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Role of cyclins and cyclin-dependent kinases in pluripotent stem cells and their potential as a therapeutic target

Siwanon Jirawatnotai¹, Stephen Dalton², Methichit Wattanapanitch^{3,*}

¹Siriraj Center of Research for Excellence for Systems Pharmacology, Department of Pharmacology, Faculty of Medicine, Siriraj Medical School, Mahidol University, Bangkok 10700, Thailand. ²Center for Molecular Medicine and Department of Biochemistry and Molecular Biology, 325 Riverbend Road, The University of Georgia, Athens, GA 30602, USA. ³Siriraj Center for Regenerative Medicine, Research Department, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

Abstract

Over the last 15 years connections between cell cycle control, maintenance of pluripotency, and control of cell fate decisions have been firmly established. With the emergence of powerful tools, such as highly-specific small molecule inhibitors for cyclin-dependent protein kinase (CDK) activity and single-cell imaging technologies, the mechanistic links between cyclins, CDKs and regulation in PSCs in mechanistic detail has been made possible. In this review, we discuss new developments that mechanistically link the CDK regulatory network to control of cell fate decisions, including maintenance of the pluripotent state. Overall, these findings have potential to impact the translational applications of stem cells in regenerative medicine, drug discovery and cancer treatment.

Keywords

pluripotent stem cells; cyclin; cyclin-dependent kinases; CDK inhibitor

Introduction

Multicellular organisms develop and maintain the critical number of cells needed for tissue homeostasis through the cell division cycle. This cycle of events is regulated by a group of core proteins centered around cyclin-dependent kinases (CDKs), cyclins, and their negative regulatory subunits, the cyclin-dependent kinase inhibitors (CDKIs). The cell cycle can be separated into four phases, Gap 1 (G1), DNA replication (synthesis phase, S-), Gap 2 (G2) and mitosis (M-phase). G1 phase is the period when cells sense growth signals and ‘decide’

*Corresponding author: methichit.wat@mahidol.ac.th.

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whether they will commit to another round of cell division or, exit to the quiescence state (G0).

During late pre-implantation and early post-implantation stages of mammalian embryonic development, pluripotent cells divide rapidly but this is followed by asynchronous cell divisions coinciding with progression through gastrulation (1). One consequence of rapid cell division at this stage of development is to rapidly increase the number and volume of pluripotent stem cells (PSCs) before they commit to one of the embryonic germ layers during gastrulation. The mean cell cycle length in the murine embryonic epiblast is around 5 hours. Within a small region of the embryo called the “proliferative zone”, which constitutes about 10% of the whole epiblast, cell generation time can be as short as 2–3 hours, while those in the other regions such as extra-embryonic tissues can be ~6.5 hours (2). Once the PSCs differentiate into one of the germ layers (mesoderm, ectoderm or endoderm), the cycle length extends to 16 hours or more due to the extension of the gap phases (2). Changes in proliferative rates and cell cycle control have also been described as uncommitted cells/nuclei transition towards a differentiated state in *Drosophila* (3), zebrafish (4) and *Xenopus* (5). This suggests a conserved, fundamental role for the cell cycle machinery in control of cell identity and fate. For a detailed description of the cell cycle in PSCs see (6, 7).

Due to ethical concerns and technical difficulties in studying the cell cycle in human embryos, other *in vitro* human models have been developed. Cultured PSCs are an excellent *in vitro* model to understand peri-implantation development, including mechanisms that link the cell cycle regulation to cell identity. Importantly, PSCs undergo symmetric self-renewal in culture while retaining the ability to differentiate into all of the adult lineages. These properties are generally shared by embryonal carcinoma cells (ECCs), embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and epiblast stem cells (EpiSCs) (8). From a translational perspective, PSCs have several advantages over adult stem cells that have limited proliferative capacity and restricted differentiation potential *in vitro*.

Mouse ESCs (mESCs) are derived from the inner cell mass (ICM) of pre-implantation epiblast at E3.5 – E4.5, representing a “naïve-state” of pluripotency. mESCs require two important signals, leukemia inhibitory factor (LIF) and bone morphogenetic protein 4 (BMP4), to promote their self-renewal and block their differentiation (9–11). More recently, another type of early epiblast-like population representing a “ground-state” pluripotency was established (12). This type of ground-state pluripotent cells is considered as being earlier stage of pluripotency than naïve cells according to their epigenetic and transcriptional profiles (6). In contrast, human ESCs (hESCs) are derived from the inner cell mass (ICM) of pre-implantation human blastocysts at E5.5. hESCs exhibit a morphology and molecular signature similar to those of mouse EpiSCs, which are derived from post-implantation epiblasts. Both hESCs and mEpiSCs require FGF2 signaling to sustain their self-renewal and represent a “primed-state” of pluripotency, corresponding to primitive ectoderm in the post-implantation epiblast (10, 13). Upon withdrawal of LIF and activation of Activin A and FGF2 signaling, naïve mESCs can be transformed into mEpiSCs and acquire the primed state of pluripotency. On the other hand, inhibition of glycogen synthase kinase 3 (GSK3) and MEK/ERK pathways using two small molecule inhibitors or 2i, CHIR99021 and PD0325901, plus LIF promote ground-state pluripotency of mESCs (10). Recent studies

demonstrated that primed hESCs can be reverted to naïve pluripotent cells using different protocols such as overexpression of *KLF2* and *NANOG* and culture in a medium containing the 2i plus LIF and Go6963 (14) or culture in a medium containing five kinase inhibitors plus LIF, activin and FGF2 (15) (Figure 1).

Recent reports demonstrate that extrinsic factors that control naïve and primed pluripotency impact cell cycle regulation in these cell types. Most studies of the cell cycle with mESCs have relied on culture in serum where they exhibit their characteristics similar to PSCs from post-implantation embryonic stages. Under more defined culture conditions, such as in the presence of 2i, mESCs exhibit their full pluripotent capacity and more closely resemble pluripotent ESCs from the ICM (ground-state), as evidenced in chimera formation (9, 10, 16). A recent study using the Fluorescence Ubiquitination Cell Cycle Indicator (FUCCI) reporters in mESCs compared cell cycle characteristics in serum vs 2i conditions. This study showed that the short G1-phase in the mESCs cultured in serum is due to lack of G1-phase control i.e. lack of MAPK, CDK/cyclin D and pRB control (9). In contrast, the mESCs cultured in 2i have a more extended G1-phase with hypophosphorylated retinoblastoma protein (RB) and a shorter G2-phase. A fundamental finding here is that PSCs establish a functional G1 checkpoint as they transition from the ground state to the primed state (17).

