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Extra Views

Chk1 versus Cdc25

Chking One's Levels of Cellular Proliferation

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NOTE ADDED IN PROOF

Recently, Krämer et al. have reported that the mitotic phosphatase Cdc25B may also be regulated by Chk1 to ensure proper timing of cell division.20 These data, therefore, suggest that all currently known Cdc25 phosphatase family members are regulated by Chk1 and that Cdc25 deregulation in a likely mechanism for the observed Chk1 haploinsufficiency phenotype in vivo.

ABSTRACT

This review summarizes recent studies which have provided new insight into the mechanisms by which the DNA damage response kinase, Chk1 inhibits the dual specificity phosphatase, Cdc25, and thereby regulates cell cycle progression. Recently, Chk1 has been shown to not only regulate Cdc25A degradation but also its ability to interact with various Cdk complexes through phosphorylation of the carboxy-terminus of the phosphatase. Surprisingly, these effects appear to be specific for Chk1, but not Chk2, which may explain the recently reported in vivo haploinsufficiency phenotype observed in the mammary gland using a Chk1 conditional mouse model.

Cell cycle transitions are tightly regulated by checkpoint pathways. Central to these stress response circuits are the serine/threonine transducer kinases, which act as tumor suppressors in a "sentry-like" fashion, preventing cellular proliferation in the presence of deleterious genetic lesions. One such kinase, Chk1, is capable of exerting critical regulatory control over the Cdc25 phosphatase family of proto-oncogenes, thus preventing inappropriate cell cycle progression. Accordingly, Chk1 has been implicated in regulating entry into S-phase, plays a critical role in monitoring the fidelity of DNA replication, and prevents entry into mitosis in the presence of DNA damage.

A highly conserved eukaryotic protein, Chk1, following activation, acts to repress several key agonists of the cell cycle. An increase in Chk1 activity results in the covalent modification of either Cdc25A or Cdc25C, which are thought to have roles in S phase and G_2/M progression, respectively.[1-3] Phosphorylation is thought to result in the degradation of Cdc25A and sequestration of Cdc25C to the cytoplasm.[1-5] These events disrupt the ability for Cdc25 to have access to their Cdk/cyclin substrates. The importance of Chk1 is evidenced by the embryonic lethality observed in Chk1 null mice. Therefore, a mouse model was created in which Chk1 could be conditionally deleted in somatic cells, in order to provide further insight into the mechanisms by which Chk1 regulates cell cycle progression in vivo.

By employing such a mouse model, our laboratory has demonstrated that haploinsufficiency for Chk1 results in severe cell cycle mis-coordination through checkpoint disruption.[6] Chk1 heterozygous tissues displayed characteristics of early S phase and G_2/M simultaneously. Chk1 heterozygosity resulted in haploinsufficiency, as indicated by a compromised S-phase checkpoint, where cells exhibited a six-fold increase in DNA replication after improperly traversing the G_1/S transition. Furthermore, these tissues simultaneously condensed their chromatin prematurely prior to the completion of S-phase, and greater than one-third of these cells arrested in S-phase due to high levels of endogenous DNA damage. In addition, the mitotic kinase complex, Aurora B/INCENP was sequestered to heterochromatin only in those cells with an activated DNA damage response. Homozygous disruption of both Chk1 alleles resulted in a similar, but more severe phenotype, resulting in cell lethality. These results demonstrated a critical role for Chk1 in maintaining checkpoint homeostasis in somatic cells, which was not compensated for by the presence of the closely related kinase, Chk2.

Recently, studies from N. Sagata and his colleagues have provided an explanation for this apparent lack of compensation by Chk2. These investigators have shown that *Xenopus* Chk1, but not Chk2, can inhibit the interaction between Cdc25 and its targets Cdk2/cycE (S phase) and Cdk1/cycB1 (mitosis). This inhibition is brought on by phosphorylation of a Chk1-specific residue, Thr504 (homologous to T507 in humans), present at the carboxy-terminus of xCdc25A.[7] These studies complement an earlier report by Chen et al.,[8] which showed that human Chk1 phosphorylates T507 preventing hCdc25A from associating with Cdk1/cycB1. This suggests that Chk1, in a two-pronged

