, 2000.
11. Takahashi K and Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126: 663-676, 2006.
12. Wu T, Wang H, He J, Kang L, Jiang Y, Liu J, Zhang Y, Kou Z, Liu L, Zhang X and Gao S: Reprogramming of trophoblast stem cells into pluripotent stem cells by Oct4. Stem Cells 29: 755-763, 2011.
13. Tsai SY, Bouwman BA, Ang YS, Kim SJ, Lee DF, Lemischka IR and Rendl M: Single transcription factor reprogramming of hair follicle dermal papilla cells to induced pluripotent stem cells. Stem Cells 29: 964-971, 2011.
Liu YY, Tachiki KH and Brent GA: A targeted thyroid hormone receptor antagonist dominant-negative mutation (P989H) selectively inhibits gene expression in differentiated embryonic stem cells. Endocrinology 143: 2664-2672, 2002.

Van Oudenhove JJ, Grandy RA, Ghule PN, Del Rio R, Lian JB, Stein JL, Zaidi SK and Stein GS: Lineage-specific early differentiation of human embryonic stem cells requires a G2 cell cycle pause. Stem Cells 34: 1765-1775, 2016.

Gonzales KA and Liang H: Transcriptomic profiling of human embryonic stem cells upon cell cycle manipulation during pluripotent state dissolution. Genom Data 6: 118-119, 2015.

Gonzales KA, Liang H, Lim YS, Yan YS, Yeo JC, Tan CP, Gao B, Le B, Tan ZY, Low KJ, et al.: Deterministic restriction on pluripotent state dissolution by cell-cycle pathways. Cell 162: 564-579, 2015.

Islam MS, Stemig ME, Takahashi Y and Hui SK: Radiation response of mesenchymal stem cells derived from bone marrow and human pluripotent stem cells. J Radiat Res 56: 269-277, 2015.

Rebuzzini P, Pignolosa D, Mazzini G, Di Liberto R, Coppola A, Terranova N, Magi R, Redi CA, Zuccotti M and Garagna S: Mouse embryonic stem cells that survive γ-rays exposure maintain pluripotent differentiation potential and genome stability. J Cell Physiol 227: 1242-1249, 2012.

Rebuzzini P, Fassina L, Mulas F, Bellazzi R, Redi CA, Di Fronzo R, Magi R, Redi CA, Zuccotti M and Garagna S: Mouse embryonic stem cells irradiated with γ-rays differentiate into cardiomyocytes but with altered contractile properties. Mutat Res 756: 37-45, 2013.

Fluckiger AC, Marcy G, Marchand M, Négre D, Cosset FL, Mitalipov S, Wolf D, Saviatier P and Dehays C: Cell cycle features in long-term human embryonic stem cell lines. Stem Cells 24: 547-556, 2006.

Becker KA, Stein JL, Lian JB, van Wijnen AJ and Stein GS: Establishment of histone gene regulation and cell cycle checkpoint control in human embryonic stem cells. J Cell Physiol 210: 517-526, 2007.

Ko J, Madrigal P, Bertero A and Vallier L: Initiation of stem cell differentiation involves cell cycle-dependent regulation of developmental genes by Cyclin D. Genes Dev 30: 421-433, 2016.

Zhang X, Neganova I, Przyborski S, Yang C, Cooke M, Zglinicki T, O’Connor JE, Burks D, Jones R, Armstrong L and Jones R, Armstrong L and Jones R, Armstrong L and Jones R: Developmental activation of the Rb-E2F pathway and establishment of naïve embryonic stem cell pluripotency. Stem Cell Res 1: 181-191, 2013.

De Luca C, Raya A, Consiglio A, Edel MJ: The cell-cycle state of stem cells determines cell fate propensity. Cell 155: 135-147, 2013.

Han SM, Han SH, Coh YR, Jiang G, Chan Ra J, Kang SK, Lee HW and Youn HY: Enhanced proliferation and differentiation of Oct4- and Sox2-overexpressing human embryonic stem cells. Exp Mol Med 46: 10014.

Li P, Ma X, Adams JR and Yuan P: A tight control of Rip1 by Oct4 and Smad3 is critical for mouse embryonic stem cell stability. Cell Death Dis 6: e1588, 2015.

