

CDC25A Phosphatase: a Rate-Limiting Oncogene That Determines Genomic Stability

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Abstract

CDC25A is a critical regulator of cell cycle progression and checkpoint response. Overexpression of this cyclin-dependent kinase phosphatase occurs often in human cancers. Our recent genetic studies in the mouse indicate that restricting CDC25A can limit tumorigenesis induced by the HER2/*neu*-RAS oncogenic pathway without compromising normal cell division or viability. These findings offer a sound foundation to justify development of CDC25A inhibitors for antitumor therapy. [Cancer Res 2008;68(5):1251–3]

Introduction

Deregulated cell cycle progression is a hallmark of cancer (1). Regulatory components of the cell cycle machinery often become targets of genetic alterations during carcinogenesis. Cyclin-dependent kinases (CDK) play central roles in promoting cell cycle progression, and uncontrolled activation of CDKs is the driving force of cancer cell proliferation. Overexpression of CDKs and cyclins, as well as down-regulation of CDK inhibitors, is found in a majority of human cancers. High levels of functional redundancy seem to exist among the components of the CDK system, according to remarkably mild developmental phenotypes of mice deficient for each component, such as cyclin D1, D2, D3, E1, E2, CDK2, CDK4, or CDK6 null mice (2). However, recent studies showed critical rate-limiting roles for each of cyclin D1, cyclin E, and CDK4 in transformation and tumorigenesis mediated by other oncogenes (e.g., RAS; refs. 3–6). These observations imply that oncogenic cell cycle progression during tumor initiation requires nonredundant regulation of specific CDK complexes or higher capacity of total CDK activity. CDK activation depends not only on the association with a cyclin subunit but also on proper phosphorylation of the catalytic subunit. CDK-activating kinases mediate phosphorylation of a threonine residue located in the T-loop domain to activate CDKs. In contrast, phosphorylation of the tyrosine and threonine residues located in the ATP binding domain, i.e., Tyr¹⁵ and Thr¹⁴ of CDK1 and CDK2, inhibits the CDK activity. This inhibitory phosphorylation is mediated by the WEE1 and MYT1 kinases, and subsequently eliminated (dephosphorylated) by the CDC25 dual specificity phosphatases (7). Whereas the levels of cyclin B/CDK1 complexes accumulate progressively from early G₂ phase, CDK1 activation at the G₂-M transition depends on the activity of CDC25 phosphatases. Thus, CDC25 phosphatases are critical for timely CDK activation in cell cycle progression. Mammalian cells have three CDC25 proteins, CDC25A, B, and C. The expression patterns of these proteins are

mostly overlapping, and the knowledge on the function of each CDC25 protein has been limited. Studies using cultured cells suggest that CDC25A regulates both G₁-S and G₂-M transitions, whereas CDC25B and CDC25C are involved only in G₂-M regulation.

Recent studies provided convincing evidence that the regulation of CDC25 proteins is important for cell cycle checkpoint in response to DNA damage, acting as effective targets of the checkpoint-signaling pathways. Damage caused by ionizing irradiation, UV light, oxidative stress, and other DNA-damaging agents leads to activation of ataxia-telangiectasia-mutated (ATM) and ATR (ATM- and Rad3-related) kinases, which then activate the checkpoint transducer kinases, Chk1 and Chk2. Chk1 phosphorylates CDC25A on multiple sites including Ser⁷⁶, Thr¹²⁴, and Ser¹⁷⁸. Whereas Ser¹⁷⁸ phosphorylation creates a docking site for 14-3-3 proteins, Ser⁷⁶ phosphorylation promotes subsequent phosphorylation of Ser⁸² and Ser⁸⁸ by undefined kinase(s) (refs. 8, 9). Phosphorylation of Ser⁸² and Ser⁸⁸ facilitates the association with the SCF^{β-TrCP} ubiquitin ligase complex. Thus, the Chk1-mediated priming phosphorylation of CDC25A initiates the multistep process of ubiquitin-mediated proteasomal degradation. The Chk1-dependent degradation of CDC25A is now thought to be a major p53-independent mechanism of cell cycle arrest or delay in G₁, S, and G₂, given the role for CDC25A in activating CDKs throughout the cell cycle. CDC25B and CDC25C are also substrates of CHK1 and/or CHK2. Whereas the significance of CDC25B phosphorylation remains unclear, CHK2-mediated phosphorylation leads CDC25C to association with 14-3-3 and cytoplasmic sequestration.