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fashion in response to DNA damage, can quickly squelch Cdk activity during either S phase or G_2/M, while mediating longer term repression through Cdc25A degradation, to facilitate repair. Furthermore, it appears that there are conserved Thr504-like binding sites present in all Cdc25 isoforms from yeast to human.[7] These results support the in vivo haploinsufficiency data, and suggest that a reduction in the amount of Chk1, but not Chk2, results in the inability to properly regulate the interaction between Cdc25A and its substrates rendering them active during the wrong phase of the cell cycle (Fig. 1). We postulate that the increased levels of Cdc25A observed in Chk1 heterozygous animals resulted in premature chromatin condensation (PCC), possibly as a consequence of erroneous activation of Cdk1/cycB complexes, by Cdc25A during S-phase. Accordingly, Pwinica-Worms and colleagues also found that human cells transfected with a Cdc25A T507A mutant prematurely condensed their chromatin in a manner similar to that observed in our study in Chk1 conditional heterozygous tissues. Considering these results, it seems plausible that Chk1's reduced ability to phosphorylate Thr507 of Cdc25A may both be necessary and sufficient to cause the in vivo haploinsufficiency phenotype observed in Chk1 conditional heterozygous mice.

In our studies, we reasoned that due to the lower levels of Chk1 protein present in tissues, the endogenous amount of Chk1 present was insufficient to saturate all the Chk1 specific phosphorylation sites on Cdc25A resulting in elevated levels of the phosphatase. This assumption is supported by the work of Uto et al., using *Xenopus* eggs expressing various Cdc25A mutants which could only be phosphorylated, presumably by Chk1, at single residues when all other other sites were changed to alanines. The kinetics of degradation for these Cdc25A mutants varied, suggesting that even if one residue remains unphosphorylated, the physiological consequence may result in accumulated Cdc25A and also the retained ability to interact with Cdk complexes.

Interestingly, our data and that of Uto et al., suggest that Chk2 may play less of a role in monitoring S phase progression than previously thought. This is due in part to the observation that in Chk1 conditional heterozygous tissues, the wild type levels of Chk2 are not able to halt DNA synthesis in the presence of DNA damage. Additionally, *Xenopus* and human Chk2 were not able to phosphorylate Thr504 and Thr507 on Cdc25A, respectively.[8] This suggests that Chk1 is the main regulatory kinase of the Cdc25 pathway during both checkpoint intervention and normal cell cycle.

Cdc25C is also regulated by Chk1-mediated Ser216 phosphorylation, resulting in the generation of a 14-3-3 binding pocket.[4] The binding of 14-3-3 proteins to Cdc25C results in its export out of the nucleus. This is thought to prevent Cdk1 activation during G_2/M. Unfortunately, we were unable to directly determine the phosphorylation status of Cdc25C from tissue lysates using currently available reagents. However, it will be important, in future studies to examine the levels, the phosphorylation state, and the localization of Cdc25C in Chk1 heterozygotes. Thus, Cdc25C phosphorylation, at Ser216 by Chk1, may also be playing a critical role in regulating cell cycle progression, but this remains to be substantiated in our mouse model. Importantly, if there is a Thr504-like binding site in Cdc25C, the premature chromatin condensation observed may be due in part to the inappropriate activation of Cdc25C and Cdc25A, both of which are thought to activate Cdk1/cycB complexes during G_2/M. Additionally, it should be noted that several kinases, notably Chk2, have been shown to phosphorylate Cdc25C at Ser216 suggesting that Chk1 may not be essential for regulation of this phosphatase