Gonzana N, Villeva F, Atkinson SP, Lloret M, Passos JF, von Kaurianen M, Hennings C, Hennings C, Hennings C and Hennings C: Transcriptome profiling of human embryonic stem cells upon cell cycle manipulation during pluripotent state dissolution. Genom Data 6: 118-119, 2015.
differentiation in the respiratory epithelium
Bruno MD and Whitsett JA:
Tompkins DH, Besnard V, Lange AW, Lee SH, Oh SY, Do SI, Goga A:
Huskey NE, Guo T, Evason KJ, Li Z, Xiao W and Zhang W:
Li L, Wang J, Hou J, Zmoos AF, Cecchinii MJ, Spache DB, Latfa LF, O’Brien M, et al: Inhibition of pluripotency networks by the Rb tumor suppressor restricts reprogramming and tumorigenesis. Cell Stem Cell 16: 39-50, 2015.

Vilas JM, Ferreirós A, Carneiro C, Morey L, Da Silva-Alváez S, Fernandes T, Abad M, Di Croce L, García-Caballero T, Serrano M, et al: Transcriptional regulation of Sox2 by the retinoblastoma family of pocket proteins. Oncotarget 6: 4092-4102, 2015.

Kelleher FC and O'Sullivan H: FOXM1 in sarcoma: Role in cell cycle, pluripotency genes and stem cell pathways. Oncotarget 2992-3002, 2015.

Schoeftner S, Scarola M, Comisso E, Schneider C and Benetti R: E2f1-3 switch from activators in progenitor cells to repressors in differentiating cells. Cell Stem Cell 4: 374-378, 2009.

Walter RA and Alves J: Transcription factor FOXM1c is repressed by Rb and activated by cyclin D1/Cdk4. Biol Chem 387: 949-962, 2006.

Chong JL, Wenzel PL, Sáenz-Robles MT, Nair V, Ferrey A, Hagan JP, Gomez YM, Sharma N, Chen HZ, Osseph M, et al: E2F1-3 switch from activators in progenitor cells to repressors in differentiating cells. Nature 462: 930-934, 2009.

Schoefnfer S, Scaroila M, Comissio E, Schneider C and Benetti R: An Oct4-4rb axis, controlled by Mrir35, integrates stem cell self-renewal and cell cycle control. Stem Cells 31: 717-728, 2013.

Doonan JH and Morris NR: The bimG gene of Aspergillus niduallas an example of a self-renewal and cell cycle control. Stem Cells 31: 717-728, 2013.

Sáenz-Robles MT, Nair V, Ferrey A, Dumitru R, Gama V, Fagan BM, Sandoval JL, Gallo P, et al: Nuclear accumulation of 53bp1 in differentiating cells. Cell Death Dis 3: 1244, 2012.

Fernandes T, Abad M, Di Croce L, García-Caballero T, Kareta MS, Gorges LL, Hafeez S, Maimets T, Neganova I, Armstrong L and Lako M: Activation of Rb/53BP1 signaling pathway as a major target of p53 in murine embryonic stem cells. Int J Biochem Cell Biol 43: 2317-2324, 2011.

van Harn T, Foeijer F, van Vugt M, Banerjee R, Yang F, Oostra A, Justilien H and te Riele H: Loss of Rb proteins causes genomic instability in the absence of mitogenetic signaling. Genes Dev 24: 1377-1388, 2010.

Karantza V, Maroo A, Fay D and Sedivy JM: Overproduction of Rb protein after the G1/S boundary causes G2 arrest. Mol Cell 13: 6640-6652, 1995.

Sage J and Straugh AF: RB’s original CIN? Genes Dev 24: 1329-1333, 2010.

Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, van Berkum NL, Ebmeier CC, Goossens J, Rahl PB, Levine SS, et al: Mediator and cohesin connect gene expression and chromatin architecture. Nature 467: 430-435, 2010.

Hu G, Kim J, Xu Q, Leng Y, Orkin SH and Ellelge SJ: A genome-wide RNAi screen identifies a new transcriptional module required for self-renewal. Genes Dev 23: 837-848, 2009.

Ding L, Paszkowski-Rogacz M, Nitzsche A, Slabicki MM, Heninger AK, de Vries I, Kütter R, Junqueira M, Shevchenko A, Schaaf H, et al: A genomewide RNAi screen for Oct4 modulators defines a role of the Paf1 complex for embryonic stem cell identity. Cell Stem Cell 4: 403-415, 2009.

Fazzio TG and Panning B: Condensin complexes regulate mitotic progression and interphase chromatin structure in embryonic stem cells. J Cell Biol 227: 481-503, 2010.