Specific mode of cell cycle control by cyclins in PSCs

Cell fate decision are frequently made from the G1 phase of the cell cycle (18). The common key regulators of cell cycle progression that impact these decisions are cyclins and CDKs. CDK-cyclin complexes are the master regulators of cell cycle progression and sit at the top of the regulatory hierarchy that links the cell cycle to cell fate decisions. In mammalian somatic cells, the CDK-cyclin complexes exhibit an oscillatory activity and regulate cell cycle transitions from one phase to another (11, 19). Upon growth factor stimulation, cyclin D (cyclin D1, D2, or D3) is upregulated and binds to their catalytic partner CDK4 or CDK6 (CDK4/6). In turn, the binding of cyclin regulatory subunits combined with post-translational modifications activate CDK catalysis and initiate phosphorylation of the pocket proteins pRB (p110), p107, and p130 (20). Mono-phosphorylation of pocket proteins by CDK-cyclin D complexes partially de-repress E2F target genes, that are required for the activation of genes involved in the transition from G1-into S-phase. Later in G1, the E2F target genes cyclin E1,2 are activated allowing for the formation of CDK2-cyclin E complexes and further phosphorylation on the mono-phosphorylated pocket protein (21). Consequently, E2F target genes are fully de-repressed and a new wave of transcription drives the G1-to S-phase transition. The cyclin E-CDK2 complex also controls initiation of DNA synthesis by promoting assembly of a pre-replication complex at replication origins. As the cell passes from the G1 to the S phase, the cyclin A-CDK2 complex replaces cyclin E-CDK2 complex when it terminates the assembly of the pre-replication complex thereby blocking further origin firing (22). As the cell cycle progresses through G2/M, the level of cyclin A declines and cyclin B-CDK1 begins to orchestrate progression from G2- to M-phase. During the onset of the G2 to the M phase, CDK1 associates with cyclin A or cyclin B. Both types of the complex are essential for timely M-phase progression of the cell (23). As such, substrates of CDK1 specifically target activities required for chromosome segregation (24).

Activation of the CDK activity is ensued by the availability of cell cycle phase-specific cyclin partners. Cyclin D is activated by mitotic signals available from the cellular environment. Cyclin E is downstream of E2F and MYC (25–27), hence its transcription starts during G1 following the Cyclin D-dependent E2F phosphorylation. At this point, the expression of cyclin E marks cell independence from the extracellular growth signal. Cyclin E levels then decline during late S phase by a mechanism involving E3-ubiquitin ligase FBXW7-dependent protein degradation (28). In addition, the expression of cyclin E protein is also restricted by miR-15a (28). Although cyclin A is also transcriptionally induced by E2Fs at the restriction point, cyclin A protein does not accumulate until the late G1/S phase transition due to ubiquitination by the anaphase promoting complex (APC) and subsequent proteolysis by the 26S proteasome (29). This elaborate network of regulation ensures the dynamic control of CDK activity and smooth progression between the different cell cycle phases.

Cell cycle in the PSCs operates by the same principles as that described for somatic cells although there are subtle differences. For example, PSCs *in vitro* and *in vivo* spend a high proportion of time in S-phase (50–70%) and only a short time in G1-phase (1, 6). As previously described, PSCs undergo rapid cell/nuclear divisions with cell cycles lacking fully-formed gap phases (both G1 and G2). Cultured naïve ESCs are one of the most well-studied models for cell cycle in PSCs. mESCs divide very rapidly and have a truncated G1 phase of <3 h (30, 31). These cells express high levels of cyclins E, A and B proteins and corresponding CDK1 and CDK2 kinase activities (1, 32). Interestingly, elevated levels of cyclins E and A can be observed throughout the cell cycle, in contrast to their phase-specific expression in somatic cells (1, 33). The only CDK activity that shows phase-specific regulation in naïve mESCs is the CDK1-cyclin B complex. Expression levels of cyclin B and CDK1 are still periodic, peaking during mitosis albeit higher than the level in differentiated cells (34). As a consequence of hyperactivated CDK1 and CDK2 kinases, RB1 is constitutively phosphorylated and inactivated throughout the cell cycle, and E2F activity is constitutively de-repressed. This is an important determinant of rapid transition through G1-phase (Figure 1).

The special mode of the cell cycle in PSCs is a result of several adaptations over the canonical somatic cell mechanism. First, the activity of the APC/C (anaphase-promoting complex/cyclosome) enzyme is attenuated by high levels of the EMI1 (early mitotic inhibitor-1) protein. EMI expression during S through prophase blocks APC substrate binding to cyclin A and B, allowing for the high expression levels of cyclin A and B in PSCs (35, 36). Likewise, expression of FBXW7 and miR-15a, negative regulators of cyclin E are intrinsically low in PSCs compared to that in differentiated cells (37, 38). Second, in PSCs, transcription of the cyclin E gene is positively controlled by pluripotency transcription factors. The 5' regulatory region of CCNE1, a gene encoding cyclin E1 contains binding sites for ESRRB, KLF4, and TFCP211. Thus, elevated expressions of the key pluripotent factors such as ESRRB, KLF4, TFCP211 and MYC drive constitutive expression of cyclin E in PSCs (38). This firmly establishes that the unique mode of cell cycle regulation in PSCs is hard-wired into the pluripotency program. Finally, the constitutive activity of several CDKs in PSCs is reinforced by the degradation of CDK inhibitory proteins such as CDK2 p27^{KIP1},

caused by overexpression of SKIP2, another one of the APC targets (39). In sum, PSCs have an unusual cell cycle, most notably highlighted by rapid cell division and a short G1-phase.

Direct regulation/maintaining of pluripotency by cyclins

In hESCs, regulators of G1 and G2/M transition are required for the maintenance of pluripotency and cell fate decisions by modulating transcription factors, signaling pathways and epigenetics (40). Cyclin Ds (D1, D2 and D3) and Cyclin E as well as the activities of their catalytic partners (CDK2, 4 and 6) vary by cell cycle phase-specific mechanisms. In undifferentiated hESCs, the expression of Cyclin D1–3 peak at late G1 and G1/S phases (7, 41, 42). The function of cyclin D protein in pluripotency was investigated using shRNAs to knock down the expression of the three cyclin Ds in all possible combinations (41). Single-knockdown of cyclin D resulted in a moderate increase in lineage markers, including mesodermal and endodermal genes although hESCs were able to self-renew. Double-knockdown of cyclin D resulted in spontaneous differentiation toward the endodermal lineage but the capacity to differentiate into the neuroectodermal lineage was decreased. When the activity of all three cyclin Ds was decreased, hESCs lost their self-renewal capacity and differentiated into endoderm (41).

CDK2 also plays an important role in cell cycle regulation and pluripotency in hESCs. Knockdown of CDK2 results in cell morphology changes and cell cycle arrest in G1 phase. Downregulation of CDK2 also decreases the expression of pluripotency factors including OCT4, SOX2, NANOG and SSEA-4, resulting in differentiation towards extra-embryonic lineages (42). Similarly, CDK1 is required for self-renewal of hESCs and hiPSCs. Furthermore, CDK1-depleted cells accumulate DNA damage as evidenced by a higher number of double-stranded breaks (DSBs) and abnormality in mitotic organelles and karyotype as well as genomic instability (34, 43). This indicates that DNA damage-induced checkpoint controls are not fully active in PSCs. Moreover, CDK1 is required for PI3K-Akt kinase signaling and maintenance of pluripotency (34).

Recently seminal work by Liu *et al.* demonstrated a direct link between cell cycle machineries and the key pluripotency factors (31). Genetic experiments performed in a mouse recently showed that G1 cyclins (D1, D2, D3, E1, and E2) are dispensable for mESC proliferation but are essential for self-renewal, as indicated by their differentiation to the trophectodermal lineage (31). A possible mechanism to explain the dependency of G1-CDK activities in maintenance of pluripotency comes from observations that G1 cyclins–CDKs directly phosphorylate NANOG, OCT4 and SOX2 *in vitro*. *In vivo* G1-CDKs stabilize NANOG, OCT4 and SOX2 by promoting their interaction with Pin1, thereby protecting them from polyubiquitylation and proteasomal degradation (30). An independent study also showed that CDK2 is required for optimal SOX2 activity through phosphorylation at S39 and S253 (39). The function of CDKs in maintenance of self-renewal appears to be specific to G1 cyclins, since the elimination of cyclins A1,2 has no detectable effect (40). It is still not clear whether cyclin A-CDK2 complex phosphorylates the pluripotent factors and stabilizes them, and why pluripotency is not compromised in the cyclin A null stem cell population. Potentially, cyclin A-CDK2 activity in these cells was compensated for by other activities such as cyclin E-CDK2.

