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ABSTRACT

Cancer research has been devoted toward an understanding of the molecular regulation and functional significance of cell-cycle regulators in the pathogenesis and development of cancers. Cyclin-dependent Kinase 2-associated Protein 1 (CDK2AP1) is one such cell-cycle regulator, originally identified as a growth suppressor and a prognostic marker for human oral/head and neck cancers. Functional importance and the molecular mechanism of CDK2AP1-mediated cell-cycle regulation have been documented over the years. Recent progress has shown that CDK2AP1 is a competency factor in embryonic stem cell differentiation. Deletion of CDK2AP1 leads to early embryonic lethality, potentially through altered differentiation capability of embryonic stem cells. More intriguingly, CDK2AP1 exerts its effect on stem cell maintenance/differentiation through epigenetic regulation. Cancer cells and stem cells share common cellular characteristics, most prominently in maintaining high proliferative potential through an unconventional cell-cycle regulatory mechanism. Cross-talk between cellular processes and molecular signaling pathways is frequent in any biological system. Currently, it remains largely elusive how cell-cycle regulation is mechanistically linked to epigenetic control. Understanding the molecular mechanism underlying CDK2AP1-mediated cell-cycle regulation and epigenetic control will set an example for establishing a novel and effective molecular link between these two important regulatory mechanisms.

KEY WORDS: Cyclin-dependent kinase 2-associated protein 1 (CDK2AP1), cell cycle, epigenetic regulation, oral/head and neck cancer, embryonic stem cells, cancer stem cells.

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Double Edge: CDK2AP1 in Cell-cycle Regulation and Epigenetic Regulation

INTRODUCTION

Recent progress in cancer research has unveiled many important cellular processes potentially related to the pathogenesis of cancers. Despite significant advances in understanding the biology of cancer development, we still need to unravel cellular and molecular mechanisms that will be essential to the invention of clinical strategies for the prevention, treatment, and cure of cancers. It is widely accepted that cancer is a disease of cellular differentiation. Two of the major alterations of differentiation are seen phenotypically as uncontrolled proliferation of cells and/or deregulation of programmed cell death (Hanahan and Weinberg, 2011). Altered proliferation and apoptosis have been significantly correlated to the early pathogenesis of many cancers. As predicted, the most prominent feature of cellular/molecular alterations in cancers is related to cell-cycle regulatory machinery. Molecular mechanisms governing these cellular processes have been extensively studied, and the functional consequences of their deregulation have been demonstrated. However, cellular and molecular heterogeneity in cancers makes it difficult to establish a generalized mechanism that can be utilized for understanding cancer development, and in designing therapeutic strategies for a cure.

Recently, the concept of cancer stem cells has begun to emerge (Visvader and Lindeman, 2008). Cancer cells and stem cells share many key biological properties. A cardinal trait possessed by both cell types is the extensive proliferative potential essential for embryogenesis and tumor development. In this review, we will examine the dual regulatory roles of CDK2AP1 in cell-cycle regulation and in epigenetic control, linking these two important cellular and molecular processes in cancer development and stem cell maintenance. The resemblance in molecular mechanisms between these two biological models and the utility of understanding cell-cycle and epigenetic regulation with a stem cell model will be discussed toward advancing our knowledge in cancer research, in view of the genesis of cancer stem cells.

CDK2AP1 IN CANCERS

The hallmark of cancer is rapid and uncontrolled growth. In head and neck cancers, several key cell-cycle regulatory molecules have been implicated in its pathogenesis. An essential component in cell-cycle transition is the cyclin-CDK complex and Retinoblastoma Protein (RB). Upon RB phosphorylation by the cyclin/CDK complexes on RB, there is a release of E2F, subsequently allowing E2F to transcribe the necessary components for the cell to continue through the G1/S transition. Specifically, RB function is mediated by cyclin E/CDK2 activity through E2F transcriptionally regulating cyclin E. In contrast, CDK4 and CDK6 act upstream of RB, and they inhibit RB function by phosphorylation (Hinds *et al.*, 1992, 1994; Sherr, 1993). Loss of RB function has mainly been implicated in retinoblastomas; however, both down- and up-regulation of RB

