Molecular Pathways

The tumor suppressor CHK2: regulator of DNA damage response and mediator of chromosomal stability

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Abstract

CHK2 represents a multi-organ tumor susceptibility gene that encodes for a serine/threonine protein kinase, which is involved in the response to cellular DNA damage. After ATM mediated phosphorylation, the activated Chk2 kinase can act as a signal transducer and phosphorylates a variety of substrates including the Cdc25 phosphatases, p53, PML, E2F-1 and Brca1, which has been associated with halting the cell cycle, the initiation of DNA repair and the induction of apoptosis after DNA damage. In addition, most recent work has revealed another, DNA damage independent function of Chk2 during mitosis that is required for the proper mitotic spindle assembly and the maintenance of chromosomal stability. This novel role involves a mitotic phosphorylation of the tumor suppressor Brca1 by the Chk2 kinase. Based on its role during DNA damage response, Chk2 has been suggested as an anti-cancer therapy target, but given its recently discovered new function and its role as a tumor suppressor it is questionable whether inhibition of Chk2 is indeed beneficial for anti-cancer treatment. However, loss of CHK2 in human tumors might be exploited for novel therapies that are based on synthetic lethal interactions.
Background

The DNA damage checkpoint

In order to maintain genome integrity eukaryotic cells have evolved signaling pathways that are activated in response to genotoxic damage. These so-called checkpoint pathways are responsible to halt the cell cycle to provide extra time for DNA repair or, if the damage cannot be repaired, to induce apoptosis (1). Failure to properly respond to genotoxic insults inevitably results in an accumulation of genetic alterations, which is directly associated with tumorigenesis. The DNA damage checkpoint pathway involves the function of the checkpoint kinase Chk2 (also designated as hCds1 or Chek2) and the structurally distinct, but functionally similar Chk1 kinase (2).

The Chk2 kinase functions in the DNA damage response pathway

Dependent on the type of DNA damage, the Chk2 and Chk1 kinases are phosphorylated and thereby, activated by the ataxia telangiectasia mutated (ATM) or ATM- and Rad3-related (ATR) kinases, respectively. These sensor kinases are recruited to DNA strand breaks by the DNA damage sensor complexes, the so-called MRN (Mre1-Rad50-Nbs1)- and ATRIP (ATR-interacting protein)-complex. While ATR mainly phosphorylates and activates Chk1 after single strand breaks, Chk2 is mainly activated by ATM in response to double strand breaks and this is mediated by phosphorylation of threonine-68 of Chk2. After the initial phosphorylation of Chk2 by ATM, Chk2 homo-dimerizes and achieves its full activation by trans-phosphorylation of the threonine-383 and -387 residues within the activation loop of the kinase (Figure 1) (3, 4).

Once activated, Chk2 can phosphorylate several key substrates including Cdc25C, Cdc25A, p53, Brca1, the promyelocytic leukemia protein (PML) and E2F-1, which is required to mediate cell cycle arrest, DNA repair and apoptosis (2-4). Chk2 phosphorylates the dual specificity phosphatase Cdc25C on serine-216, which promotes its binding to the 14-3-3 protein and results in its sequestering into the cytoplasm. Since Cdc25C is required to activate CDK1 at the G2/M transition in the nucleus this leads to a cell cycle arrest in G2 phase and protects cells from entering mitosis in the presence of DNA damage (5). Similarly, Chk2 phosphorylates the related CDK2 phosphatase Cdc25A resulting in a cell cycle arrest in G1. In this scenario, Chk2 phosphorylates Cdc25A on serine-123, -178 and -292, which promotes its binding to the SCFβ-TrCP -ubiquitin ligase complex and causes its subsequent proteasomal degradation preventing the activation of CDK2 at the G1/S transition (6).

Interestingly, despite this reported significance of Chk2 for the G1 and G2 cell cycle arrest no gross effect on the cell cycle arrest after DNA damage is observed in CHK2-deficient mice, suggesting that this role of Chk2 is not essential (7). Moreover, the function of Chk2 in G1...
and G2 has been questioned in human colon carcinoma cells where no effect on the cell cycle arrest nor on the stability of Cdc25A was observed after homozygous deletion or siRNA mediated depletion of CHK2 (8, 9). A possible explanation for these observations could be the partially redundant function of Chk1 that might share overlapping substrates including Cdc25A and Cdc25C.

The tumor suppressor p53 has been reported to be another key target of Chk2 in response to DNA damage. In fact, knockout mice have been employed to demonstrate that Chk2 phosphorylates p53 on serine-20 and this phosphorylation was shown to disrupt the p53-MDM2 interaction leading to the stabilization and accumulation of p53 after DNA damage (10, 11). Thus, Chk2 has been implicated as a direct regulator of p53 and was suggested to mediate the p53 dependent cell cycle arrest and apoptosis after genotoxic damage. However, other studies using knockout mice or CHK2-deficient human cell lines challenged these results and showed no requirement of Chk2 for the stabilization of p53 after DNA damage (8, 12, 13). Thus, due to these conflicting results the role of Chk2 for the regulation of Cdc25 phosphatases or p53 is presently unclear and suggests that Chk2 is not essential for the cell cycle arrest in response to genotoxic damage.