Altogether, these recent reports underpin a close relationship between cell cycle regulators and pluripotency regulation, placing the cell cycle machinery centrally in the mechanisms that control stem cell pluripotency and self-renewal. Although further research is still required to understand this relationship in detail, the findings provide a solid foundation on which to explore the link between CDK-cyclin complexes and cell fate decisions. This information also opens an opportunity to manipulate stem cells through the small molecule CDK inhibitors.

Cyclins and somatic cell reprogramming

Induced pluripotent stem cells (iPSCs) can be generated from a range of mouse or human somatic cells such as fibroblasts or circulating mononuclear cells by introducing four pluripotency-associated transcription factors, OCT3/4, SOX2, KLF4 and MYC. The resulting iPSCs acquire ESC characteristics at the morphological and molecular level and acquire self-renewal capacity and broad differentiation potential (44, 45). Thus, human iPSCs represent an alternative source of unlimited PSCs for cell replacement therapy generated without the ethical concerns regarding embryo destruction. In addition, generation of patient-specific iPSCs provides valuable disease models to study underlying disease pathogenesis (46).

Studies of the molecular mechanisms underpinning reprogramming have identified critical roles for the cell cycle machinery. During the later stages of reprogramming, de-differentiating cells acquire a cell cycle structure that is characteristic of PSCs, including a truncated G1-phase and accelerated cell proliferation rates (47). Of the four reprogramming factors, MYC is most closely linked to cell cycle control where it impacts targets such as cyclin regulatory subunits and additionally, extends the proliferative capacity of cells (48). Consistent with these findings, reprogramming efficiency can be enhanced in the cells with rapid cell proliferation, mediated by over-expressing cyclin B1-CDK1(34), cyclin D-CDK4/6 and cyclin E/A-CDK2 or, by down-regulating pRb (47). Conversely, reprogramming efficiency can be diminished when the cell cycle is blocked, following ectopic expressions of the cell cycle inhibitors such as p15, p16, or p21 (47), or treatment with small molecule CDK2 and CDK4/6 inhibitors (49). Likewise, genetic deletions of p21, p27 and p18 also enhance reprogramming efficiency (49) (Figure 2). This evidence suggests that the reprogramming process and pluripotency network can be controlled by modulating the activity of cell cycle regulators. However, the exact mechanisms that connect cell proliferation to reprogramming efficiency remain to be elucidated.

Cyclins and stem cell differentiation

Upon exposure to differentiation cues, mouse and human PSCs respond by activating developmental genes in G1, implicating this stage of the cell cycle as being a “decision-making” period with regards to developmental fate. Previous studies indicate that cells are most responsive to inductive signals in G1-phase (41, 50), establishing it as period when cells are in a permissive state to differentiate (8, 41). One possible explanation to explain why PSCs are prone to initiate differentiation in G1-phase could be related to a permissive chromatin environment that exists at this time. This would potentially allow the loading of

new transcription factors and chromatin remodeling required for the establishment of new developmental programs (51, 52). Once cells commit to one of the three embryonic germ layers and exit the pluripotent state, the length of G1-phase increases considerably and this, in turn, increases the duration of cell division (8, 9). Interestingly, a cell cycle arrest triggered by overexpressing cell cycle inhibitors such as p15, p16 or p21 promotes spontaneous differentiation (Figure 2). Ultimately, a prolonged cell cycle arrest induction can drive hESCs toward irreversible differentiation (47).

A unique opportunity to study the link between cell cycle control and mechanisms of cell fate commitment has recently been made possible by the availability of the FUCCI reporter system. The strength of this tool is that it allows the cell cycle to be monitored in live cells at the single-cell level. One outcome of the FUCCI system has been to show that G1 can be compartmentalized so that endodermal differentiation occurs in early G1 and neuroectodermal differentiation can preferentially occur in late G1. Furthermore, differentiation efficiency can be enhanced by synchronizing hESCs in the early G1-phase prior to directed differentiation into endoderm derivatives and insulin-expressing or hepatocyte-like cells (41). These observations reveal a deep mechanistic link between the G1-phase of the cell cycle and developmental decisions made by PSCs.

Further investigation using the FUCCI system into mechanisms underlying cell fate decisions reveal the importance of the cell cycle regulators such as cyclin Ds, which play a central role in coordinating G1 progression together with CDK4/6. Cyclin Ds recruit transcriptional corepressors and coactivator complexes at developmental genes, thereby having the potential to impact developmental programs (53). In this context, levels of cyclin Ds show dynamic, germ layer-specific changes during differentiation suggesting a mechanism for how cell cycle regulation could impact chromatin and developmental gene activity. Upon neuroectodermal differentiation in which Activin A/Nodal signaling is inhibited, cyclin Ds are highly expressed. In contrast, during endodermal or mesodermal differentiation which requires Activin A/Nodal signaling, levels of cyclin Ds are low. Functional analyses revealed a non-cell cycle role of cyclin D-CDK4/6 complexes that work by inhibiting the transcriptional activity generated by Activin/Nodal signaling. This impacts the cellular localization of Smad2/3, which serves as the main effector in this pathway. Further studies demonstrated that efficient differentiation of hESCs to endodermal lineages, that usually require Activin A/Nodal signaling, can be achieved by inhibiting the activity of cyclin D-CDK4/6 without the need of exogenous Activin A (41).

A more recent study showed that other cell cycle regulators also are required for efficient mesodermal differentiation. Inhibition of the G2/M regulator CDK1 resulted in a decrease in bone morphogenetic protein (BMP) signaling activity, which is required for mesodermal specification, thus blocking the lateral plate mesoderm (LPM) formation. In addition to CDK1, CDK2 and phosphorylation of pRb are required for mesodermal differentiation. This notion was supported by the loss of *Brachyury* (*T*) expression after inhibition of CDK2 and pRb during LPM induction (40). These data suggest that the cell cycle regulators are lineage-specific and context-dependent. Therefore, manipulation of cell cycle regulators can enhance lineage specification and cell fate decisions by controlling the activity of key

developmental pathways. The knowledge obtained from these studies will be useful and applicable to the development of specialized cells for cell-based therapies.

Role of cyclin B-CDK1 in mitotic bookmarking

During mitosis, global transcription decreases due to disengagement of many transcription factors from condensed chromatin. In such circumstances, an important challenge for PSCs is how to maintain their stem cell identity after each cell cycle. Thus, there is a requirement for a process that ensures accurate transmission of mother cell identity to the daughter cell. One potential mechanism could be “mitotic bookmarking”, which refers to the retention of epigenetic marks during mitosis that enables rapid gene activation upon entry into the next G1 phase. In principle, this mechanism establishes how a stem cell ‘remembers’ what it is, by transmitting epigenetic information corresponding to cell identity from one cell cycle to the next (54). Genes that are ‘bookmarked’ can rapidly reactivate upon entry into the G1 phase because marked genomic sites are associated with DNase-hypersensitivity that marks promoters and enhancers. DNase-hypersensitive sites are established by chromatin modifiers and transcription factors that bind to and bookmark mitotic chromatin (54). In PSCs, well-known stem cell transcription factors including ESRRB, SOX2, OCT4, KLF4, bookmark mitotic chromatin in order to reestablish the pluripotency program upon exit from M-phase (55–58). The bookmarking by these factors emphasize that the memory of stemness is highly-regulated throughout the cell cycle, even during mitosis when gene expression is limited.