function has been observed in head and neck cancer, conferring a greater degree of malignancy and aggressiveness, dependent upon cellular context (Pavelic *et al.*, 1996; Regezi *et al.*, 1999). Down-regulation of RB function obviously allows the cell cycle to remain unchecked and leads to continual cell division and cell proliferation; however, up-regulation may also be detrimental, leading to a decrease in pro-apoptotic signals that are triggered during the cell cycle. In either case, changes in the RB pathway alter cell-cycle transition and allow for greater cancer cell survival. Alteration of these cyclins and CDKs has been observed in head and neck cancers as well.

Nevertheless, there are negative regulators of CDK activity that are an integral part of the cell cycle and the RB pathway. These inhibitors belong to either the CIP/KIP family or the INK family, and function by arresting the cell cycle in the G1 phase. Members of the CIP/KIP family include proteins such as p21^{WAF1}, p27^{KIP-1}, and p57, and members of the INK family include proteins such as p16^{INK4a}, p18^{INK4C}, p19^{INK4d}, and p14^{ARF} (Sherr and Roberts, 1999). For example, p21^{WAF1} functions as a cell-cycle regulator by binding to and inhibiting complex formation of CDK2 and CDK4 with their respective cyclins, thereby down-regulating CDK activity. p21^{WAF1} as a CDK2 inhibitor specifically disrupts the RB pathway, with subsequent effects on down-regulation of E2F activity, leading to cell-cycle inhibition. However, these molecules may have multiple roles—for example, p21^{WAF1} also functions as an inhibitor of DNA synthesis by competing for a binding partner to DNA polymerase δ (Abbas and Dutta, 2009). Down-regulation of these inhibitors has been implicated in oral/head and neck cancer, such as reduction of p27^{KIP-1} or loss of p16^{INK4a} function (Timmermann *et al.*, 1997; Schoelch *et al.*, 1999). However, context may be important for these regulators as well, since p21^{WAF1} has been shown to have both tumor-suppressive and oncogenic properties in oral cancers (Abbas and Dutta, 2009), the tumor-suppressive effects coming from the obvious inhibition of the cell cycle and tight regulation by p53. Nevertheless, cellular senescence by p21^{WAF1} can also lead to the inhibition of apoptosis, since an active cell cycle is necessary for the proper apoptotic signals, similar to the up-regulation of RB discussed above (Abbas and Dutta, 2009), thus allowing head and neck cancer (HNC) another mechanism of altering CDK activity and abrogating proper cell-cycle control. Alteration in cell-cycle control is one of the mechanisms involved in promoting a cancer phenotype. As discussed above, cell-cycle alteration can be accomplished by mutating or altering levels of various molecules in this pathway. Recent studies have implicated a new molecule, CDK2AP1, which is now coming to the forefront as a key cell-cycle regulator that is altered in head and neck cancer (Zolochovska and Figueiredo, 2009a,b, 2010, 2011; Zheng *et al.*, 2011).

Human CDK2AP1 is a highly conserved, ubiquitously expressed gene located on chromosome 12q24 and is a 115-aa nuclear polypeptide that is down-regulated in ~70% of oral cancers (Tsuiji *et al.*, 1998; Shintani *et al.*, 2001). Murine *Cdk2ap1*, with only 3 amino acid deviations from the human *CDK2AP1*, is located at chromosome 5 (Kim *et al.*, 2001, 2005). *In vitro* studies have unveiled cellular roles of CDK2AP1. It functions as an S-phase regulator through 2 important cellular partners:

CDK2 and DNA polymerase- α /primase (Matsuo *et al.*, 2000). CDK2AP1 has also been shown to play a role in TGF- β -induced growth arrest, cisplatin-induced genotoxicity, and apoptosis (Kohno *et al.*, 2002; Hu *et al.*, 2004; Kim *et al.*, 2005). CDK2AP1 has been shown to form a homodimer, which is the active form for its growth-suppressing effect (Buajeeb *et al.*, 2004; Kim *et al.*, 2005). We have shown that *Cdk2ap1* expression in a xenograft mouse model of head and neck cancer *in vivo* inhibits tumor growth by reducing proliferation and increasing apoptotic indices (Figueiredo *et al.*, 2005).