The cell cycle-promoting action of CDC25A and its role as a p53-independent checkpoint target suggest that the expression level of this phosphatase must be tightly controlled in normal cells. Misregulation of CDC25A levels results in impaired checkpoint response to DNA damage with aberrantly high CDK activity. Impaired checkpoint could lead to genomic instability, a hallmark of cancer. Indeed, CDC25A overexpression has been reported in a variety of human cancers, including breast, liver, esophageal, endometrial, and colorectal cancers, and non-Hodgkin lymphomas (7, 10). Furthermore, CDC25A overexpression often correlates with more aggressive diseases and poor prognosis. Recent studies on *in vivo* roles of the checkpoint pathways suggest that genomic instability caused by checkpoint defects could be a major mechanism of tumor initiation and progression (11), although it is still controversial. CDC25A is an E2F target gene, and its high expression in cancer tissues may simply reflect enhanced proliferation of cancer cells. Therefore, the central questions that awaited experimental evaluation were whether deregulated overexpression of CDC25A is tumorigenic *in vivo* and how altered regulation of CDC25A levels affects cell cycle control and genomic stability during tumor initiation. We addressed these questions by generating and characterizing mouse mammary tumor virus (MMTV)-*CDC25A* transgenic mice and *Cdc25A*-knockout mice, as reported in recent two articles (12, 13).

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CDC25A Overexpression Cooperates with HER2/*neu* or RAS in Mammary Tumorigenesis

MMTV-*CDC25A* transgenic mice exhibit modest alveolar hyperplasia but no spontaneous tumorigenesis in mammary glands, suggesting that *CDC25A* overexpression alone is insufficient for tumor initiation (12). However, MMTV-*CDC25A*;MMTV-*H-ras* double transgenic mice develop mammary tumors with much shorter latency than MMTV-*H-ras* mice (12 versus 20 weeks). This is consistent with a previous study on transformation of cultured fibroblasts (14). The tumor latency in MMTV-*CDC25A*;MMTV-*neu* double transgenic mice is comparable with that in MMTV-*neu* mice; however, mammary tumors grow faster with more invasive characteristics in MMTV-*CDC25A*;MMTV-*neu* double transgenic mice. Importantly, tumor cells with *CDC25A* overexpression display miscoordination of S phase and mitosis, and have severe genomic instability indicated by aneuploidy, deletion of fragile chromosomal regions (e.g., a telomeric region of chromosome 4 including the *Trp73* and *Casp9* loci), and other chromosomal aberrations. The chromosome 4 locus is homologous to human chromosome 1p31-36, a hotspot for cancer-associated alterations. Although it is yet to be determined how these chromosomal aberrations are involved in the aggressive phenotype of *CDC25A*-overexpressing tumors, this study clearly indicates that *CDC25A* cooperates with the *neu-ras* oncogenic

pathway in mammary tumor development and presents the first *in vivo* evidence of oncogenic function of *CDC25A*. Mammary tissues in *Chk1* heterozygous (+/-) mice exhibit similar cell cycle miscoordination with increased expression of *CDC25A* (15), and *Chk1* (+/-) mice are more susceptible to MMTV-*wnt*-induced tumorigenesis (16). These observations support the notion that *CDC25A* down-regulation is a major mechanism of CHK1-mediated checkpoint response and critical for tumor suppression *in vivo*.

CDC25A Is Rate-Limiting for Tumorigenesis Induced by HER2/*neu*-RAS Pathway

To obtain further insight into the roles of *CDC25A* in tumorigenesis, we recently generated a *Cdc25A*-null mouse strain (13). *Cdc25A*-homozygous (-/-) mice die *in utero* by embryonic day 7, indicating that *Cdc25A* is an essential gene for early embryogenesis. This lethal phenotype is in sharp contrast with viable *Cdc25B* (-/-) and *Cdc25C* (-/-) mice with limited developmental defects (17, 18). *Cdc25A*-heterozygous (+/-) mice do not show appreciable developmental defects. The expression levels of *CDC25A* protein in *Cdc25A* (+/-) mouse embryonic fibroblasts (MEF) were significantly reduced and correlated with increased Tyr¹⁵ phosphorylation of CDK1 and CDK2. Although *Cdc25A* (+/-) MEFs display normal kinetics of cell cycle progression, they have enhanced G₂ checkpoint,