Solozobova V, Rolletschek A and Blatter C: Nuclear accumulation and activation of p53 in embryonic stem cells after DNA damage. BMC Cell Biol 10: 46, 2009.

Dumitru R, Gama V, Fagan BM, Bower JJ, Swahari V, Penwy LH and Deshmukh M: Human embryonic stem cells have constitutively active p53. Mol Cell 15: 15768-15773, 2006.

Van Hoof D, Ioanou G, Skepper RN, Pinkwe MW, Linding R, Heck AJ, Mummery CL and Krijgsfeld J: Phosphorylation dynamics during early differentiation of human embryonic stem cells. Cell Stem Cell 5: 214-226, 2009.

Li L, Wang J, Hou J, Wu Z, Zhuang Y, Lu M, Zhang Y, Zhou X, Li L, Xiao W and Zhang W: Cdk1 interplays with Oct4 to repress differentiation of embryonic stem cells into trophoderm. J Cell Biol 204: 4007-4017, 2012.

Zhao Z, Deibel RW, Leroux PH, Ballabeni A, Heffner GC, Heninger AK, de Vries I, Kittler R, Junqueira M, Shevchenko A, Schaaf H, et al: A genomewide RNAi screen for Oct4 modulators defines a role of the Paf1 complex for embryonic stem cell identity. Cell Stem Cell 4: 403-415, 2009.

Fazzio TG and Panning B: Condensin complexes regulate mitotic progression and interphase chromatin structure in embryonic stem cells. J Cell Biol 227: 481-503, 2010.

Solozobova V, Rolletschek A and Blatter C: Nuclear accumulation and activation of p53 in embryonic stem cells after DNA damage. BMC Cell Biol 10: 46, 2009.

Dumitru R, Gama V, Fagan BM, Bower JJ, Swahari V, Penwy LH and Deshmukh M: Human embryonic stem cells have constitutively active p53. Mol Cell 15: 15768-15773, 2006.

Hadjal Y, Hadehode O, Yazidi CE, Barruet E and Binétruy B: A p38MAPK-p53 cascade regulates mesodermal differentiation and neurogenesis of embryonic stem cells. Cell Death Dis 4: e1070, 2013.

Heo SH, Cha Y and Park KS: Hydroxyurea induces a hypersensitive apoptotic response in mouse embryonic stem cells through p38-dependent acetylation of p53. Stem Cell Dev 23: 2435-2442, 2014.