Modulation of TGF- β and downstream effector molecules have been shown to alter cell proliferation, survival, and differentiation (Massagué, 2008; Padua and Massagué, 2009). A majority of oral carcinomas exhibit reduced expression of TGF- β receptor II (TGF β RII), and only a few tumors show mutations in the members of the TGF- β pathway (Prime *et al.*, 2004). Numerous studies have shown that most primary oral squamous cell carcinomas retain TGF- β signaling, which diminishes as the disease progresses from carcinoma to metastasis (Meng *et al.*, 2011). During this transition, there is a significant decrease in TGF β RII expression, resulting from down-regulation of gene expression without detectable mutations (Paterson *et al.*, 2001). The significance of CDK2AP1 in TGF- β signaling has been examined in normal and malignant keratinocytes (Fig. 1). TGF- β 1 treatment of normal human keratinocytes increased cellular levels of CDK2AP1 mRNA and protein (Hu *et al.*, 2004). Analysis of the data suggests that SMAD induced by TGF- β 1 binds at the proximal promoter of the *CDK2AP1* gene. A significant correlative expression of TGF- β receptor II (TGF β RII) and CDK2AP1 has been found in human oral squamous cell carcinoma (OSCC) tissues (Peng *et al.*, 2006). There was a loss of expression of CDK2AP1 and p21^{WAF1} in OSCC. A decrease in TGF β RII expression had a statistically significant correlation with decreased CDK2AP1 and p27^{KIP-1} expression. Further *in vitro* studies showed that there is a correlative decrease in TGF β RII, CDK2AP1, SMAD2, and pSMAD2/3 in human OSCC lines. It has also been found that OSCC lines resistant to TGF- β 1 failed to induce pSMAD2/3 and expression of CDK2AP1 (Peng *et al.*, 2006). These results provide evidence of a link between the disrupted TGF- β -SMAD signaling pathway and loss of the cell-cycle-inhibitory proteins CDK2AP1, p21^{WAF1}, and p27^{KIP-1} in OSCC, which may lead to resistance of the TGF- β 1 growth-inhibitory effect in OSCC.

The importance of CDK2AP1 is also being recognized in cancers outside of the head and neck. In prostate cancer (PCa), CDK2 inhibitors have been associated with a decrease in PCa cell growth. PCa cells naturally decrease CDK2AP1 expression to confer survival. To understand the mechanisms behind this down-regulation, investigators forced PCa cells to overexpress CDK2AP1, which led to cell-cycle arrest, apoptosis, and reduced invasion capacity (Zolochovska and Figueiredo, 2009a). Also, CDK2AP1 enhanced demethylation at the Androgen Receptor (AR) promoter, subsequently leading to an increase in AR expression and enhanced AR transcriptional activity (Zolochovska and Figueiredo, 2009a). Analysis of these data suggests that CDK2AP1 may restore a tumor-suppressive effect of the AR by modulation of specific transcriptional functions

involved in the AR pathway. Furthermore, CDK2AP1 was shown to reduce chemotaxis to osteoblasts and reduce osteoblastic proliferation (Zolochovska and Figueiredo, 2011). Also altered were signaling pathways involved in prostate cancer/bone cross-talk, suggesting a role of CDK2AP1 in altering the bone microenvironment in favor of a benign state (Zolochovska and Figueiredo, 2011).