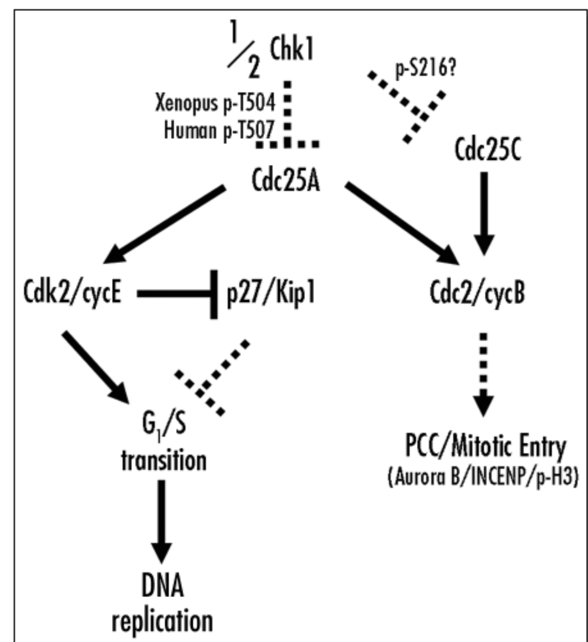

Figure 1. Haploinsufficiency for Chk1 results in Cdc25 deregulation and cell cycle mis-coordination. Dashed lines indicate partial activation or inhibition.

during G_2/M.[4,9,10] The mitotic phosphatases Cdc25A and Cdc25C may be improperly activated in Chk1 heterozygous cells resulting in the incomplete destruction or inactivation of these proto-oncogenes.

Additionally, our studies, performed using the Chk1 conditional mice, have also revealed a potentially interesting role for Aurora B kinase during an activated checkpoint response. Since Aurora B kinase was sequestered to heterochromatic regions only in those nuclei containing DNA damage foci, this implies that sequestration of Aurora B may provide a mechanism to prevent mitotic progression in the presence of DNA damage. The PCC observed may be a direct result of Aurora B being concentrated to these particular regions due to its role in H3 Ser10 phosphorylation during chromatin compaction.[11-13] It is intriguing to speculate that the Aurora B relocalization seen during exposure to DNA damage, not only averts mitotic progression, but also induces mitotic catastrophe due to lagging uncondensed DNA,[11,14] if the G_2/M checkpoint is overridden. These results support a model in which Cdk1 activity is partially upregulated in Chk1 heterozygous mammary epithelial cells due to the elevated levels of Cdc25A, ultimately leading to the presence of localized chromatin condensation as revealed by the presence of Aurora B and phospho-H3 containing foci (Fig. 1). Thus, Aurora B, and possibly phospho-H3 may potentially be used as markers for premature chromatin condensation in mammalian cells during exposure to DNA damage.

The Chk1 inhibitor, UCN-01, is currently in Stage III FDA trials as a potential anti-cancer therapy.[15-17] However, it is obvious from the preceding discussion that endogenous levels of Chk1 and its inherent kinase activity are necessary for cellular homeostasis. Application of Chk1 inhibitors may potentially have deleterious cell cycle effects, not only in tumors cell, but also in "normal" cells, where a 50% reduction of Chk1 activity may induce the haploinsufficiency phenotype described herein. Such an inhibitor may potentially affect highly proliferative cells in organs such as the intestine or the immune system. Alternatively, the application of chemical agents which stall DNA replication, in addition to Chk1 inhibitors

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may circumvent this problem by resulting in the death of both unstable proliferating normal and tumor cells.[18] Of course, appropriate doses will be necessary to limit the toxicity of these inhibitors against wildtype cells. In addition, the interaction between the C-terminus of Cdc25A (hThr507) and the Cdk complexes, which need this region for potential tumorigenic activation, may provide a new molecular target for anti-cancer therapies. As an example, one can envision small peptide mimetics of the Cdc25A C-terminus blocking the binding of Cdc25A to Cdk complexes in tumor cells. Clearly, the haploinsufficiency phenotype observed in conditional Chk1 mice should be taken into account when using UCN-01 and other Chk1 specific inhibitors.[19]

As discussed above, evidence from frog, mouse and human has shown that Chk1 is necessary for the inactivation of phosphatase activity for Cdc25. This finding also supports our in vivo phenotype in that haploinsufficiency for Chk1 may not only lead to an accumulation of Cdc25A but may also prevent efficient phosphorylation of Thr504 on Cdc25A. This indicates that Chk1 acts to rapidly halt the activation of Cdk/cyclin complexes, through Cdc25, presumably, for example, to quickly suspend DNA replication during S phase, in addition to its role in degrading this proto-oncogene in the event of checkpoint activation. It appears that perturbation of either Chk1 or Cdc25A levels results in a situation that primes vertebrate cells towards cell cycle mis-coordination, genomic instability, and subsequently tumorigenesis.

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