During development, asymmetric (biased) bookmarking plays role to control the differential expressions of gene sets that determine self-renewal or fate commitment. For example, biased bookmarking has been described at genomic sites bookmarked by the nuclear receptor ESRRB. In mESCs, genes proximally bound to ESRRB during mitosis are also expressed in pluripotent cells of the embryo, whereas regions losing ESRRB during mitosis are associated with trophoblastic genes, an extra-embryonic lineage in which ESRRB functions later during development (55).

Evidences suggested that the bookmarking stem cell transcription factors may be subjected to a distinct mode of regulation for their roles in bookmarking. Firstly, only a subset of the promoters/enhancers occupied by the stem cell transcription factors during interphase are bookmarked during M phase. Secondly, the transcription factors bookmarking the sites during M phase appeared to be at a reduced amount and with the altered component compared to interphase (59, 60). For example, ESRRB, OCT4, KLF4 and SOX2, have been shown to bind in mitosis only to their most active targets in interphase, including to clusters of enhancers located in the vicinity of cell identity genes (55, 57, 61). Other stem cell factors found to bind the mitotic chromosome include MYC, RBPI, and GATA1 (60, 62, 63).

Given the crucial role of the mitotic bookmarking in a mitotic memory of gene regulation, understanding of how the bookmarking is regulated during the cell cycle could be very important and might have an impact especially in the stem cell reprogramming. Cyclin B1-CDK1 is known to play a critical role in M-phase progression (64, 65) but recently, it has also been implicated to play a key role in the bookmarking mechanism. For example, mitotic

bookmarking of OCT4 on lineage-specific genes for trophectoderm has been shown to be negatively regulated by cyclin B-CDK1. CDK1 activity facilitates Aurora kinase B-mediated OCT4 S229 phosphorylation that results in OCT4 dissociation from the mitotic DNA (66). This interaction is perhaps important for the selected daughter cell, whose pluripotency is to be retained. Contrary to this finding, recent studies reported that core transcription factors, including OCT4, in PSCs are associated with chromatin during mitosis (56, 57). It is still not clear whether the contrasting results are from a technical discrepancy. Both observations could hold true, however. For example, OCT4 regulated by cyclin B-CDK1 could selectively bind to a set of pluripotent genes and dissociates from another set of cell fate genes. Cyclin B-CDK1 was also shown to promote SOX2 nuclear localization and DNA binding during mitosis. Here, SOX2 nuclear localization was impaired by the CDK1 small molecule inhibitor RO-3306 (67), suggesting that cyclin B-CDK1 might also involve with the SOX2 proximity to the mitotic chromosomes and the bookmarking activity of SOX2. Cyclin B-CDK1 has long been known as a regulator of stem cell asymmetric cell division (68). Therefore, it may indirectly regulate the selective bookmarking process during mitosis through the asymmetric cell division as well. This needs to be validated experimentally.

Stem cell-based cancer treatment by blocking cyclin-CDK activities

Understanding the role of cell cycle machinery in the self-renewal and differentiation of stem cell opens an immense therapeutic opportunity, not only for the regenerative medicine, but also for the treatment of the disease driven by stem cells, such as cancer.

The concept of cancer ‘initiating’ cells or cancer stem cells (CSCs) has become well-accepted over the last decade. It describes a population of cancer cells that possesses characteristics associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer sample. CSCs also exhibit limitless replication potential. They contribute as an origin of cancer and fuel tumor development including metastasis and contribute to the resistance of tumor cells to anti-cancer therapies (69).

The origin of CSCs is still unclear and is likely to vary from cancer to cancer. One hypothesis presents that somatic mutations can occur in resident stem cells, which in turn bestows cancerous features on the stem cells. Alternatively, cancer cells may acquire stemness characteristics *via* trans-differentiation.

CSCs are anticipated to share some common characteristics with other stem cell populations, including the expressions of key stem cell regulators. As such, genes usually associated with PSCs have been observed in multiple types of CSCs (70–72). Expressions of *NANOG*, *OCT4*, *SOX2*, and the signaling networks essential for the maintenance of the stemness are also observed in the CSCs.

CSCs may be viewed as a reservoir of the tumor, any therapies that only target cells of the bulk tumor and leave CSCs intact will not be successful. In this scenario, therapeutics would kill the majority of cancer cells and induce temporary regression of gross tumor lesions but, fail to prevent disease relapse and metastatic dissemination. Thus, addition of therapeutics that target CSCs into the cancer treatment regimen should be considered. The current

understanding of stem cell regulation by the cell cycle machinery has also opened up a new opportunity to target the CSCs. From a therapeutic perspective, a big challenge in dealing with CSCs is their heterogeneity. With no universal marker identified to date, targeting CSCs have been problematic (73–77), although targeting the proliferative properties of CSCs by cell cycle inhibition could be a potential strategy.

Only recently, emergence of highly specific CDK4/6 small molecule inhibitors has made it possible to block the cell cycle machinery. Examples include Palbociclib, Ribociclib, and Abemaciclib which have quickly become among the most effective small molecule anti-cancer therapeutics available (Figure 3). These inhibitors are being used as a first-line treatment for advanced hormonal receptor-positive breast cancer and are being trialed in tested tumors with a disturbed p16-CDK4/6-pRB-E2F pathway (78, 79). The target specificity and relatively safe clinical profile of the CDK4/6 inhibitors allows their use as a unique tool to; i) validate the role of CSCs in cancer, and ii) directly manipulate the CSC cell cycle in the animal models or patients.

Recent findings provided clues on the involvement of CDK4/6 activity relating to *in vivo* tumor growth driven by CSCs (80, 81). These studies showed that the anti-cancer activity of CDK4/6 inhibitors was greatly enhanced in the *in vivo* milieu, which is permissive for CSC population (82). *In vitro* treatment of basal-B triple-negative breast cancer cells, enriched for CD44⁺/CD24[−] breast CSCs, by the CDK4/6 inhibitors converted the cells from the mesenchymal phenotype to a more differentiated epithelial- and luminal-like phenotype (83). In addition, the inhibition of CDK4/6 activity in the tumor also reduces cancer drug resistance or sensitizes the CSC-rich cancer cell lines to irradiation or cytotoxic T cell-mediated killing (83–86). Analyses on CSC populations in several breast cancer cell lines of different subtypes revealed that CDK4/6 inhibition, by small molecule inhibition or siRNA, depletes the CSC populations in the culture (87), indicating that activities of CDK4/6 are rate-limiting factor for breast CSC maintenance.

As described in the previous section, the length of the G1 is a determinant of cell fate during neuronal development. Shortening the length of G1 by ectopic elevation of CDK activity delays neural commitment, while the opposite is true when CDK activity is reduced (86). This mutual relationship between cell fate decisions and cell cycle progression in neural cells was recently demonstrated to underline the resistance of MYC-driven medulloblastoma to JQ-1, an inhibitor that works by targeting the BET-bromodomain. JQ-1 resistant medulloblastoma cells adopted a new epigenetic program resembling that of the committed neuron stem cell i.e. high levels of CDK4/6 activity and NEUROGs/NEURODs/MYC expression that allows them to escape gene suppression by the BET inhibitor. This cell fate resetting and the JQ-1 resistance could be recapitulated by overexpression of the catalytic partners of CDK4/6, cyclin D2 or cyclin D3. Likewise, the elevated CDK activity has been proposed to dedifferentiate neuronal cells (88). Concurring with these findings, CDK4/6 inhibition effectively prevented JQ-1 resistance (84), indicating that the cell cycle regulators are the drivers of neuronal stem cell fate remodeling and drug resistance in cancer. These observations also show that CDK inhibition not only blocks cancer cell proliferation but, that it also interferes with stem cell-mediated cancer phenotypes, creating a two-pronged angle of attack for cancer therapy.