In gastric cancer, CDK2AP1 was negatively correlated with stage and invasiveness (Choi *et al.*, 2009). Patients with CDK2AP1 present in their gastric tissue samples had a better life expectancy, while p53 status made no difference (Choi *et al.*, 2009). In another study, CDK2AP1 was shown to play a role in epithelial-to-mesenchymal transition (EMT), determining both cell fate and differentiation (Tsuji *et al.*, 2008). This was determined in the context of hamster pouch carcinoma cells, where CDK2AP1 induction was growth-inhibitory yet increased EMT. This is contrary to our overall understanding of CDK2AP1 as a tumor suppressor. Therefore, cellular context is important in determination of the role of CDK2AP1 as a tumor suppressor or an oncogene.

The role of CDK2AP1 has been delineated in colon cancer. Most colorectal cancers are characterized by alterations in the length of their DNA repeat sequences, known as microsatellite instability. This instability has been considered to alter growth-regulatory elements, allowing for the progression of a cancerous phenotype. Microsatellite-unstable colorectal cancer lines were studied, and an inverse relationship between microsatellite stability and *CDK2AP1* status was found, suggesting that *CDK2AP1* helped keep these regions of DNA stable (Yuan *et al.*, 2003). Analysis of the data suggests that CDK2AP1 has a tumor-suppressor function in colon epithelial cells. CDK2AP1 has been shown to be a key molecule in the cell cycle and may function as a growth suppressor in various cancer settings. It will be essential to assess how CDK2AP1 functions in a developmental context to determine how it is involved in cell-cycle regulation and differentiation. It is in this light that we have recently unraveled a dual role of CDK2AP1 functions in stem cells and in epigenetic control.

CDK2AP1 IN ESC DIFFERENTIATION

Embryonic stem cells (ESCs) possess an unlimited potential for self-renewal and the capacity to differentiate into 3 primary germ layers. It is not surprising that ESCs should have a cell-cycle profile that is significantly different from that of somatic cells, since ESCs have a unique biological function of balancing self-renewal and differentiation. Previous studies have revealed that mouse embryonic stem cells (mESCs) possess (1) a distinctive cell-cycle profile, (2) a rapid rate of cell division, (3) a lack of the RB pathway, (4) different cyclin-CDK levels, and (5) elevated cell-cycle-independent CDK activity (Savatier *et al.*, 1994; Stead *et al.*, 2002). The distinct cell-cycle profile of mESCs includes a truncated G1 phase and a relatively longer S phase (Faast *et al.*, 2004). Cell-cycle time of mESCs is approximately 8–11 hrs, which is only half to a third of the cell-cycle time of somatic cells (Stead *et al.*, 2002). The prominent feature of cell-cycle regulation in mESCs is the lack of the RB pathway

Figure 1. CDK2AP1 in cell-cycle regulation. Studies have shown that TGF- β induces CDK2AP1, and this blocks CDK2 from allowing the cells to pass the G1/S checkpoint. Normally, cyclin D and cyclin E, in conjunction with their respective CDKs, will allow hyperphosphorylation of RB releasing E2F and progression through the cell cycle. CDK2AP1 in this context blocks CDK2's activities in RB phosphorylation, thereby inhibiting the cell cycle. (Adapted from Hu *et al.*, 2004.)

involvement. Hypo-phosphorylated RB is virtually undetectable during the truncated G1 phase in mESCs (Savatier *et al.*, 1994). Moreover, mESCs show a low level of cyclin D and CDK4-associated kinase activity, but show constitutive cyclin E/CDK2 kinase activity (Savatier *et al.*, 1996). During differentiation, there is a robust expression of D-type cyclins and CDK4-associated kinase activity, along with a reduction of cyclin E/CDK2 kinase activity, resulting in G1/S control by the RB pathway (Savatier *et al.*, 1996). Furthermore, mESCs show that individual cyclins stay activated throughout the cell cycle and do not oscillate, and CDK activity is elevated significantly with the absence of CDK-inhibitory molecules (Savatier *et al.*, 1994; Stead *et al.*, 2002; Faast *et al.*, 2004). From these findings, it is suggested that the cell cycle plays a major role in ESC self-renewal and differentiation.