**The role of Chk2 in DNA repair and apoptosis**

In human cells, Chk2 appears to be involved in DNA repair by phosphorylating and regulating the tumor suppressor breast cancer 1 (Brca1). Upon DNA damage, Chk2 phosphorylates Brca1 on serine-988 and this causes its dissociation from nuclear foci. The soluble and active Brca1 then mediates the error free homologous recombination (HR) DNA repair pathway, while repressing the error prone non-homologous end joining (NHEJ) (14-16). To facilitate DNA repair via HR Brca1 forms a protein complex together with Brca2, which can directly interact with the Rad51 recombinase, a key component of the HR DNA repair pathway (17-19). Presumably, the regulation of Brca1 by Chk2 assists the switch from NHEJ to HR (15). However, this pathway operates only during S-Phase and G2 when the DNA is duplicated and sister chromatids are available. Interestingly, Brca1 also associates with DNA mismatch repair proteins, such as the Msh2-Msh6-complex (20) and Chk2 also interacts with Msh2 (21) suggesting a possible, but yet undefined involvement of Chk2 and Brca1 in DNA mismatch repair.

When DNA damage cannot be repaired the damaged cell can initiate apoptosis, which might also be regulated by the Chk2 kinase. In fact, by regulating p53 it was suggested that Chk2 is required for the induction of p53 dependent apoptosis (12). In addition, Chk2 might also support p53 independent apoptosis by phosphorylating the transcription factor E2F-1 on serine-364, which is associated with its stabilization, transcriptional activation and the induction of apoptosis in a p53-independent manner (22). Moreover, Chk2 can also
phosphorylate the tumor suppressor PML on serine-117, which promotes its pro-apoptotic activity in a p53 independent manner (23).

**Chk2 is required for the maintenance of chromosomal stability and functions during mitotic spindle assembly**

In addition to the established role of Chk2 after DNA damage most recent work from our laboratory revealed a new and DNA damage independent function of the Chk2 kinase in mitosis that is required for the maintenance of chromosomal stability (Figure 1) (24). This novel function of Chk2 might be of particular interest since chromosomal instability (CIN), which is defined as the perpetual gain or loss of whole chromosomes, is a major characteristic of human cancer and can directly contribute to tumorigenesis and tumor progression (25). Importantly, the loss of CHK2 or impairment of its kinase activity is sufficient to induce CIN in diploid human somatic cells, thereby placing CHK2 into the squad of the very few genes associated with CIN in human cancer (24). Since chromosomal segregation defects take place during mitosis it is conceivable that Chk2 might fulfill an important role during mitotic cell division. In fact, Chk2 is required for the proper and timely assembly of the mitotic spindle apparatus, which is a prerequisite for both, the accurate attachment of chromosomes to the mitotic spindle and the following faithful segregation of sister chromatids onto the two daughter cells (24). Thus, CHK2 represents a key tumor suppressor gene involved in the proper assembly of mitotic spindles and the maintenance of chromosomal stability.

Interestingly, the tumor suppressor protein Brca1 is a direct target of the Chk2 kinase and is phosphorylated on serine-988 not only after DNA damage, but also during mitosis in the absence of damage. Intriguingly, this mitotic phosphorylation of Brca1 mediates the mitotic role of Chk2. Indeed, loss of BRCA1 or impairment of its Chk2 mediated phosphorylation causes an improper mitotic spindle assembly and induces CIN in human somatic cells (24). In line with a possible mitotic role, Brca1 localizes to mitotic centrosomes where it might regulate centrosome integrity and spindle assembly, possibly by regulating the ubiquitination of γ-tubulin (26, 27).

**CHK2 alterations in human cancer**

Several studies identified CHK2 as a multi-organ cancer susceptibility gene, which is mutated in both, somatic and hereditary human cancers including breast, colon, prostate and lung carcinomas amongst others, albeit at low frequencies (3, 28). In addition, a loss of the CHK2 locus on chromosome 22q13 has been reported in breast, colorectal, ovarian and brain tumors (29-31) and epigenetic silencing of CHK2 expression was shown in lung cancer (32). Point mutations at I157T and the deletion mutation 1100delC encoding a truncated
Chk2 protein with a reduced or absent kinase activity were shown to be main mutations in human tumors, increasing the risk to develop breast and prostate cancers (33-35) as well as thyroid, bladder, kidney, ovarian and colorectal cancers (36-38). Furthermore, germline mutations of CHK2 have been found in families with the Li-Fraumeni syndrome (LFS) that do not harbor mutations in TP53, suggesting that Chk2 could act as an upstream regulator of p53 (39). However, CHK2 mutations do not account for the cancer predisposition phenotype of LFS as originally thought (40) and concomitant mutations in CHK2 and TP53 have been reported in colon and breast cancer arguing against an exclusive role upstream of p53 (41, 42). Supporting this, the mitotic function of Chk2 required for the maintenance of chromosomal stability appears also to be independent of p53 (24). Furthermore, the loss of CHK2 was found in the majority of human lung adenocarcinomas (24) and this result might be of particular importance, because lung adenocarcinomas are exceedingly induced after the experimental induction of CIN in various mouse models (43).