Although stem cell-targeting by cell cycle inhibition has significant potential, there are many outstanding questions that need to be addressed. Inhibition of cell cycle regulators such as CDK4/6 or CDK2 will create cell cycle quiescence, which could antagonize the effect of chemotherapeutics that target S-phase cancer cells when simultaneously administered (89, 90). Inhibition of CDK4/6 was shown to promote HSC preservation, in a 5-FU-induced HSC exhaustion mouse model, in which CDK4/6 inhibition permitted the rapid recovery of peripheral blood cells after rounds of the chemotherapy (91). This finding indicates that CDK4/6 inhibition may hinder tumor elimination. It remains unclear how best to combine these two classes of drugs to avoid their potentially antagonistic effects. Innovative regimens for combination strategies should be tested between cell cycle inhibitors and other classes of chemotherapeutics. Some regimens have already been shown to be effective. For example, the simultaneous combination of Palbociclib with Paclitaxel exerted an antagonistic effect whereas sequential treatment inhibited cell proliferation and increased cell death more efficaciously than single treatments (89).

The mechanism beyond the CSC cell fate alteration by CDK4/6 inhibitors is still unclear. The proposed model would be that CDK4/6 along with CDK2 might exert their stem cell regulatory function through the direct phosphorylation on OCT4, SOX2, and NANOG, resulting in their stabilization, similar to that found in PSCs (31, 92). However, this model still has to be demonstrated in the contexts of CSCs. An alternative mechanism was recently reported in cervical cancer where cyclin D1-CDK4/6 activates NANOG expression, through the pRB phosphorylation/E2F-*regulated* transcriptional pathway (Figure 3). This causes cancer cells to acquire a stem cell-like phenotype and evasion of cytotoxic lymphocytes (86).

Lastly, emerging evidence indicates that in several adult tissues both quiescent (out of cell cycle) and active (in cell cycle) stem cell subpopulations may coexist, in separate yet adjoining locations (93). How would these populations respond to the cell cycle inhibitors is still unclear. Therefore, further research on how cell cycle proteins control the CSCs is needed before applying the CDK inhibitors in the clinical setting.

Concluding remark

During the last decade, roles for the cell cycle machinery in regulating developmental genes and executing cell fate decisions have been established. However, recent studies supported the more extensive role of cyclins and CDKs in the stem cell biology, such as mitotic bookmarking, supporting the notion that cell cycle proteins are major regulators for stem cell homeostasis. Although, a complete view of how the cell cycle machinery cooperates with stem cell elements during development and disease progression is far from clear, several basic principles underpinning this have been identified. For example, manipulating the activity of specific cell cycle proteins is sufficient to modulate aspects of stem cell behavior, as demonstrated in the process of pluripotent induction, and cancer stem cell inhibition. In the near future, such applications will be expanded when more mechanistic insights are revealed.

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Abbreviations:

APC	anaphase promoting complex
BMP4	bone morphogenetic protein 4
CDK	cyclin-dependent kinases
CDKIs	cyclin-dependent kinase inhibitors
CSCs	cancer stem cells
ESCs	embryonic stem cells
ECCs	embryonal carcinoma cells
EpiSCs	epiblast stem cells
FUCCI	Fluorescence Ubiquitination Cell Cycle Indicator
GSK3	glycogen synthase kinase 3
LIF	Leukemia inhibitory factor
iPSCs	induced pluripotent stem cells
ICM	inner cell mass
PSCs	pluripotent stem cells

References

1. Stead E, White J, Faast R, Conn S, Goldstone S, Rathjen J, et al. Pluripotent cell division cycles are driven by ectopic Cdk2, cyclin A/E and E2F activities. *Oncogene*. 2002;21(54):8320–33. [PubMed: 12447695]
2. Snow MHL. Gastrulation in the mouse: Growth and regionalization of the epiblast. *Journal of Embryology and Experimental Morphology*. 1977;42(1):293.
3. Edgar BA, Lehner CF. Developmental control of cell cycle regulators: a fly's perspective. *Science* (New York, NY). 1996;274(5293):1646–52.
4. Yarden A, Geiger B. Zebrafish cyclin E regulation during early embryogenesis. *Developmental dynamics : an official publication of the American Association of Anatomists*. 1996;206(1):1–11. [PubMed: 9019240]
5. Murray AW, Kirschner MW. Cyclin synthesis drives the early embryonic cell cycle. *Nature*. 1989;339(6222):275–80. [PubMed: 2566917]

6. Boward B, Wu T, Dalton S. Concise Review: Control of Cell Fate Through Cell Cycle and Pluripotency Networks. *Stem cells* (Dayton, Ohio). 2016;34(6):1427–36.
7. Liu L, Michowski W, Kolodziejczyk A, Sicinski P. The cell cycle in stem cell proliferation, pluripotency and differentiation. *Nature cell biology*. 2019;21(9):1060–7. [PubMed: 31481793]
8. Dalton S Linking the Cell Cycle to Cell Fate Decisions. *Trends Cell Biol*. 2015;25(10):592–600. [PubMed: 26410405]
9. Coronado D, Godet M, Bourillot PY, Taponnier Y, Bernat A, Petit M, et al. A short G1 phase is an intrinsic determinant of naive embryonic stem cell pluripotency. *Stem cell research*. 2013;10(1):118–31. [PubMed: 23178806]
10. Nichols J, Smith A. Naive and primed pluripotent states. *Cell stem cell*. 2009;4(6):487–92. [PubMed: 19497275]
11. Zaveri L, Dhawan J. Cycling to Meet Fate: Connecting Pluripotency to the Cell Cycle. *Frontiers in Cell and Developmental Biology*. 2018;6(57).
12. Ying QL, Wray J, Nichols J, Battle-Morera L, Doble B, Woodgett J, et al. The ground state of embryonic stem cell self-renewal. *Nature*. 2008;453(7194):519–23. [PubMed: 18497825]
13. Pastor WA, Chen D, Liu W, Kim R, Sahakyan A, Lukianchikov A, et al. Naive Human Pluripotent Cells Feature a Methylation Landscape Devoid of Blastocyst or Germline Memory. *Cell stem cell*. 2016;18(3):323–9. [PubMed: 26853856]
14. Takashima Y, Guo G, Loos R, Nichols J, Ficiz G, Krueger F, et al. Resetting transcription factor control circuitry toward ground-state pluripotency in human. *Cell*. 2014;158(6):1254–69. [PubMed: 25215486]
15. Theunissen TW, Powell BE, Wang H, Mitalipova M, Faddah DA, Reddy J, et al. Systematic identification of culture conditions for induction and maintenance of naive human pluripotency. *Cell stem cell*. 2014;15(4):471–87. [PubMed: 25090446]
16. Alexandrova S, Kalkan T, Humphreys P, Riddell A, Scognamiglio R, Trumpp A, et al. Selection and dynamics of embryonic stem cell integration into early mouse embryos. *Development* (Cambridge, England). 2016;143(1):24.
17. Ter Huurne M, Chappell J, Dalton S, Stunnenberg HG. Distinct Cell-Cycle Control in Two Different States of Mouse Pluripotency. *Cell stem cell*. 2017;21(4):449–55.e4. [PubMed: 28985526]
18. Blomen VA, Boonstra J. Cell fate determination during G1 phase progression. *Cellular and molecular life sciences : CMLS*. 2007;64(23):3084–104. [PubMed: 17891333]
19. Hindley C, Philpott A. The cell cycle and pluripotency. *The Biochemical journal*. 2013;451(2):135–43. [PubMed: 23535166]
20. Hydbring P, Malumbres M, Sicinski P. Non-canonical functions of cell cycle cyclins and cyclin-dependent kinases. *Nat Rev Mol Cell Biol*. 2016.
21. Narasimha AM, Kaulich M, Shapiro GS, Choi YJ, Sicinski P, Dowdy SF. Cyclin D activates the Rb tumor suppressor by mono-phosphorylation. *Elife*. 2014;3.
22. Coverley D, Laman H, Laskey RA. Distinct roles for cyclins E and A during DNA replication complex assembly and activation. *Nature cell biology*. 2002;4(7):523–8. [PubMed: 12080347]
23. Errico A, Deshmukh K, Tanaka Y, Pozniakovskiy A, Hunt T. Identification of substrates for cyclin dependent kinases. *Adv Enzyme Regul*. 2010;50(1):375–99. [PubMed: 20045433]
24. Petrone A, Adamo ME, Cheng C, Kettenbach AN. Identification of Candidate Cyclin-dependent kinase 1 (Cdk1) Substrates in Mitosis by Quantitative Phosphoproteomics. *Mol Cell Proteomics*. 2016;15(7):2448–61. [PubMed: 27134283]
25. Santoni-Rugiu E, Falck J, Mailand N, Bartek J, Lukas J. Involvement of Myc activity in a G(1)/S-promoting mechanism parallel to the pRb/E2F pathway. *Mol Cell Biol*. 2000;20(10):3497–509. [PubMed: 10779339]
26. Geng Y, Eaton EN, Picon M, Roberts JM, Lundberg AS, Gifford A, et al. Regulation of cyclin E transcription by E2Fs and retinoblastoma protein. *Oncogene*. 1996;12(6):1173–80. [PubMed: 8649818]
27. Koff A, Giordano A, Desai D, Yamashita K, Harper JW, Elledge S, et al. Formation and activation of a cyclin E-cdk2 complex during the G1 phase of the human cell cycle. *Science* (New York, NY). 1992;257(5077):1689–94.