During the normal cell cycle, CDK2AP1 functions as an inhibitor of G1/S transition through down-regulation of CDK2 (Fig. 1) (Matsuo *et al.*, 2000). mESCs with disrupted expression of *Cdk2ap1* showed an altered cell-cycle profile along with increased CDK2 activity, increased proliferation, loss of stem cell differentiation potential, and an altered pRB phosphorylation (Kim *et al.*, 2005). *Cdk2ap1* KO mESCs showed no significant change in G1 population (2–3% alteration), but there was a significant increase in the S phase (up to 13.3%, $p < 0.0001$) and a decrease in the G2/M phase (up to 90%, $p < 0.0003$) (Kim *et al.*, 2005). Compared with WT cells, *Cdk2ap1* KO mESCs showed a two-fold increase in proliferation (Kim *et al.*, 2005). Perhaps more interestingly, *Cdk2ap1* KO mESCs resulted in abrogation of leukemia-inhibitory factor (LIF) withdrawal-induced differentiation and showed a dramatic increase in the phosphorylated

form of RB (Kim *et al.*, 2009a). Furthermore, teratoma formation analysis showed that tumors from *Cdk2ap1* KO mESCs contained only mesoderm lineage tissues and failed to differentiate properly into all 3 lineages (Kim *et al.*, 2009b). Based on these findings, we conclude that CDK2AP1 plays a major role in mESCs differentiation. These findings also explain, in part, the lethality of *Cdk2ap1* KO mice during embryonic development.

EPIGENETIC CONTROL AND CROSS-TALK WITH THE CELL CYCLE

Epigenetics has become a growing field in the scientific community in recent years. Several groups are studying how cells alter expression and phenotype without altering the DNA sequence itself but modifying the DNA with methylation or accessibility due to chromatin structure *via* histone modification (Egger *et al.*, 2004; Jiang *et al.*, 2004). DNA methylation is mainly associated with a down-regulation in gene expression (Tate and Bird, 1993; Newell-Price *et al.*, 2000; Jaenisch and Bird, 2003). Histones that are involved in the overall chromatin structure can be acetylated to allow for a more open structure, or have various degrees of methylation signifying various heterochromatic states and transcriptional status (Bradbury, 1992; Brownell and Allis, 1996). Alteration of these epigenetic marks by various proteins that modify histones as well as alter DNA methylation have been shown to cause cells to both differentiate and de-differentiate, based on cellular context and the types of modifications involved. Acetylation on histones brings a negative charge to histones, which are positively charged (Allfrey *et al.*, 1964). This, in turn, decreases the interaction between the N termini of histones and the negatively charged phosphate groups of DNA. The weakened interaction relaxes the condensed chromatin into a more relaxed structure, which may lead to greater levels of gene transcription (Wolffe and Pruss, 1996). The process of acetylation and deacetylation is catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively. HATs are known to modulate cell-cycle progression through p300/CBP (transcriptional co-activators with the HAT domain) and P300/CBP-associated factor (PCAF) (Nakatani *et al.*, 1996). HDACs have also been implicated in the regulation of the cell cycle (Kouzarides, 1998). However, it is not totally clear how these epigenetic modifying proteins function as a whole, and, depending upon context, the same enzyme can be functioning either in pluripotency or differentiation; one such example is with histone deacetylating enzymes, where inhibition in one context can lead to stem cell renewal, and inhibition in another context can lead to mesenchymal stem cell differentiation (Cho *et al.*, 2005; Dovey *et al.*, 2010). All the mechanisms involved in epigenetic regulation and controlling cell fate are not known, yet there is a growing body of evidence suggesting that cell-cycle regulators may play a partial role in controlling epigenetic status.