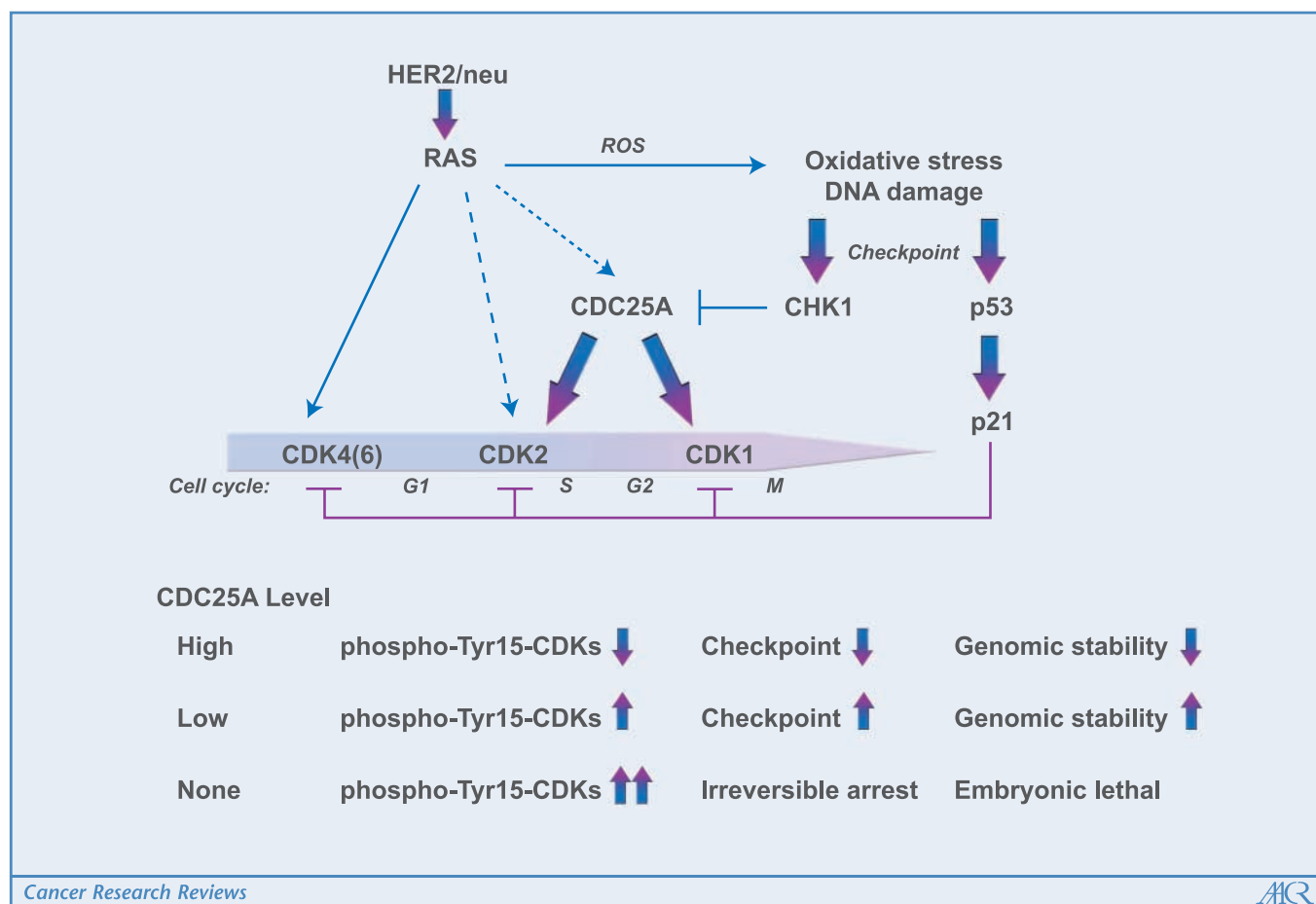


Figure 1. Model for the role of *CDC25A* in proliferation and checkpoint. *CDC25A* phosphatase, which activates CDK2 and CDK1 by removing inhibitory Tyr¹⁵ phosphorylation, plays a key role in the RAS oncogenic pathway. RAS activation results in activation of CDK4(6) and CDK2 and promotes cell cycle progression, which could up-regulate *CDC25A* levels. Aberrant RAS activation also causes checkpoint response with accumulation of ROS, often resulting in cellular senescence. Generally, damage from oxidative stress activates the ATR(ATM)-CHK1 (CHK2) pathway as well as the p53/p21^{CIP1} pathway. CHK1 targets *CDC25A* to ubiquitin-mediated degradation. Thus, the *CDC25A* level is tightly controlled by multidirectional mechanisms. The studies using MMTV-*CDC25A* mice and *Cdc25A*-knockout mice suggest that the *CDC25A* level is a determining factor for the efficacy of checkpoint response, genomic stability, and tumorigenesis induced by the HER2/*neu*-RAS pathway.