28. Siu KT, Rosner MR, Minella AC. An integrated view of cyclin E function and regulation. *Cell cycle (Georgetown, Tex)*. 2012;11(1):57–64.
29. Hsu JY, Reimann JD, Sorensen CS, Lukas J, Jackson PK. E2F-dependent accumulation of hEmi1 regulates S phase entry by inhibiting APC(Cdh1). *Nature cell biology*. 2002;4(5):358–66. [PubMed: 11988738]
30. Savatier P, Huang S, Szekely L, Wiman KG, Samarut J. Contrasting patterns of retinoblastoma protein expression in mouse embryonic stem cells and embryonic fibroblasts. *Oncogene*. 1994;9(3):809–18. [PubMed: 8108123]
31. Liu L, Michowski W, Inuzuka H, Shimizu K, Nihira NT, Chick JM, et al. G1 cyclins link proliferation, pluripotency and differentiation of embryonic stem cells. *Nature cell biology*. 2017;19(3):177–88. [PubMed: 28192421]
32. Fujii-Yamamoto H, Kim JM, Arai K, Masai H. Cell cycle and developmental regulations of replication factors in mouse embryonic stem cells. *The Journal of biological chemistry*. 2005;280(13):12976–87. [PubMed: 15659392]
33. White J, Stead E, Faast R, Conn S, Cartwright P, Dalton S. Developmental activation of the Rb-E2F pathway and establishment of cell cycle-regulated cyclin-dependent kinase activity during embryonic stem cell differentiation. *Molecular biology of the cell*. 2005;16(4):2018–27. [PubMed: 15703208]
34. Wang XQ, Lo CM, Chen L, Ngan ES, Xu A, Poon RY. CDK1-PDK1-PI3K/Akt signaling pathway regulates embryonic and induced pluripotency. *Cell Death Differ*. 2017;24(1):38–48. [PubMed: 27636107]
35. Reimann JD, Freed E, Hsu JY, Kramer ER, Peters JM, Jackson PK. Emi1 is a mitotic regulator that interacts with Cdc20 and inhibits the anaphase promoting complex. *Cell*. 2001;105(5):645–55. [PubMed: 11389834]
36. Reimann JD, Gardner BE, Margottin-Goguet F, Jackson PK. Emi1 regulates the anaphase-promoting complex by a different mechanism than Mad2 proteins. *Genes Dev*. 2001;15(24):3278–85. [PubMed: 11751633]
37. Takeishi S, Nakayama KI. Role of Fbxw7 in the maintenance of normal stem cells and cancer-initiating cells. *Br J Cancer*. 2014;111(6):1054–9. [PubMed: 24853181]
38. Gonnot F, Langer D, Bourillot PY, Doerflinger N, Savatier P. Regulation of Cyclin E by transcription factors of the naive pluripotency network in mouse embryonic stem cells. *Cell cycle (Georgetown, Tex)*. 2019;18(20):2697–712.
39. Bar-On O, Shapira M, Skorecki K, Hershko A, Hershko DD. Regulation of APC/C (Cdh1) ubiquitin ligase in differentiation of human embryonic stem cells. *Cell cycle (Georgetown, Tex)*. 2010;9(10):1986–9.
40. Yiangou L, Grandy RA, Osnato A, Ortmann D, Sinha S, Vallier L. Cell cycle regulators control mesoderm specification in human pluripotent stem cells. *The Journal of biological chemistry*. 2019;294(47):17903–14. [PubMed: 31515269]
41. Pauklin S, Vallier L. The Cell-Cycle State of Stem Cells Determines Cell Fate Propensity. *Cell*. 2014;156(6):1338. [PubMed: 28898638]
42. Neganova I, Zhang X, Atkinson S, Lako M. Expression and functional analysis of G1 to S regulatory components reveals an important role for CDK2 in cell cycle regulation in human embryonic stem cells. *Oncogene*. 2009;28(1):20–30. [PubMed: 18806832]
43. Neganova I, Tilgner K, Buskin A, Paraskevopoulou I, Atkinson SP, Peberdy D, et al. CDK1 plays an important role in the maintenance of pluripotency and genomic stability in human pluripotent stem cells. *Cell death & disease*. 2014;5:e1508. [PubMed: 25375373]
44. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126(4):663–76. [PubMed: 16904174]
45. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131(5):861–72. [PubMed: 18035408]
46. Wattanapanitch M Recent Updates on Induced Pluripotent Stem Cells in Hematological Disorders. *Stem cells international*. 2019;2019:5171032. [PubMed: 31191673]