RB is well-known as a cell-cycle regulator and tumor suppressor. However, RB has also been shown to interact with several proteins involved with heterochromatin structure, including HP1, Suv39h1, DNMT1, and the SWI/SNF chromatin remodeling complex (Siddiqui *et al.*, 2007). Knocking out RB led to

changes in levels of histone modifications as well as heterochromatin protein dynamics. Similarly, change in p53 status has been shown to alter DNA methyltransferase levels. In p53 knockout mice, DNMT levels have been shown to increase in the thymus and in the liver, which, in turn, led to an increased level of global methylation in these organs (Park *et al.*, 2005). However, in specific regions, there was hypomethylation instead of hypermethylation, suggesting that p53 was involved not only in overall DNA methylation changes, but also in maintenance of specific DNA methylation patterns (Park *et al.*, 2005). In another more molecular study, p53 was mutated and unable to suppress DNMT1 expression, leading to an overall increase in methylation and specific hypermethylation of p16INK4A (Guo *et al.*, 2008). This led to a subsequent decrease in p16INK4A gene expression and potential alteration of the cell cycle (Guo *et al.*, 2008). Analysis of these data together suggests that there is a growing link between cell-cycle molecules and epigenetic control, either by direct interaction between epigenetic regulators and cell-cycle regulators, or by cell-cycle regulators altering the expression of epigenetic regulators. Similar to the above-mentioned findings, CDK2AP1 (originally thought to be only a cell-cycle molecule) is now known to be involved in epigenetics, which will be discussed further below.

CDK2AP1 IN EPIGENETIC REGULATION

From fertilization to gastrulation, ESCs are challenged by an extensive array of signals which include: (1) gene expression changes at stage-specific embryonic development, (2) secreted small molecules and growth factors, (3) appropriate cellular responses, and (4) communication between cells and the surrounding environment (Koledova *et al.*, 2010). One possible mechanism by which ESCs can process an overwhelming number of different signals is through epigenetics. DNA methylation, genomic imprinting, and bivalent chromatin allow many important tissue-specific genes to be primed for expression but 'held back' for an appropriate development time by either DNA methylation and/or histone modification (Spivakov and Fisher, 2007). When a critical time is reached to turn a gene either on or off, DNA methylation regulatory enzymes such as DNMTs and/or nucleosome remodeling complexes such as NuRD can rapidly modify DNA and/or histone to respond to appropriate developmental cues (Tong *et al.*, 1998; Muller and Leutz, 2001; Becker and Horz, 2002). Epigenetic mechanisms are essential for determining stem cell properties by regulating gene expression without altering DNA sequence. As ESCs differentiate, pluripotency-associated factors become silenced, and tissue- and lineage-specific genes become activated. Expression of *Oct4*, for example, is under epigenetic control. *Oct4* expression is down-regulated as mESCs differentiate by increased promoter methylation. An alteration of DNA methylation and histone modification by 5-aza-2-deoxycytidine or trichostatin A (TSA) at the *Oct4* promoter lead to failed stem cell differentiation, which suggests that epigenetics plays an intricate role in stem cell regulation (Tsuji-Takayama *et al.*, 2004).

Recent studies linked CDK2AP1 to epigenetic regulation and stem cell biology. CDK2AP1 has been identified as a core

subunit of the Nucleosome Remodeling and Histone Deacetylation complex (NuRD) (Fig. 2) associated with Methyl CpG Binding Domain proteins 2 and 3 (MBD2 and MBD3) (Le Guezennec *et al.*, 2006; Spruijt *et al.*, 2010). NuRD-mediated gene-silencing has a crucial function in the lineage commitment of mESCs, since this process allows cells to exit pluripotency (Kaji *et al.*, 2006). It has been reported that mESCs lacking MBD3 (a component of NuRD) display a profound defect in differentiation that results in persistent self-renewal (Kaji *et al.*, 2006), a phenotype that is remarkably similar to *Cdk2ap1*-null mESCs. These reports suggest functional collaboration of MBD3 and CDK2AP1 in the NuRD complex toward regulation of ESC differentiation *via* epigenetic mechanisms. The epigenetic role of CDK2AP1 in mESCs is further explained in Deshpande *et al.* (2009). It was found that CDK2AP1 promoted *Oct4* promoter methylation during mESC differentiation by physically interacting with MBD3, and that deletion of *Cdk2ap1* resulted in retention of *Oct4* expression (Fig. 3) (Deshpande *et al.*, 2009). Although further study is needed before we can understand the full extent of CDK2AP1-mediated epigenetic regulation in stem cell self-renewal and differentiation, it is clear that the epigenetic regulatory role of CDK2AP1 may have global genome-wide implications beyond one, yet important, master switch, OCT4. Interestingly, a recent study showed that CDK1 and CDK2 phosphorylated Enhancer of Zeste Homolog 2 (EZH2) that led to a decrease in EZH2 methyltransferase activity, resulting in inhibition of cancer cell invasion (Wei *et al.*, 2011). This suggests that there may be a signaling link between