Lee KH, Li M, Michalowski AM, Zhang X, Liao H, Chen L, Xu Y, Wu X and Huang J: A genomewide study identifies the Wnt signaling pathway as a major target of p53 in murine embryonic stem cells. Proc Natl Acad Sci USA 107: 69-74, 2010.
99. Abdelalim EM and Tooyoma I: Knockdown of p53 suppresses Nanog expression in embryonic stem cells. Biochem Biophys Res Commun 443: 652-657, 2014.
100. Akdemir KC, Jain AK, Allton K, Aronov B, Xu X, Cooney AJ, Li W and Barton MC: Genome-wide profiling reveals stimulus-specific functions of p53 during differentiation and DNA damage of human embryonic stem cells. Nucleic Acids Res 42: 205-223, 2014.
101. Zhen HY, Zhou J, Wu HN, Yao C, Zhang T, Wu T, Quan CS and Li YL: Lidamycin regulates p53 expression by repressing Oct4 transcription. Biochem Biophys Res Commun 447: 224-230, 2014.
102. Ng WL, Chen G, Wang M, Wang H, Story M, Shay JW, Zhang X, Wang J, Amin AR, Hu B, et al: OCT4 as a target of miR-34a stimulates p53 but inhibits p53 to promote human cell transformation. Cell Death Dis 5: e1024, 2014.
103. Chen T, Du J and Lu G: Cell growth arrest and apoptosis induced by Oct4 or Nanog knockdown in mouse embryonic stem cells: A possible role of Trp53. Mol Biol Rep 39: 1855-1861, 2012.
104. Zhang ZN, Chung SK, Xu Z and Xu Y: Oct4 maintains the pluripotency of human embryonic stem cells by inactivating p53 through Sirt1-mediated deacetylation. Stem Cells 32: 157-165, 2014.
105. Yu KR, Yang SR, Jung JW, Kim H, Ko K, Han DW, Park SB, Choi SW, Kang SK, Schöler H and Kang KS: Knocking down OCT4 and SOX2 multipotency and maintains stemness through the direct regulation of OCT4 and SOX2. Stem Cells 30: 876-887, 2012.
106. Itahana Y, Zhang J, Geke J, Vardy LA, Han R, Iwamoto K, Cukuroglu E, Robson P, Pouladi MA, Colman A and Itahana K: Histone modifications and p53 binding poise the p21 promoter for activation in human embryonic stem cells. Sci Rep 6: 2812, 2016.
107. Suivorova II, Grigorash BB, Chuykin IA, Pospelova TV and Pospelov VA: G1 checkpoint is compromised in mouse ESCs due to functional uncoupling of p53:p21Waf1 signaling. Cell Cycle 15: 52-63, 2016.
108. Filion TM, Qiao M, Ghale PN, Mandeville M, van Wijnen AJ, Stein JL, Lian JB, Altieri DC and Stein GS: Survival responses of human embryonic stem cells to DNA damage. J Cell Physiol 220: 586-592, 2009.
109. Zhu H, Hu S and Baker J: JMJ55 regulates cell cycle and pluripotency in human embryonic stem cells. Stem Cells 32: 2098-2110, 2014.
110. Niculescu AB III, Chen X, Smeets M, Hengst L, Prives C and Reed SE: Effects of p21(Cip1/Waf1) at both the G1/S and the G2/M cell cycle transitions: pRb is a critical determinant in blocking DNA replication and in preventing endoreduplication. Mol Cell Biol 18: 629-643, 1998.
111. Karimian A, Ahmadi Y and Yousefi B: Multiple functions of p21 in cell cycle, apoptosis and transcriptional regulation after DNA damage. DNA Repair (Amst) 42: 63-71, 2016.
112. Chan WH, Houng WL, Lin CA, Lee CH, Li PW, Hsieh JT, Shen JL, Yeh HI and Chiang WH: Impact of dihydroxyacetic acid on mouse embryonic stem cells and related regulatory mechanisms. Environ Toxicol 28: 87-97, 2013.
113. Tahmasebi S, Alain T, Rajasekhar VK, Zhang JP, Prager-Khoutorsky M, Khoutorsky A, Dogan Y, Gkogkas CG, Petroulakis E, Sylvestre A, et al: Multifaceted regulation of somatic cell reprogramming by mRNA translational control. Cell Stem Cell 14: 606-616, 2014.
114. Zhen HY, He QH, Li Y, Zhou J, Yao C, Liu YN and Ma LJ: Lidamycin induces neural differentiation of mouse embryonic carcinoma cells through down-regulation of transcription factor Oct4. Biochem Biophys Res Commun 421: 44-50, 2012.
115. Tsai CC, Su PF, Huang YF, Yew TL and Hung SC: Oct4 and Nanog directly regulate Dnmt1 to maintain self-renewal and undifferentiated state in mesenchymal stem cells. Mol Cell 47: 169-182, 2012.
116. Lee J, Go Y, Kang I, Han YM and Kim J: Oct-4 controls cell-cycle progression of embryonic stem cells. Biochem J 426: 171-181, 2010.
117. Arabidopsis thaliana: Energy metabolism in the acquisition and maintenance of stemness. Semin Cell Dev Biol 52: 68-75, 2016.
118. Arabidopsis thaliana: Energy metabolism in the acquisition and maintenance of stemness. Semin Cell Dev Biol 52: 68-75, 2016.
119. Lunt SY, van Wijnen AJ and Stein GS: Connectivity and function of stem cells in the bone marrow. Annu Rev Cell Dev Biol 27: 441-464, 2011.
120. Lunt SY and Vander Heiden MG: Aerobic glycolysis: Meeting the metabolic requirements of cell proliferation. Science 324: 1029-1033, 2009.
121. Lunt SY and Vander Heiden MG: Analyzing metabolism with metabolomics. Science 331: 1278-1283, 2011.
122. Lunt SY and Vander Heiden MG: Mitochondrial metabolic pathways in cell proliferation. Science 324: 1029-1033, 2009.
123. Lunt SY and Vander Heiden MG: Aerobic glycolysis: Meeting the metabolic requirements of cell proliferation. Annu Rev Cell Dev Biol 27: 441-464, 2011.
124. Lunt SY and Vander Heiden MG: Aerobic glycolysis: Meeting the metabolic requirements of cell proliferation. Annu Rev Cell Dev Biol 27: 441-464, 2011.