47. Ruiz S, Panopoulos AD, Herrerias A, Bissig KD, Lutz M, Berggren WT, et al. A high proliferation rate is required for cell reprogramming and maintenance of human embryonic stem cell identity. *Current biology : CB*. 2011;21(1):45–52. [PubMed: 21167714]
48. Daksis JI, Lu RY, Facchini LM, Marhin WW, Penn LJ. Myc induces cyclin D1 expression in the absence of de novo protein synthesis and links mitogen-stimulated signal transduction to the cell cycle. *Oncogene*. 1994;9(12):3635–45. [PubMed: 7526316]
49. Zhan Z, Song L, Zhang W, Gu H, Cheng H, Zhang Y, et al. Absence of cyclin-dependent kinase inhibitor p27 or p18 increases efficiency of iPSC generation without induction of iPSC genomic instability. *Cell death & disease*. 2019;10(4):271. [PubMed: 30894510]
50. Dalton S G1 compartmentalization and cell fate coordination. *Cell*. 2013;155(1):13–4. [PubMed: 24074854]
51. Singh AM, Sun Y, Li L, Zhang W, Wu T, Zhao S, et al. Cell-Cycle Control of Bivalent Epigenetic Domains Regulates the Exit from Pluripotency. *Stem cell reports*. 2015;5(3):323–36. [PubMed: 26278042]
52. Egli D, Birkhoff G, Eggen K. Mediators of reprogramming: transcription factors and transitions through mitosis. *Nature Reviews Molecular Cell Biology*. 2008;9(7):505–16. [PubMed: 18568039]
53. Pauklin S, Madrigal P, Bertero A, Vallier L. Initiation of stem cell differentiation involves cell cycle-dependent regulation of developmental genes by Cyclin D. *Genes Dev*. 2016;30(4):421–33. [PubMed: 26883361]
54. Festuccia N, Gonzalez I, Owens N, Navarro P. Mitotic bookmarking in development and stem cells. *Development*. 2017;144(20):3633–45. [PubMed: 29042475]
55. Festuccia N, Dubois A, Vandormael-Pournin S, Gallego Tejeda E, Mouren A, Bessonard S, et al. Mitotic binding of Esrrb marks key regulatory regions of the pluripotency network. *Nature cell biology*. 2016;18(11):1139–48. [PubMed: 27723719]
56. Deluz C, Friman ET, Strebinger D, Benke A, Raccaud M, Callegari A, et al. A role for mitotic bookmarking of SOX2 in pluripotency and differentiation. *Genes Dev*. 2016;30(22):2538–50. [PubMed: 27920086]
57. Liu Y, Pelham-Webb B, Di Giammartino DC, Li J, Kim D, Kita K, et al. Widespread Mitotic Bookmarking by Histone Marks and Transcription Factors in Pluripotent Stem Cells. *Cell Rep*. 2017;19(7):1283–93. [PubMed: 28514649]
58. Teves SS, An L, Hansen AS, Xie L, Darzacq X, Tjian R. A dynamic mode of mitotic bookmarking by transcription factors. *Elife*. 2016;5.
59. Caravaca JM, Donahue G, Becker JS, He X, Vinson C, Zaret KS. Bookmarking by specific and nonspecific binding of FoxA1 pioneer factor to mitotic chromosomes. *Genes Dev*. 2013;27(3):251–60. [PubMed: 23355396]
60. Kadauke S, Udugama MI, Pawlicki JM, Achtman JC, Jain DP, Cheng Y, et al. Tissue-specific mitotic bookmarking by hematopoietic transcription factor GATA1. *Cell*. 2012;150(4):725–37. [PubMed: 22901805]
61. Festuccia N, Owens N, Papadopoulou T, Gonzalez I, Tachtsidi A, Vandoermel-Pournin S, et al. Transcription factor activity and nucleosome organization in mitosis. *Genome Res*. 2019;29(2):250–60. [PubMed: 30655337]
62. Yang J, Sung E, Donlin-Asp PG, Corces VG. A subset of Drosophila Myc sites remain associated with mitotic chromosomes colocalized with insulator proteins. *Nat Commun*. 2013;4:1464. [PubMed: 23403565]
63. Lake RJ, Tsai PF, Choi I, Won KJ, Fan HY. RBPJ, the major transcriptional effector of Notch signaling, remains associated with chromatin throughout mitosis, suggesting a role in mitotic bookmarking. *PLoS Genet*. 2014;10(3):e1004204. [PubMed: 24603501]
64. Brandeis M, Rosewell I, Carrington M, Crompton T, Jacobs MA, Kirk J, et al. Cyclin B2-null mice develop normally and are fertile whereas cyclin B1-null mice die in utero. *Proceedings of the National Academy of Sciences of the United States of America*. 1998;95(8):4344–9. [PubMed: 9539739]
65. Santamaria D, Barriere C, Cerqueira A, Hunt S, Tardy C, Newton K, et al. Cdk1 is sufficient to drive the mammalian cell cycle. *Nature*. 2007;448(7155):811–5. [PubMed: 17700700]

66. Kim HJ, Shin J, Lee S, Kim TW, Jang H, Suh MY, et al. Cyclin-dependent kinase 1 activity coordinates the chromatin associated state of Oct4 during cell cycle in embryonic stem cells. *Nucleic Acids Res.* 2018;46(13):6544–60. [PubMed: 29901724]
67. Ravindran Menon D, Luo Y, Arcaroli JJ, Liu S, KrishnanKutty LN, Osborne DG, et al. CDK1 Interacts with Sox2 and Promotes Tumor Initiation in Human Melanoma. *Cancer Res.* 2018;78(23):6561–74. [PubMed: 30297536]
68. Ishidate T, Elewa A, Kim S, Mello CC, Shirayama M. Divide and differentiate: CDK/Cyclins and the art of development. *Cell Cycle.* 2014;13(9):1384–91. [PubMed: 24675894]
69. Lytle NK, Barber AG, Reya T. Stem cell fate in cancer growth, progression and therapy resistance. *Nat Rev Cancer.* 2018;18(11):669–80. [PubMed: 30228301]
70. Clarke MF, Fuller M. Stem cells and cancer: two faces of eve. *Cell.* 2006;124(6):1111–5. [PubMed: 16564000]
71. Wong DJ, Liu H, Ridky TW, Cassarino D, Segal E, Chang HY. Module map of stem cell genes guides creation of epithelial cancer stem cells. *Cell stem cell.* 2008;2(4):333–44. [PubMed: 18397753]
72. Zhang J, Wang X, Li M, Han J, Chen B, Wang B, et al. NANOGP8 is a retrogene expressed in cancers. *FEBS J.* 2006;273(8):1723–30. [PubMed: 16623708]
73. Nechushtan H, Hamamreh Y, Nidal S, Gotfried M, Baron A, Shalev YI, et al. A phase IIb trial assessing the addition of disulfiram to chemotherapy for the treatment of metastatic non-small cell lung cancer. *Oncologist.* 2015;20(4):366–7. [PubMed: 25777347]
74. Fu Y, Chang H, Peng X, Bai Q, Yi L, Zhou Y, et al. Resveratrol inhibits breast cancer stem-like cells and induces autophagy via suppressing Wnt/beta-catenin signaling pathway. *PLoS One.* 2014;9(7):e102535. [PubMed: 25068516]
75. Schech A, Kazi A, Yu S, Shah P, Sabnis G. Histone Deacetylase Inhibitor Entinostat Inhibits Tumor-Initiating Cells in Triple-Negative Breast Cancer Cells. *Mol Cancer Ther.* 2015;14(8):1848–57. [PubMed: 26037781]
76. Shi P, Liu W, Tala, Wang H, Li F, Zhang H, et al. Metformin suppresses triple-negative breast cancer stem cells by targeting KLF5 for degradation. *Cell Discov.* 2017;3:17010. [PubMed: 28480051]
77. Wu YH, Chiu WT, Young MJ, Chang TH, Huang YF, Chou CY. Solanum Incanum Extract Downregulates Aldehyde Dehydrogenase 1-Mediated Stemness and Inhibits Tumor Formation in Ovarian Cancer Cells. *J Cancer.* 2015;6(10):1011–9. [PubMed: 26366215]
78. Otto T, Sicinski P. Cell cycle proteins as promising targets in cancer therapy. *Nat Rev Cancer.* 2017;17(2):93–115. [PubMed: 28127048]
79. Schettini F, De Santo I, Rea CG, De Placido P, Formisano L, Giuliano M, et al. CDK 4/6 Inhibitors as Single Agent in Advanced Solid Tumors. *Front Oncol.* 2018;8:608. [PubMed: 30631751]
80. Sittithumcharee G, Supramote O, Vaeteewoottacharn K, Sirisuksakun C, Jamnongsong S, Laphanuwat P, et al. Dependency of Cholangiocarcinoma on Cyclin D-Dependent Kinase Activity. *Hepatology.* 2019.
81. Witkiewicz AK, Borja NA, Franco J, Brody JR, Yeo CJ, Mansour J, et al. Selective impact of CDK4/6 suppression on patient-derived models of pancreatic cancer. *Oncotarget.* 2015;6(18):15788–801. [PubMed: 26158861]
82. Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumour formation by single human melanoma cells. *Nature.* 2008;456(7222):593–8. [PubMed: 19052619]
83. Dai M, Zhang C, Ali A, Hong X, Tian J, Lo C, et al. CDK4 regulates cancer stemness and is a novel therapeutic target for triple-negative breast cancer. *Sci Rep.* 2016;6:35383. [PubMed: 27759034]
84. Bandopadhyay P, Piccioni F, O'Rourke R, Ho P, Gonzalez EM, Buchan G, et al. Neuronal differentiation and cell-cycle programs mediate response to BET-bromodomain inhibition in MYC-driven medulloblastoma. *Nat Commun.* 2019;10(1):2400. [PubMed: 31160565]
85. Han YK, Lee JH, Park GY, Chun SH, Han JY, Kim SD, et al. A possible usage of a CDK4 inhibitor for breast cancer stem cell-targeted therapy. *Biochem Biophys Res Commun.* 2013;430(4):1329–33. [PubMed: 23261434]