Figure 2. CDK2AP1 in the nucleosome remodeling complex. A hypothetical model can be established describing how CDK2AP1 may be involved in altering transcription globally in the cell. In this model, a target gene is normally transcribed (top); however, this transcription may shut down due to the incorporation of methylation marks on CpG sites (middle). The establishment of these marks is uncertain, but may involve the function of various DNMTs. CDK2AP1's interaction with members of the nucleosome remodeling complex (NuRD), including MBD2 and 3, HDAC1 and 2, and RB, may alter the chromatin landscape (middle and bottom), subsequently leading to a closed chromatin structure and down-regulation of transcription (bottom).

Figure 3. Schematic for the role of CDK2AP1 as the epigenetic regulator of *Oct4*. A hypothetical model can be established where CDK2AP1 is a core component of the NuRD complex, and it has the ability to down-regulate *Oct4* expression by binding to methylated CpG islands (filled circle = methylated CpG site; open circle = unmethylated CpG site) during mESC differentiation. In undifferentiated mESCs, the NuRD complex is in its inactive state, the promoter of *Oct4* is hypomethylated, and *Oct4* expression remains high (left). Upon LIF removal, mESCs differentiate into embryoid bodies (EBs), leading to *Oct4* promoter hypermethylation, an active NuRD complex, and *Oct4* down-regulation (right). The role of CDK2AP1 as an epigenetic regulator may not be limited to *Oct4*, but could play a global role in both cancer signaling pathways and the pluripotency of stem cells. (Adapted from Deshpande *et al.*, 2009.)

CDKs and EZH2, which epigenetically controls osteogenic differentiation of mesenchymal stem cells (Wei *et al.*, 2011). It will be intriguing to see if CDK2AP1-mediated epigenetic control has any functional correlation to its cell-cycle-regulatory role (Kim *et al.*, 2005).

SUMMARY

In this review, we have examined several lines of evidence demonstrating the significance of cell-cycle regulatory mechanisms in cancer development as well as in stem cell maintenance and differentiation. It is generally considered that cancer cells are quite different from stem cells, since they are generated from fully differentiated cells. However, two important cellular and molecular similarities between cancer cells and stem cells provide scientific justification for how studying stem cell models can be beneficial in understanding cancer biology. First, cancer cells share many molecular pathways that are important in the maintenance and differentiation of stem cells. Second, evidence supports that genesis of cancer cells may involve a de-differentiation process, which eventually causes terminally differentiated cellular phenotypes to revert to a stem-cell-like state. This involves reactivation or inactivation of key molecular and cellular pathways that resemble those in stem cells. In addition, the emerging concept of cancer stem cells provides us with sufficient justification to use stem cells as a proper experimental model to understand the mechanism of the genesis and development of cancers. As reviewed in this article, CDK2AP1 is involved in both cancer development and stem cell fate. Moreover, CDK2AP1 is emerging as one of the key molecules involved in the interplay between cell-cycle regulation and epigenetic regulation. CDK2AP1 may potentially be regulating molecular networks, rendering mutual intra-regulatory influences. It is intriguing that a given cell-cycle regulator, such as CDK2AP1, has a dual role in cell-cycle and epigenetic control.

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