**Clinical-Translational Advances**

**Targeting the Chk2 kinase for anti-cancer therapy**

Based on its reported functions during the cellular DNA damage response, it was shown that inhibition of Chk2 might increase the therapeutic index of DNA damaging drugs. In fact, antisense inhibition of CHK2 was shown to enhance the apoptotic activity of γ-irradiation and of treatment with the topoisomerase I inhibitor camptothecin (44). Similarly, Chk2 inhibition using siRNA or dominant-negative mutants has been reported to enhance adriamycin induced apoptosis in a colon carcinoma xenograft model by preventing the release of survivin from the mitochondria (45). According to these results, it is expected that small molecule inhibitors of Chk2 including NSC-109555, debromohymenialdisine (DBH), VRX0466617 and EXEL-9844 might also show therapeutic efficacy during anti-cancer treatment (46-49) and in fact, several Chk2 inhibitors such as AZD7762, PF447736 and XL844 were evaluated in phase I clinical studies (48). Unfortunately, most Chk2 inhibitor compounds suffer from unspecificity and inhibit also the Chk1 kinase, which fulfills distinct functions in the G2 DNA damage checkpoint (50, 51). Thus, the anti-cancer efficacy of Chk1/Chk2 inhibitors might not be related to a sole inhibition of Chk2. In contrast to a possible role of Chk2 inhibition in enhancing chemotherapy responses it has also been demonstrated inhibition of Chk2 can lead to a protection from radio- or chemotherapy (46, 52), which might indicate that targeting of Chk2 is not of benefit for anti-cancer treatment. Furthermore, based on the latest results on the mitotic role of Chk2 (24) it should also be considered that the inhibition of Chk2 might be associated with an increase in chromosome missegregation, which may contribute to de novo tumorigenesis in response to therapy.
Treatment of CHK2-deficient human tumors

Despite the conflicting results regarding the therapeutic value of Chk2 inhibition, a key issue is whether the frequent loss of CHK2 in human cancer, especially in human lung adenocarcinomas (24), can be exploited for therapeutic purpose. One possibility may be the use of poly-(ADP-ribose) polymerase (PARP) inhibitors that prevent the repair of DNA single strand breaks via base excision repair and trigger instead the Brca1-mediated homologous recombination (HR) pathway of DNA repair. If both repair pathways are suppressed cells cannot respond to DNA damage anymore and undergo apoptosis. This concept known as “synthetic lethality” has been validated by the use of small-molecule inhibitors for PARP (KU0058684 and KU0058948) that selectively inhibit the cell growth of BRCA1-deficient cells (53). Moreover, since the function of Brca1 in HR requires its phosphorylation by Chk2 (15), PARP inhibitors can act synthetic lethal with CHK2 deficiency (54). Thus, lung adenocarcinomas with their frequent loss of CHK2 might in particular benefit from a treatment with PARP inhibitors, a notion that remains to be tested in clinical trials.

Given the novel function of Chk2 in mitotic spindle assembly, anti-mitotic drugs that target the dynamics of microtubules might also exhibit synergistic effects with CHK2 deficiency in cancer cells. Those drugs include taxanes, epothilones and Vinca alkaloids and are frequently used for anti-cancer treatment (55). It will be of great interest to investigate whether these drugs show an enhanced efficacy in CHK2 deficient cancer cells that already show an impaired formation of mitotic spindles. This attractive hypothesis should be addressed in future experiments and possibly in clinical trials.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
References


Figure Legend

Fig. 1 The role of Chk2 in the DNA damage response and in the regulation of mitosis.
Left panel: The Chk2 kinase is activated by the ATM kinase by phosphorylation of the threonine-68 residue in response to DNA double strand breaks (DSBs). Chk2 achieves its full activation after homo-dimerization by trans-phosphorylation of the threonine-383 and -387 residues located within the activation loop. Subsequently, Chk2 can phosphorylate several key substrates including Cdc25C (on Ser-216), Cdc25A (on Ser-123, Ser-178, Ser-292), p53 (on Ser-20), PML (on Ser-117), E2F-1 (on Ser-364) and Brca1 (on Ser-988) and these phosphorylations are required to mediate cell cycle delay, DNA repair and apoptosis in response of DNA damage.
Right panel: During mitosis and in the absence of DNA damage the active Chk2 kinase can phosphorylate the tumor supressor Brca1 on serine-988. This phosphorylation promotes the accurate assembly of a normal mitotic spindle, which is a prerequisite for a faithful segregation of the sister chromatids and for the maintenance of chromosomal stability.
# Clinical Cancer Research

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