86. Oh SJ, Cho H, Kim S, Noh KH, Song KH, Lee HJ, et al. Targeting Cyclin D-CDK4/6 Sensitizes Immune-Refractory Cancer by Blocking the SCP3-NANOG Axis. *Cancer Res.* 2018;78(10):2638–53. [PubMed: 29437706]
87. Kishino E, Ogata R, Saitoh W, Koike Y, Ohta Y, Kanomata N, et al. Anti-cell growth and anti-cancer stem cell activity of the CDK4/6 inhibitor palbociclib in breast cancer cells. *Breast Cancer.* 2019.
88. Ali F, Hindley C, McDowell G, Deibler R, Jones A, Kirschner M, et al. Cell cycle-regulated multi-site phosphorylation of Neurogenin 2 coordinates cell cycling with differentiation during neurogenesis. *Development (Cambridge, England).* 2011;138(19):4267–77.
89. Cretella D, Fumarola C, Bonelli M, Alfieri R, La Monica S, Digiacomio G, et al. Pre-treatment with the CDK4/6 inhibitor palbociclib improves the efficacy of paclitaxel in TNBC cells. *Sci Rep.* 2019;9(1):13014. [PubMed: 31506466]
90. Jin D, Tran N, Thomas N, Tran DD. Combining CDK4/6 inhibitors ribociclib and palbociclib with cytotoxic agents does not enhance cytotoxicity. *PLoS One.* 2019;14(10):e0223555. [PubMed: 31600301]
91. He S, Roberts PJ, Sorrentino JA, Bisi JE, Storrie-White H, Tiessen RG, et al. Transient CDK4/6 inhibition protects hematopoietic stem cells from chemotherapy-induced exhaustion. *Sci Transl Med.* 2017;9(387).
92. Ouyang J, Yu W, Liu J, Zhang N, Florens L, Chen J, et al. Cyclin-dependent kinase-mediated Sox2 phosphorylation enhances the ability of Sox2 to establish the pluripotent state. *The Journal of biological chemistry.* 2015;290(37):22782–94. [PubMed: 26139602]
93. Li L, Clevers H. Coexistence of quiescent and active adult stem cells in mammals. *Science (New York, NY).* 2010;327(5965):542–5.

Highlights

- The cell cycle machinery has a prominent role in stem cell pluripotency and self-renewal.
- Cyclin-dependent protein kinases and their effectors have key roles in control of cell fate decisions.
- The availability of highly-specific inhibitors for components of the cell cycle machinery opens up opportunities for control of stem cell characteristics, reprogramming and disease treatment.

Figure 1. Cell cycle control of somatic cells and pluripotent stem cells.

Cell cycle can be separated into four phases, Gap 1 (G1), DNA replication (synthesis phase, S), Gap 2 (G2) and mitosis (M). A. In mammalian somatic cells such as mouse embryonic fibroblasts (MEFs), the G1 phase is extended and the CDK-cyclin complexes exhibit an oscillatory activity and regulate cell cycle transitions from one phase to another. B. Naïve and primed pluripotent cells undergo rapid cell division with abbreviated G1 phase. Naïve pluripotent cells (mESCs cultured in the presence of LIF and serum) have elevated levels of cyclins E and A throughout the cell cycle. The only CDK activity that show phase-specific regulation is the CDK1-cyclin B complex. When being cultured in the defined medium in the presence of two small-molecule inhibitors of MEK/ERK and GSK3 signaling (2i), naïve mESCs can be converted to the ground-state pluripotent cells and display longer G1 phase with a functional G1 checkpoint. Upon withdrawal of LIF and activation of Activin A and FGF2 signaling, naïve mESCs can be transformed into mEpiSCs and acquire the primed state of pluripotency, which display phase-dependent CDK activity. On the other hand, the primed-state pluripotent cells can be converted to naïve-state pluripotent cells by overexpressing KLF2 and NANOG in the presence of 2i plus LIF and Go6963.

Figure 2. Roles of cell cycle regulators in cell fate commitment in pluripotent stem cells (PSCs). PSCs have abbreviated G1 phase and short duration of cell cycle. The self-renewal property is regulated by cyclin Ds and cyclin E, and their catalytic partners, CDK2, CDK4, and CDK6. PSCs are more responsive to differentiation cues in G1 phase due to a permissive chromatin environment whereas they are refractory to differentiation signals in S, G2 and M phases. Once the cells differentiate toward embryonic germ layers and exit the pluripotent state, their length of G1 phase and cell cycle increases. Spontaneous differentiation can be promoted by cell cycle inhibitors such as p15, p16 or p21. On the other hand, reprogramming efficiency can be enhanced by overexpressing cyclin B1-CDK1, cyclin D-CDK4/6 and cyclin E/A-CDK2 or diminished by overexpressing cell cycle inhibitors such as p15, p16 and p21.

Figure 3: Targeting CSC for cancer treatment by inhibiting CDK4/6 kinase activity.

A. Cyclin D and the kinase partners CDK4/6 promote cancer cell proliferation, and maintain a self-renewal population of cancer cell, the cancer stem cell (CSC). Cyclin D-CDK4/6 through the inactivation of pocket proteins (pRB) allow cancer cell proliferation. In addition, cyclin D-CDK4/6 may facilitate tumor relapse and cancer drug resistance by maintaining the expression of the core stem cell factors, such as OCT4, SOX2 and NANOG. NANOG promoter is controlled by two E2F-binding sites and positively regulated by cyclin D-CDK4/6 activity. Expression of cyclin D in cancer also prevents terminal differentiation of the cancer cells. B. Inhibition of CDK4/6 activity by specific CDK4/6 small molecule inhibitors results in cancer cell division halt and reduction of the self-renewal population of cancer cell.