arresting more efficiently in response to ionizing irradiation. Importantly, *Cdc25A* (+/-) MEFs show markedly reduced efficacy in undergoing transformation upon expression of activated RAS and a dominant-negative p53 mutant. These data suggest that CDC25A plays a crucial role in RAS-mediated oncogenic transformation. Consistently, *Cdc25A* (+/-) mice exhibit significant resistance to tumorigenesis involving RAS activation. The latency of mammary tumorigenesis induced by MMTV-*neu*- or MMTV-*ras* transgene is markedly prolonged in the *Cdc25A* (+/-) background. Decreased proliferation and increased Tyr¹⁵ phosphorylation of CDK1/2 are observed in premalignant mammary tissues of *Cdc25A* (+/-);MMTV-*neu* mice, compared with those in *Cdc25A* (+/+);MMTV-*neu* mice. Taken together, we concluded that the expression level of CDC25A is rate limiting for *in vivo* tumorigenesis induced by activation of the HER2/*neu*-RAS oncogenic pathway.

CDC25A: a Determining Factor for Genomic Stability and Tumor Progression?

These studies suggest that cells must control CDC25A levels within an appropriate range so that the coordination of cell cycle progression and checkpoint response is maintained (Fig. 1). Aberrantly high levels of CDC25A cause deregulated CDK activation with decreased Tyr¹⁵ phosphorylation and impair checkpoint response, which cooperate with the oncogenic action of RAS. Lower levels of CDC25A result in increased Tyr¹⁵ phosphorylation of CDKs, enhanced checkpoint response, and relative resistance to RAS-mediated tumor initiation. It is well-known that activation of RAS triggers not only proliferation-stimulatory signals (e.g., extracellular signal-regulated kinase activation, p27^{Kip1} down-regulation, and Cyclin D1 up-regulation) but also proliferation-inhibitory or senescence-inducing signals (e.g., p53 activation and p21^{Cip1} up-regulation; ref. 19). The senescence-like response of primary cells to RAS activation is likely to result from oxidative stress with accumulated reactive oxygen species (ROS). RAS activation in p53-deficient MEFs can cause transformation, with the p53-dependent senescence checkpoint abrogated. We hypothesize that RAS activation affects CDC25A levels with a balance of conflicting signals. RAS may activate CDK4/6 and CDK2 by Cyclin D1 up-regulation and p27^{Kip1} down-regulation, respectively. As a consequence, E2F transcription factors are activated, which may transactivate CDC25A expression. On the other hand, RAS-induced oxidative stress may activate not only the p53-dependent pathway but also the CHK1-dependent (p53-independent) pathway, which leads to CDC25A degradation. CHK1 is also an E2F target gene (20)

and seems to down-regulate CDC25A protein levels during S and G₂ even during unperturbed proliferation. If the CHK1-dependent signaling pathway or the CDC25A ubiquitination process is somehow perturbed during RAS-mediated tumorigenesis, CDC25A should become robustly overexpressed at both transcriptional and posttranscriptional levels, and genomic stability will be severely compromised. This hypothesis is consistent with the clinical correlations between CDC25A expression and aggressive forms of cancers. Thus, it is critical to fully define the mode of CDC25A activation during RAS-mediated tumor initiation and the mechanisms of CDC25A overexpression in human cancers.

The rate-limiting role for CDC25A in tumor initiation and progression provides an encouraging scientific support to the ongoing efforts in developing therapeutic strategies targeted on CDC25 phosphatases (7). Multiple CDC25 inhibitory compounds have been developed, such as para-quinonoid compounds derived from vitamin K. The potent para-quinonoid compounds NSC663284, N82685, and IRC083864 can inhibit proliferation of cancer cells and *in vivo* growth of xenografted human tumors. More characterizations about selectivity and toxicity are needed before clinical trials of such compounds are validated. It is plausible that pharmacologic inhibition of CDC25 phosphatases *in vivo* would induce senescence-like irreversible cell cycle arrest, especially in cancer cells that accumulate ROS due to aberrant oncogene activation. Also, partial inhibition of CDC25A might be beneficial for genomic stability and tumor suppression of normal tissues. Another implication from our studies is regarding recent clinical trials of CHK1 inhibitors such as UCN-01 and XL-844. Although these compounds could be a new class of drugs to sensitize cancer cells in patients to cytotoxic drugs or radiation, the inhibition of CHK1 is likely to increase the CDC25A level not only in cancerous tissues but also in normal tissues. This may lead to decreased genomic stability and potential secondary carcinogenesis, together with DNA damage induced by chemotherapy or radiation therapy. Further investigations are needed to fully understand the pathophysiologic roles of the CHK1-CDC25A pathway and better design targeted intervention.

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